AN ABSTRACT OF THE DISSERTATION OF

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Title: Influence of Soft Wheat Characteristics on Quality of Batter-based Products.

Abstract approved:

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Wheat is a globally traded staple crop. Wheat is important in human diets because of its agronomic adaptability, physical characteristics, functionality for the production of leavened products and nutritional value. Two significant characteristics make wheat an important staple food-crop. First, the proteins present in wheat endosperm have attributes that enable gas retention after the proteins are hydrated and mechanically worked during dough production. Second, a wider variety of products can be made out of wheat compared to other cereals. Wheat quality is defined in terms of suitability for specific end-uses. This is important for breeders, farmers, flour millers, and food producers and consumers. In the U.S. Pacific Northwest (PNW) climatic conditions favor production of soft wheat. Three soft wheat types are planted in the PNW, soft white winter (SWW), soft white spring (SWS), and club (CLUB). Batterbased products are important applications for soft wheats and include a wide range of products such as pancakes and waffles, cakes, and coatings. Pancakes are produced from fluid batters using a single step mixing process and contain sugar concentrations < 30% in their formulations. Cakes are complex food systems where their

classification is based on mixing process to produce the batters and the sugar-flour ratio concentrations in their formulations.

This dissertation is focused on the functionality, analysis, and selection of soft wheat quality traits that affect end-product performance and also developing a methodology to rapidly predict cake quality.

The first study (Chapter 3) was concerned with the functionality of SWW wheats in pancake making. The aim of this study was to observe the differences in genotype and protein concentration on batter flow and pancake making performance of a collection of SWW wheats. Two formulations were used in the study: one based on Finnie et al (2006) called "old" and another based on the AACC-I Approved Method 10-80.01 called "new". The "new" lean formulation had an improved ability to distinguish the performance of different flours compared with the "old" as a result of wider range of pancake diameters. This study showed that pancake making performance would not be optimized by conventional superior high-quality soft wheat flours with soft kernel texture, high break flour yield, and low water-, carbonate-, and sucrose SRCs. From our results it appears that for unchlorinated flours, at least for thicker pancakes, the most appropriate flour would have higher water and sucrose SRCs and be grown under management conditions conductive to higher protein.

The second study (Chapter 4) was a meta-analysis of data collected by the USDA Western Wheat Quality Laboratory (Pullman, WA). This study was done to advance understanding soft wheat quality traits that differentially affect sugar-snap cookie diameter (CODI) and Japanese sponge cake (SC) volume (CAVOL). Principal component analysis (PCA) and partial least square (PLS) regression models were used to obtain useful actionable information from the data. The overall data showed that break flour yield (BKFY) was the single most important trait positively associated with both CAVOL and CODI. SWW wheats showed CVs > 10% for kernel hardness (SKHRD), grain and flour protein concentrations, ash, sucrose-, and lactic acid SRCs. These observations suggested that hardness, protein, ash, and the two SRCs were more sensitive to G&E effects than were the end-product traits that had CVs < 10%.

The third study (Chapter 5) was built on the second study by adding two additional quality tests, oxidative gelation capacity (PeakOXI) and median particle size, to the potential prediction of CODI and CAVOL. Similar to the second study, BKFY was the single most important trait positively associated with both CAVOL and CODI. Virtual selection of SWWs based on either BKFY or SKHRD alone showed (in both the second and third studies) that using these enabled a gain of 134 mL for CAVOL and 0.6 cm for CODI using SKHRD and 122 mL for CAVOL and 0.58 cm for CODI using BKFY (Chapter 5). PeakOXI was significantly correlated with CODI but not with CAVOL. This contrasted with our hypothesis that PeakOXI would affect both products similarly. Notably 13 SWW samples had PeakOXI values higher than 800 cP. PeakOXI values this high have never been observed in soft wheats prior to this

study. This is a valuable genetic resource for further studies that may lead to ways to better exploit oxidative gelation.

The fourth study (Chapter 6) expanded the concepts in previous studies and included the use of a test to measure cake-batter viscosity in an attempt to predict cake quality. This study investigated the relationships between wheat quality traits, cake batters, and cake making quality in three cake types: SC, layer cake (LC), and pound cake (PoC). This study differed from the studies in Chapters 4 and 5 and was similar to Chapter 3 as the samples were fewer but specifically chosen to span the entire range of typical SWW quality. In this study we also developed a viscosity-based method to predict SC and LC quality that takes only eight minutes. This could be useful for screening or selection for cake quality in soft wheat breeding programs. In SC, there were no significant differences in cake quality traits between varieties. However, SC volume had a strong negative association with PeakOXI. For LC, the variety Tubbs, with harder kernel and higher absorption characteristics, had the largest LC volume. In contrast to SC, LC volume was significantly and positively associated with PeakOXI. In PoC, Kaseburg, with the highest protein content, had the largest cake volume. PoC was significantly and positively associated with flour protein concentration suggesting that flour proteins were important for larger volumes and confirming other observations in the literature. In contrast to LC and SC, PoC was not significantly associated with PeakOXI.

The overall impact of the studies reported is:

- For pancakes, the most important soft wheat trait is flour protein concentration. Water-, and sucrose SRCs were potentially useful parameters for predicting pancake quality.
- For SC and sugar-snap cookies, break flour yield was the most important single trait in predicting higher SC volumes and larger cookie diameters.
 Therefore, selection in soft wheat breeding should be focused on kernel hardness and break flour yields as primary factors.

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Influence of Soft Wheat Characteristics on Quality of Batter-based Products

by

Carlos A. Fajardo Centeno

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APPROVED:

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

Carlos A. Fajardo Centeno, Author

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CONTRIBUTION OF AUTHORS

Chapter 3.

Dr. Andrew Ross guided the project and contributed to the manuscript.

Chapter 4.

Dr. Andrew Ross guided the project and contributed to the manuscript. The USDA Western Wheat Quality Laboratory created the data.

Chapter 5.

Dr. Andrew Ross guided the project and contributed to the manuscript. Mr. Carlos Fajardo performed or supervised measurements of oxidative gelation capacity and median particle size parameter in the Oregon State University Cereal Quality Program. The USDA Western Wheat Quality Laboratory created the balance of the data.

Chapter 6.

Dr. Andrew Ross guided the project and contributes to the manuscript. The Wheat Marketing Center assisted with milling the soft wheat samples in the study. The Wheat Marketing Center assisted with manufacturing of Japanese sponge cake and with cake measurements.

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Chapter : 1 General Introduction

1.1 Background

Wheat is cultivated worldwide and is one of the "cereals", a classification that also includes rice, barley, sorghum, rye, oats, and millet. Wheat has been, and remains, important in human diets because of its agronomic adaptability, physical characteristics, functionality for the production of leavened products and nutritional value. Two significant characteristics make wheat an important staple food-crop. First, the proteins present in wheat endosperm have attributes that enable gas retention after the proteins are hydrated and mechanically worked during dough production. Second, a wider variety of products can be made out of wheat compared to other cereals (Curtis, 2002; Wrigley, 2009). Wheat quality is defined in terms of suitability for specific end-uses. This is important for breeders, farmers, flour millers, and food producers and consumers. In soft wheats, the important quality traits include kernel hardness, protein concentration in grain and flour, milling yields, and absorption characteristics of the flour. Kernel hardness is the most fundamental quality factor in soft wheats and produces flours with finer particles and low damaged starch during the milling process. Protein concentration in grain and flour tend to be low in soft wheats. Also, the formation of a viscoelastic gluten network is not desirable for soft wheat end-products such as cookies and cakes and low inherent gluten strength and low protein assist in avoiding this outcome. In the U.S. Pacific Northwest (PNW) climatic conditions favor production of soft wheat. Three soft wheat types are planted in the PNW, soft white winter (SWW), soft white spring (SWS), and club (CLUB). Soft wheat in the PNW is important for export markets such as South

Korea and Japan where one of the important applications of soft wheat is in sponge cake manufacture.

This dissertation is focused on the functionality, analysis, and selection of soft wheat quality traits that affect end-product performance and also developing a methodology to rapidly predict cake quality performance.

Soft wheat applications

Soft wheat applications include a wide range of products such as cookies, crackers, noodles cakes, donuts, pancakes or waffles, and coatings. Water content in soft wheat product formulations range from low to high, therefore creating process intermediates that range from doughs to semi-viscous or viscous batters (Kiszonas et al., 2015; Bettge et al., 2007; Choi et al 2013; Pareyt et al., 2008). Sugar-snap cookies are used to test the quality of soft wheats. These are dough-based products. Flour, sugar, and fat are the main ingredients and sugar-snap cookies have low water content (Pareyt et al., 2008)

Batter-based products include a wide range of products such as pancakes and waffles, cakes, and coatings. Pancakes are produced from fluid batters and are ideally made from soft wheat flours and sugar concentrations < 30% (Kweon et al. 2011). In appearance, pancakes are round and flat with an average height of 1 to 2 cm and a diameter between 10 to 20 cm (Finnie et al., 2006). Cakes are also batter-based products. Consumer acceptance is based on sweetness of taste, and a light and fluffy structure (Chesterton et al., 2013). No specific definition of cakes is agreed on because of the variety of

ingredients and processes used to make these products (Tireki, 2008). However, cakes can be classified using two categories: the ingredients used in the formulation and the mixing process (Wilderjans et al., 2013). Based on ingredients, cakes can be classified as high and low ratio. The high ratio classification is based on the amount or ratio of sugar being higher than the amount or ratio of flour in the formulation. Examples of high ratio cakes are Japanese sponge cakes and layer cakes. Japanese sponge cakes are important because they are also used to test the quality of soft wheat flours. The low ratio classification is based on the amount or ratio of sugar being lower or equal than the amount of ratio of flour in the formulation. In this category pound cakes are an example. Based on mixing processes, cakes can be classified as creaming type or foaming (meringue) type. This classification is based on how the sugar is dissolved or dispersed to produce the batter (Wilderjans et al., 2010). In the creaming process, sugar and fat are first whipped to form a light and aerated paste before the eggs, flour, and remaining ingredients are added and mixed to form a batter. In the foaming process, there are two steps: first, eggs and sugar are whipped to produce a thick foam, and second, sifted flour is folded into the system to form a batter.

Control of batter viscosity is very important because it impacts end-product performance. Flour absorption characteristics are crucial parameters and have a significant influence on viscosities of doughs and batters.

Solvent retention capacity

Solvent retention capacity (SRC) is a method (AACC International Approved Method 56-11.02) developed to measure flour absorption characteristics and to predict flour performance on baked products. The SRC test measures the individual contributions of the major polymer components by exploiting their capacity to solvate and entangle, rather than dissolve, in a suitable solvent. These entangled polymeric networks can swell to various degrees, and to different degrees in different solvents and the extent of swelling can be measured by changes in volume or weight. In the case of SRC on flour the increase in weight of retained solvent is measured (Kweon et al., 2011). The SRC test uses four different solvents: water, 50% w/w sucrose, 5% w/w sodium carbonate, and 5% w/w lactic acid (Kweon et al., 2011). Water-SRC is associated with the overall swelling properties of the polymers. Sucrose-SRC is associated with swelling properties of arabinoxylans (AX) and gliadins. Sodium carbonate-SRC is associated with swelling properties of damaged starch. Lactic acid-SRC is associated with swelling properties of glutenin (Kweon et al., 2011). The liquids are called "solvents", rather than solutions, because of evidence that for example, the 50% w/w sucrose-water solvent gives optimum swelling of AX in a non-linear fashion compared to very low or very high sucrose-inwater w/w concentrations (Kweon et al., 2011; Levine and Slade, 1994). The SRC method has been applied to describe performance of soft wheat applications. Superior quality soft wheat is focused on low absorption characteristics of water-, sodium carbonate-, and sucrose-SRCs (Ross et al., 2014)

Oxidative gelation capacity

The oxidative gelation (OG) capacity of AX under oxidative conditions has been widely studied (Morita et al., 1974; Hoseney and Faubion., 1981; Morita et al., 1974; Bettge and Morris, 2007; Carvajal-Millan et al., 2006; Ross et al., 2014). OG appears to be an important contributor in viscosity of soft wheat flours and hence affect product quality in dough-based and batter-based products (Kweon et al., 2009; Bettge and Morris, 2007; Ross et al., 2014). Different methods have been developed to measure the OG in flour slurry conditions. Recently, Ross et al. (2014) reported a new methodology to measure OG using the Rapid Visco Analyzer (RVA) with practical advantages compared to other methods such as Bostwick.

In this thesis, in chapter 3 measured OG capacity test using the method from Ross et al. (2014) to observe relationships of a batter-based product with sucrose-syrup concentration < 30% w/w with a single mixing method. Sugar-syrup concentrations are an important distinction un soft wheat applications (Kweon et al., 2011). In chapter 4 the potential for predicting sugar-snap cookies and Japanese sponge cake quality using standard and routine soft wheat quality tests applied in breeding programs was examined. The sucrose-syrup concentration for these products was > 30%. Chapter 5 was built on Chapter 4 by adding two additional quality tests, OG capacity and direct particle size, to the potential prediction of sugar-snap cookie and Japanese sponge cake quality. Chapter 6 expanded the concepts in previous chapters including standard quality tests, OG capacity, and particle size, and include the use of a test to measure cake-batter viscosity to predict

cake quality. Chapter 6 expanded the concept of cake quality beyond the Japanese sponge cakes including layer and pound cakes.

1.2 Objectives and hypotheses

Chapter 2

Chapter 2 is a review of literature and provides a detailed description of wheat classification, kernel anatomy, kernel composition, kernel physical texture, flour milling, and flour chemical composition (starch, non-starch polysaccharides, proteins, and lipids). Chapter 2 also discusses the control of viscosity in soft wheat applications and gives details of how SRC and OGC measures flour absorption characteristics. Chapter 2 also describes classifications of batter-based products and the major ingredients in their formulations (Eggs, sugar, and chemical leavening agents).

Chapter 3

This study is concerned with functionality of soft wheats in pancake making. The aim of this study was to observe the influence of differences in genotype (variety) and protein concentration on batter flow and pancake making performance of a collection of SWW wheats. Samples for this study were selected to express a contrasting absorption characteristics and OG potentials. Pancakes were processed with two formulations, one ("old") with egg, soy, dairy based on Finnie et al. (2006) and one ("new") without based on AACCI Approved Method 10-80.01. Our hypotheses were that pancake performance would not be optimized by the use of conventionally high quality soft wheat flours with

low water, carbonate, and sucrose SRCs, high break flour yield, and low protein concentration. We also hypothesized that oxidative gelation capacity and flour pasting characteristics would be associated with pancake performance.

Chapter 4

This is a meta-analysis of the USDA Western Wheat Quality Laboratory Generic and Environmental study from 2001 to 2014. The objective of this study was to identify environmental and genetic factors that impacted soft white winter wheats across these years of data. This study was done in part to advance understanding soft wheat quality traits that differentially affect sugar-snap cookie diameter and Japanese sponge cake volume. This study was also conducted in an effort to improve breeding outcomes for the Oregon State University (OSU) soft white winter wheat breeding program. The hypotheses were that environmental and genetic control traits had the same influence for both cookie diameter (CODI) and Japanese sponge cake (SC) volume (CAVOL) in SWW.

Chapter 5

For this study we aim to investigate the relationships between oxidative gelation capacity, particle size distribution, other routinely tested soft wheat quality traits, Japanese sponge cake volume, and sugar-snap cookie diameter. Another goal was to investigate the traits that most affected or could predict either or both Japanese sponge cake volume and sugar-snap cookie diameter. Our hypothesis was that oxidative gelation capacity had the same effect on both sugar-snap cookies and SC.

Chapter 6

In this study we measured cake batter properties and aimed to develop a method to predict cake volume using three different cake-types. The goal of this study was to investigate the relationships between wheat quality parameters, cake batters, and cake making quality in three different cakes: SC, layer cake, and pound cake. Similar to Chapter 3 and in contrast to Chapters 4 and 5, the sample set was chosen to express contrasts in absorption characteristics and oxidative gelation potentials. The study reported in Chapter 6 also investigated the application of novel methods for predicting cake performance across three cake types, rather than the previous focus on SC. Our hypothesis was that cake batter and cake making quality for SC, layer cake, and pound cake could be predicted using a single test. We also hypothesized that batter viscosity and OG capacity would be associated with cake making quality.

Chapter : 2 Literature review

2.1 Wheat.

Wheat is cultivated worldwide except in Antarctic. Wheat belongs to the larger classification "cereals" that also includes maize, rice, barley, sorghum, oats, millet, and rye (Kiszonas, 2012). Throughout history wheat has been an important component of the human diet due to its agronomic adaptability, physical characteristics, and functionality for the production of leavened foods. Wheat also gained importance as a result of its free-threshing trait that made the grain easy to harvest (Wrigley, 2009).

Wheat domestication goes back over 10 millennia. For example, ancient Egyptian graves contained traces of wheat. In ancient Greek culture wheat was crucial, having its own deity, Demeter (Wrigley, 2009). In this sense wheat has become one of the most important food-crops in human history, up to the present.

Two significant characteristics make wheat an important staple food-crop. Firstly, the proteins present in wheat endosperm have attributes that enable gas retention after the proteins are hydrated and mechanically worked during dough production. Secondly, a wider variety of products can be made out of wheat compared to other cereals (Curtis, 2002; Wrigley, 2009).

Wheat belongs to the genus *Triticum* and is a polyploid species, a common outcome of plant evolution. The evolution of wheat was partly a consequence of series of a natural

hybridization events that eventually led to hexaploid wheat (Gustafson et al., 2009; McIntosh, 2004). This evolution goes through the various forms of cultivated wheat: a diploid *T. monococcum* (eikorn), tetraploids *T. turgidum subsp. dicoccum and subsp. durum* (emmer and durum), and the *T. spelta and T. aestivum* (spelt and common wheat). *T. aestivum* contains 21 chromosome pairs (2n = 42) made of three similar genomes (AA, BB, and DD), each genome with seven chromosome pairs.

In botanical terms the wheat grain or kernel is a caryopsis, a dry seeded fruit where the ovary remains attached to the seed coat (Bechtel et al., 2009). In North America wheat kernels have an average length of 8 mm and weigh approximately 35 mg. Wheat kernels vary in size and texture depending on cultivar and location (Delcour and Hoseney 2010).

2.2 Wheat classification.

Wheat is classified into three categories based on traits useful for trading or end-uses purposes: kernel hardness, growth habit, and grain color. For kernel hardness, wheat is classified as hard or soft (Feiz et al., 2008; Jolly et al., 1996; Atwell, 2001). Kernel hardness has a crucial influence on the spectrum of end products that a wheat variety can make. Milling hard wheats into flour requires more energy than milling soft wheats and creates a higher amount of damaged starch compared to soft wheats (Tanhehco and Ng, 2008). Hard wheats are also better suited for dough-based products, such as bread, as a result of higher damaged starch and higher water absorption properties in the resultant flours. In contrast, soft wheats have lower damaged starch levels and as a consequence lower water absorption, which is more desirable in soft-wheat based products such as cookies, crackers, cakes, and pastries.

Based on growing conditions, wheat is classified as winter or spring. Winter wheats are planted in fall and must experience a period of cold temperature (from 0 to 5°C), called vernalization, before they can resume growth and form heads containing the kernels (Atwell, 2001; Curtis, 2002; Worland and Snape, 2001). Spring wheats are planted in spring and do not require vernalization to form heads. Both, winter and spring, wheats are harvested during summer although spring wheats can also be harvested in early fall. In North America, the type of wheat planted depends on climatic conditions. For instance, spring wheats are mostly planted in regions with severe winters, such as the Dakotas, where the plants cannot survive the extreme weather conditions. Winter wheats are mainly planted in regions with milder winter conditions such as the central and southern plains and the Pacific Northwest where the fall planted wheats survive, but it is cold enough for vernalization (Curtis, 2002).

Based on color, wheat grain is classified into two groups: red and white. The difference in color is due to the presence or absence of pigments in the seed coat (Bechtel et al., 2009; Atwell, 2001). The presence and intensity of red color pigments is controlled by genes in the long arms of chromosomes 3A, 3B, and 3D (McIntosh et al, 1998; Himi and Noda, 2005). In the case of red wheats, there is at least one dominant allele (R) and the redness increases as the number of R increases. In contrast, white wheat carries only recessive alleles (r) and produces no seed coat pigment (Flintman, 1993; Gooding, 2009).

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The red pigments, the polyphenol compounds, phlobaphene, or proanthocyanidin, are produced via the flavonoid biosynthesis pathway (Himi and Noda, 2005).

In the U.S.A. these three traits are used to define five different market classes of wheat: hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), hard white (HW), and soft white (SW). HW and SW include both winter and spring types.

2.3 Wheat kernel anatomy.

The wheat kernel contains three main anatomical structures: endosperm, bran, and embryo. Bran is the tough outer layer of the grain and encloses the endosperm. Bran is approximately 14% of the grain mass and is made of two principal components: pericarp and seed coat (Atwell, 2001, Bechtel et al., 2009). Bran contains high amounts of fiber and ash, 10% and 6 % respectively (Delcour and Hoseney, 2010).

The endosperm is about 80 to 85% of the total grain mass. It is primarily made up of starch, proteins, and arabinoxylans. Endosperm is functionally the most important component of the wheat kernel because refined flour is obtained from this constituent and contains gluten forming proteins and starch. Endosperm consists of two main components: aleurone and starchy endosperm. The aleurone is one-cell layer thick and is located between the bran and starchy endosperm. This layer surrounds the kernel and covers the starchy endosperm and germ. The aleurone layer is high in enzyme activity, ash, protein, total phosphorus, phytate phosphorus, and lipid contents. (Atwell, 2001; Delcour and Hoseney, 2010; Bechtel et al., 2009). Starchy endosperm is the largest

component in wheat and is located in the center of the grain. Furthermore, the starchy endosperm is the component with the greatest physicochemical and caloric value. The endosperm is made of cells in which the starch granules are embedded in a protein matrix and the arabinoxylans are located in the remnant cell walls (Evers and Millar, 2002; Delcour and Hoseney 2010).

The germ is located on the lower dorsal side of the caryopsis and represents 2 to 4% of the total grain mass (Bechtel et al., 2009; Evers and Betchtel, 1998). The germ is rich in protein (25%), sugar (18%), oil (48%), and ash (5%) (Delcour and Hoseney, 2010). The germ is made of two main components: embryonic axis and scutellum. The embryonic axis is the rudimentary root and shoot. The scutellum serves as a storage organ and also transports nutrients from the endosperm to the embryo during germination (Bechtel et al., 2009; Evers and Bechtel, 1998; Posner and Hibbs 1997)

2.4 Kernel texture.

Mechanical properties of wheat grains are typically evaluated based on kernel texture, also called grain hardness. Kernel texture is defined as the physical resistance of wheat kernels to a crushing or shearing (Carson and Edwards, 2009). In other words, kernel texture is measured as the level of hardness or softness and is important in determining milling properties of wheat grains. Furthermore, kernel texture is one of the most important traits determining end-uses and commercial trade of wheat because of its effects on the functionality of wheat. Kernel texture can be measured using the single kernel characterization system (SKCS). The SKCS measures the force required to crush sample wheat kernels (50 to 300 kernels) and reports the outcome as hardness index average over all kernels (Bechtel et al., 2009; Gaines et al., 1996; AACC-I approved method 55-31.01).

Kernel texture in wheat is related to the adhesion between starch granules and the protein matrix in the endosperm. In soft-wheats this interaction is weak while in hard-wheats it is strong. This interaction is mediated through proteins called friabilins (Mikulikova, 2007). Friabilins have a molecular weight of 15K and are found on the surface of water-washed starch granules in higher concentrations in granules extracted from soft wheats (Mikulikova, 2007). Friabilin proteins are comprised of three polypeptides: puroindolines a and b (pin-a and pin-b), and grain softness protein-1 (Gsp-1). Pin-a and pin-b are the principal components of friabilins. In wheat pin-a and pin-b are located on starch granule surfaces. Previous studies showed that kernel texture in soft wheats is better correlated with pin-b than pin-a content (Igrejas et al., 2001; Mikulikova, 2007). Furthermore, the absence or modification of either or both pin-a and pin-b leads to strong starch-protein adhesion. Therefore, pin-a and pin-b polypeptides are crucial determinants of kernel texture of wheats and these granule-surface proteins are also present in higher amounts in soft wheats compared to hard wheats (Mikulikova, 2007).

2.5 Wheat flour milling.

Milling is the grinding of the grain to powder. Modern roller milling is a dry-grinding process that physically separates the three anatomical structures of grain (bran, endosperm, and germ) as cleanly as possible, concurrently reducing the endosperm into

flour sized particles less than 150 μ m (Tanheco and Ng, 2008). The milling process transforms cereal grains into desirable and palatable food ingredients and is affected by wheat quality. Therefore, selection of the appropriate wheat type is important to satisfy quality requirements of customers (Posner and Hibbs, 1999) while a production of high amounts of refined-flour from a given amount of wheat is economically desirable. This is commonly referred to as extraction rate (Bass, 1988; Posner, 2009; Tanheco and Ng, 2008).

Prior to milling, a cleaning step is necessary to remove all debris and hazardous material that could reduce flour quality or damage the mill. After cleaning, water is added to wheat kernels, which is called tempering. The objective of tempering is to toughen the bran, keeping it in larger flakes, resulting in reduced amounts of small bran particles that could later contaminate the flour (Tanheco and Ng, 2008). Also, tempering softens the endosperm so it can be reduced to flour particle size more easily. The amount of water added in tempering depends on the initial moisture and hardness of the grain. In general, hard wheats require more water and tempering time than soft wheats. Soft wheats are commonly tempered around 14 to 15% moisture whereas hard wheats are tempered around 16% moisture and tempering time is between 8 to 24 hours before milling (Tanheco and Ng, 2008; Posner and Hibbs, 1999). Tempering for experimental milling is based on approved methods (AACC-I approved method 26-10.02; AACC-I Approved method 26-95.01)

The next step is grinding. Grinding in roller milling has two principal systems running in series: break and reduction. The break system rips open the kernel and separates the endosperm from the bran and germ as cleanly as possible. This is accomplished using pairs of corrugated steel rolls (break rolls) turning at different speeds in opposite directions (Figure 2.1). Final products of the break system are bran, germ, coarse endosperm (middlings), and break flour. The particle size of break flour is lower than 150 µm. Middlings from the break system are transferred to the reduction system to reduce the particle size to flour fineness and remove the final particles of fine bran. The reduction system is similar to the break system with the difference that the rolls are generally smooth. Multiple passes through different reduction rolls are used to gradually reduce the middlings to the desired particle size (lower than 150 µm). Final products of reduction system are shorts (particles of bran) and reduction flour. The flours obtained in break and reduction system can be combined to obtain "straight grade" flour.

Figure 2.1- Diagram of Brabender Quadramat laboratory mill with different mill fractions (Ross, 2013).



During milling, a proportion of the starch granules are physically damaged. Soft wheat produces less damaged starch than hard wheats. The difference in damaged starch amongst wheats is due to the weaker interactions between starch and protein in soft wheats (explained above). Soft wheat flours have between 2 to 4% of damaged starch whereas hard wheat flours have between 6 to 12% (Stauffer, 2007).

2.6 Chemical composition of the wheat kernel.

2.6.1 Starch

Starch is a carbohydrate with unique physical, chemical, and nutritional properties that makes it different from other carbohydrates. Starch is the fundamental food reserve in plants and provides about 70 to 80% of the calories of human diet worldwide (BeMiller and Huber, 2008). In wheat, starch is the most abundant carbohydrate: approximately 60 to 70% of the dry weight of the grain mass (Lineback and Rasper 2009; Stone and Morell 2009).

In wheat, starch is found in the endosperm and is formed in amyloplasts. Each amyloplast contains one granule that could be either large or small. Large granules are 25 to 40 μ m in diameter and they are lenticular (Delcour and Hoseney, 2010; Maningat et al. 2009; Stone and Morell, 2009). Small granules are 5 to 10 μ m diameter and they are spherical. On average, large starch granules represent about 7% and small granules about 93% of the total amount of starch granules in wheat grain. However large starch granules represent in average about 73% of the total weight of starch in grain.

Starch granules have the physical property of containing both crystalline and amorphous regions in alternating layers. (Stoddard, 2004; BeMiller and Huber 2008). Starch granules are made up of amylose and amylopectin glucose polymers arranged radially. Amylose is a linear polymer that can form a helix in solution, but is amorphous in the granule. Amylopectin has clustered branches packed in double helices. The packing of the double-helices forms semicrystalline lamellae (Figure 2.2). Raw starch granules are birefringent in polarized light microscopy and this is evidence of their ordered radial arrangement (BeMiller and Huber, 2008).





Amylose and amylopectin consist solely of α -D-glucopyranose units. Glucose units can be linked in two ways: via α -1,4- glycosidic bonds resulting a linear chain, and via α -1,6glycosidic bonds branching points. Typically, wheat starch contains 25% amylose and 75% amylopectin (Stone and Morell, 2009). Amylose is fundamentally linear composed of α -D glucose linked by α -1,4- bonds. The molecular weight of amylose varies between 10^4 to about 10^6 , representing approximately 500 to 200,000 glucose units (Edwards, 2007; Delcour and Hoseney, 2010). However, it appears that some amylose molecules
are branched by α -1,6-bonds, but only as 0.3 to 0.5% of the linkages. The linear chain characteristic of amylose can form a right-handed spiral or helical shape because of the axial-equatorial position of α -1,4-bonds. In this helical shape, hydroxyl groups are present in the outer side and hydrogen bonds in the inner side (Figure 2.3). This is a particular characteristic of amylose because it is able to form complexes with iodine, organic alcohols, and fatty acids. These are helical inclusion compounds called clathrates (BeMiller and Huber, 2008; Delcour and Hoseney, 2010).





Amylopectin is a highly branched molecule composed of α -D glucose linked by linear α -1,4- bonds and branched by α -1,6-bonds (4 to 5% of total glycosidic bonds). The terminal (A) in amylopectin are between 20 to 25 glucose units long. The molecular weight of amylopectin is between 10⁷ and 10⁸ (Delcour and Hoseney, 2010, BeMiller and Huber, 2008). Amylopectin contains three types of chain. A chains are made up of α -1,4-bonds; B chains composed of α -1,4-bonds and α -1,6-bonds; and the C chain consists of α -1,4bonds and α -1,6-bonds plus the reducing end group. Therefore, A chains do not have branches, B chains have branches, and C chains contain branches as well as the reducing end group (Figure 2.4)



Figure 2.4 - Cluster structure of amylopectin. A, B, and C chains.

Starch granules are insoluble at room temperature. However, they are capable capable of absorbing water, but they retain their internal structure (BeMiller and Huber 2008; Vaclavik and Christian, 2008). When starch granules are heated in excess of water, they undergo a process called gelatinization. Gelatinization is a unique property of starch and it is defined as the disruption of the molecular order of granules (BeMiller and Huber, 2008). The primary evidence of the gelatinization process is the lose of internal structure and birefringence and the subsequent irreversible swelling of granules. A further consequence of gelatinization is leaching of amylose molecules. The temperature in which starch gelatinization occurs varies depending on factors such as water:starch ratio, granule type, homogeneity of granules, and presence of other components or co-solutes for example, sugars or lipids (BeMiller and Huber, 2008).

After continued heating of starch granules in excess of water the starch forms a viscous mass or paste as a result of complete granule swelling and disruption (Atwell et al, 2008; BeMiller and Huber, 2008). Pasting is another consequence of gelatinization (Batey, 2007). Pasting temperature also varies depending on amylose:amylopectin ratio, degree of crystallinity, and presence of non-starch polymers such as arabinoxylans (BeMiller and Huber, 2008; Stoddard, 2004; Tester and Morrison, 1990).

Upon cooling, a hot starch paste generally forms a firm, viscoelastic gel. Amylose and amylopectin are able to re-associate into ordered crystalline structures through the formation of junction zones (Atwell, 2001; BeMiller 2007). This process is called retrogradation, and this process occurs faster in amylose due to its physicochemical characteristics. The junction zones will continue to grow and may form a three-dimensional structure. This effect leads to precipitation or gelation depending on factors such as concentration of starch, rate of cooling, and presence of other components (BeMiller and Hubber 2008; Tainter, 2000). As the junction zones grow, water trapped in the system will be expelled by syneresis (Stoddard, 2004). In foods, defects can appear as a consequence retrogradation, for example bread staling, loss of viscosity in food products, and precipitation in soups and sauces.

2.6.2 Non-starch polysaccharides

Non-starch polysaccharides are a group of carbohydrate polymers found in cereal cell walls. In grains, the main types of non-starch polysaccharides are cellulose, arabinoxylans, β -glucans, and arabinogalactans (Delcour and Hoseney, 2010). Non-starch

polysaccharides contrast with amylose and amylopectin in some or all the characteristics: monosaccharide composition, glycosidic linkages, and physicochemical properties. Because of the nature of the glycosidic bonds, non-starch polysaccharides are not digestible by enzymes in the human gastrointestinal tract and thus they are considered to be dietary fiber (Lineback and Rasper, 1998; Delcour and Hoseney, 2010).

2.6.2.1 Cellulose

In plants, cellulose is the major structural polysaccharide. It is a linear homopolymer of D-glucose units linked via β-1,4 glucosidic bonds. Cellulose has a ribbon like conformation that allows a parallel packing of the chains in a three-dimensional structure, which is stabilized through intermolecular hydrogen bonding and van der Waals interactions. This high degree of order makes cellulose very insoluble and with β-linkage resistant to hydrolysis (Delcour and Hoseney, 2010; Stone and Morell, 2009). Cellulose can be dissolved with strong hydrogen-bond-breaking reagents such as lithium chloride (LiCl) in dimethylacetamide (DMA) or N-methylmorpholino-N-oxide (Stone and Morell, 2009). Cellulose is present in wheat grain in a concentration about 2% (dw) and approximately 0.3% (dw) in wheat flour. The concentration in wheat flour depends on the flour extraction from milling process. For instance, patent flour, flour composed of basically pure endosperm, contains little if any cellulose. (Delcour and Hoseney, 2010; Lineback and Rasper, 2009; Saulnier et al., 2007).

2.6.2.2 Arabinoxylans

Arabinoxylans (AX) are the predominant non-starch polysaccharides in wheat grain at a concentration about 6 to 7% of the total mass (Delcour and Hoseney, 2010). AX are found in the cell walls. AX are sometimes referred to as pentosans because they are primarily made up of pentose sugars (Delcour and Hoseney, 2010; Stone and Morrel, 2009; Mares and Stone, 1973). In wheat grain, the bran contains a higher concentration of AX than the endosperm. Wheat bran is between 23 to 32% (dw) AX. In contrast, wheat endosperm is between 1.5 to 2.5% (dw) AX (Pomeranz, 1998).

The AX backbone chain is made up of β -1,4-linked D-xylopyranosyl units. These xylopyranosyl units can be unsubstituted, mono-substituted at O -3, or di-substituted at O-2 and O-3 by α –L-arabinofuranosyl residues. The relative proportions are: unsubstituted 60 to 65%, mono-substituted 12 to 20%, and di-substituted 15 to 30% (Ordaz-Ortiz and Saulnier, 2005). Depending on the tissue of origin, AX can also be substituted with other molecules such as acetic acid, ferulic acid, galactose, p-coumaric acid, and uronic acid (Atwell, 2001; Stone and Morell, 2009). The arabinose/xylose (A/X) ratio substitutions in AX determine conformation characteristics and solubility. Average A/X ratio is between 0.5 to 0.6 arabinose per one xylose molecules. However, values ranging from 0.31 to 1.06 have been reported (Courtin and Delcour, 2002; Dervilly-Pinel et al., 2001; Dervilly et al., 2000).

Based on extractability, AX can be divided into two categories: water unextractable (WU-AX) and water extractable (WE-AX) (Delcour and Hoseney, 2010; Saulnier et al.,

2007). WU-AX have large molecular weights as a result of covalent crosslinking between AX molecules. With alkaline treatment WU-AX crosslinks can be broken and the WU-AX set free from the cell wall matrix: alkali-solubilized AX (Courtin and Delcour, 2002). WE-AX are predominantly in the endosperm and are loosely bound in cell walls. It is reported that one of the main reasons the extractability of WE-AX is the lack of crosslinks with other AX molecules. WE-AX can form highly viscous solutions and this depends on AX chain length and (A/X) substitution ratios (Delcour and Hoseney, 2010; Stones and Morell, 2009; Saulnier et al., 2007).

2.6.2.3 Beta glucans

Beta glucans are linear polysaccharides in cell walls of barley, oat, and wheat (Chawla and Patil, 2010). In barley, beta glucans are the major non-starch polysaccharide and are present in a concentration between 5 to 11% (w/w). In oats beta glucans are present in a concentration between 2 to 9 % (w/w). In wheat, beta glucans are the second most important non-starch polysaccharide but are present in a concentration less than 1% (w/w) (Beredford and Stone, 1983; Lineback and Rasper, 2009; Tiwari and Cummins, 2009; Welch et al., 2000). In wheat, beta glucans are located in the aleurone layer and the starchy endosperm (Bacic and Stone 1981a;b)

Beta glucans are polymers made up of a linear chain of D-glucose linked with both β -1,4 and β -1,3 bonds. Beta glucans are linked in average 70% through β -1,4-bonds and 30% of β -1,3- bonds. In wheat, the ratio of cellotriose to cellotetrose is 4.2 to 4.5 (Li et al., 2006). Beta glucan polymers are more flexible and significantly more soluble than cellulose (Delcour and Hoseney, 2010; Stone and Morell, 2009). Beta glucans have a cylindrical shape consisting of 150,000 glucose units and an average molecular weight of 487 kDa (Chawla and Patil, 2010; Li et al., 2006). Beta glucans are either water extractable or water unextractable. It is reported that the presence of β -1,3- bonds prevents association between beta glucan molecules and thus determines solubility properties of beta glucans (Li et al., 2006). Beta glucans are capable of forming highly viscous solutions or gels at very low concentrations (Delcour and Hoseney, 2010; Anttila et al., 2005; Doublier and Wood, 1995). Beta glucans have importance in the human diet as a result of their beneficial physiological effects on human health. For example, it is reported that beta glucans lower the glycemic and insulin responses (Li et al., 2006).

2.6.2.4 Arabinogalactan-peptides

Arabinogalactan-peptides (AGP) are present in all plant tissues (Stone and Morell, 2009). AGP are water extractable polysaccharides and contain a 15-residue peptide backbone (6-8%) glycosidically linked to the polysaccharide portion (92 – 94%). AGP have a linear backbone of D-galactose with random, short D-galactose branches linked β -1,6. These branches also contain L-arabinose units linked α -1,3 (Van del Buck et al., 2002; Stone and Morell, 2009). Although the general molecular structures of AGP are known, the specific structures of AGP in wheat is still uncertain and can vary between or within varieties (Loosveld and Delcour, 2000). The concentration of AGP in wheat is 0.27 – 0.38% (dw) (Stone and Morell, 2009). Viscosities of AGP solutions remain the same under oxidative conditions such as hydrogen peroxide (Stone and Morell, 2009).

2.6.3 Proteins

Proteins are versatile macromolecules with crucial roles in biological systems. Proteins are fundamental in the human diet and cereals represent an important source of proteins. In food systems, the functionality of proteins depends on their structure and physicochemical characteristics. In the case of wheat, proteins in flour exhibit unique viscoelastic properties giving a desirable functionality in a wide diversity of foods.

Proteins are macromolecular polymers and vary in molecular weight and structure. Each protein is built up of a combination of 21 amino acids linked via peptide bonds (Delcour and Hoseney, 2010; Damoran 2008; Stryer et al., 2002). Amino acids consist of a α -carbon atom attached covalently to a hydrogen atom, amino group, carboxyl group, and side chain R group (Stryeret al., 2002, Damoran 2008, Walstra 2003, Belitz et al., 2004). Except for glycine (Gly), the α -carbon atom in amino acids is asymmetric (chiral carbon). Amino acids are commonly classified depending on their R group. Amino acids can be classified in four distinct groups; acidic (glutamic acid and aspartic acid), basic (lysine, histidine, arginine, and tryptophan), hydrophilic (glutamine, asparagine, serine, and threonine), and hydrophobic (valine, leucine, isoleucine, alanine, phenylalanine, tyrosine, cysteine, proline, methionine, and glycine).

Peptide bonds are formed as a result of condensation between the α -carbonyl group of an amino acid and the α -amino group of another amino acid. A polypeptide is formed when a large number of amino acids are linked together via peptide bonds. The free α -carbonyl group in a polypeptide is the C-terminal and the free α -amino group is the N-terminal. In

general, proteins contain n amino acids and n-1 peptide bonds (Damoran, 2008; Stryer et al., 2002).

The structure of proteins has four levels: primary, secondary, tertiary, and quaternary. The primary structure is the linear sequence of amino acids that are covalently bound via peptide bonds. The secondary structure are the special arrangements of the primary structure: e.g. alpha helixes and beta sheets. Alpha helices are flexible and helical structures form when amino acid residues are twisted. Every twist contains 3.6 amino acid residues and are stabilized via hydrogen bonds formed between the N-H group of one amino acid and the C=O of the preceding amino acid. Beta sheets are extended structures where the C=O and N-H groups are oriented perpendicular to the direction of the chain. Beta sheets are stabilized with H-bonds between segments (intersegment). Two types of β-sheets can be formed: parallel and antiparallel (Figure 2.5). Beta sheets are generally more stable than an alpha helix. For example, proteins with higher contents of beta-sheet have higher denaturation temperatures compared to proteins with higher contents of alpha-helix (Damoran 2008; Delcour and Hoseney, 2010).

Tertiary structure refers to the spatial arrangement of the secondary structures (α -helixes and β -sheets) into a three-dimensional form. In the tertiary structure, α -helixes and β sheets are folded via intra and inter molecular bonds accompanied by a reduction in protein-water interfacial area. Formation of disulphide bonds from two amino acid residues and hydrogen bonds are the major bonds stabilizing tertiary structures.



Figure 2.5 - Antiparallel (a) and parallel (b) beta sheets.

Typically, wheat grain contains between 6 to 20% protein depending on cultivar and growth conditions (Dobraszczyk, 2001, Finney and Barmore, 1948). Wheat endosperm proteins are commonly classified based on solubility and functionality (Delcour and Honesey, 2010; Goesaert et al., 2005; Jenkins and Békés, 2009). Based on solubility properties, endosperm proteins can be classified in four groups: albumins soluble in water, globulins soluble in a salt solution, gliadins soluble in aqueous 70% ethanol, and glutenins soluble in diluted acids. Osborn defined this classification in 1907 and it is still used (Delcour et al., 2012). Wheat endosperm proteins are also classified based on functionality into two groups: non-gluten and gluten-forming proteins. Non-gluten forming proteins (albumins and globulins) represent between 15 to 20% of total wheat protein. (Goesaert et al., 2005; Delcour et al., 2012). Non-gluten forming proteins are present in the outer layers of the wheat kernel and smaller concentrations in the

endosperm. For the most part, they are monomeric and part of a minor group of polymeric storage proteins in wheat (Goesaert et al., 2005).

Gluten-forming proteins have the unique ability to form strong cohesive doughs that are able to retain gas and produce a light structure after baking. Gluten-forming proteins are 80 to 85% of total wheat protein and they are the major seed storage proteins. Glutenforming proteins are present in the endosperm as a continuous matrix. Gluten-forming proteins consist of gliadins and glutenins and vary in molecular size, molecular weight, solubility, and functionality (Dobraszczyk, 2001). When these two classes of proteins are hydrated and under mechanical conditions, they can form a viscoelastic threedimensional network material called gluten. Gliadins contribute viscosity to gluten and glutenins elasticity (Ciaffi et al., 1996).

2.6.3.1 Gliadins

Gliadins are soluble in 70% (v/v) ethanol and represent about 40 to 50% of the total protein content in wheat (Qi et al., 2006; Shewry et al., 2009; Thewissen et al., 2011). Gliadins are monomeric with molecular weights between 30,000 to 80,000 (Goesaert et al., 2005; MacRitchie and Lafiandra, 1997; Delcour et al., 2012). Four different groups of gliadins can be distinguished based on their mobility in acid gel electrophoresis: α -, β -, γ -, ω -. However, due to the similarities in molecular weight between α - and β -, they are classified into one class, α -gliadins (Kuktaité, 2004). Therefore, many authors only classified gliadins into 3 groups: α -, γ -, and ω - types. Gliadins are rich in sulfur because they contain a high number of cysteine residues; α -gliadins contain six cysteine residues and γ -gliadins eight cysteine residues located in positions able to form intra-chain disulphide bonds. This prevents gliadins from forming quaternary structures (Delcour et al., 2012; Delcour and Hoseney, 2010). Conversely, ω -gliadin lacks cysteine residues and cannot form disulfide bonds (Delcour et al., 2012; MacRitchie and Lafiandra, 1997). In comparison with α - and γ -gliadin structures, ω -gliadins have relatively expanded conformations and high molecular weights (70 – 80,000) (Bietz and Wall, 1980; MacRitchie and Lafiandra, 1997). Gliadin structures contain three domains: the Nterminal region of 5 to 14 amino acids, the central domain up to 100 amino acids with repeat sequences organized into one or two motifs comprised mainly of glutamine, prolamine, and hydrophobic amino acids, and the C-terminal non-repetitive domain containing sequences rich in glutamine, lysine, and arginine that include (in the case of α -, and ω -gliadins) 6 to 8 the cysteine residues (Delcour et al., 2012).

2.6.3.2 Glutenins

Glutenins are soluble in dilute acid or alkali solutions and are 30 to 45% of the total protein content in the endosperm (Cornell, 2003). Glutenins have high molecular weights and are able to aggregate mainly through inter-chain disulfide bonds. These characteristics allow glutenins to form polymeric proteins, a profound structural differences compared to gliadins. Glutenins can be separated using a disulphide reducing agent and two fractions are obtained: low molecular weight subunit (LMW-GS) and high molecular weight subunit (HMW-GS) (Delcour et al., 2012; MacRitchie and Lafiandra, 1997). LMW-GS have molecular weight between 30,000 Da to 45,000 Da. LMW-GS have primary and secondary structures similar to α- and γ-gliadins but the difference is

that LMW-GS can form inter-chain disulphide bonds. Furthermore, based on their mobility in sodium dodecyl polyacrylamide gel electrophoresis (SDS PAGE), LMW-GS are differentiated into B, C, and D types (Delcour et al., 2012). Likewise corresponding to their first amino acid residue LMW-GS are categorized as LMW-s, LMW-m, and LMW-i (serine, methionine, or isoleucine respectively) types (Delcour et al., 2012).

HMW-GS have molecular weights between 80,000 to 160,000 Da (MacRitchie and Lafiandra 1997; Delcour et al., 2012; Payne et al., 1980). Two types of HMW-GS are identified based on molecular weight: x-type (83,000 to 88,000 Da) and y-type (67,000 to 74,000 Da) (Delcour et al., 2012). Both x- and y-types have three typical domains: small N- and C- terminals, and a large central domain. N-terminal constitutes about 42 amino acids, C-terminal between 80 to 100 amino acids, and central domain between 600 to 850 amino acid residues (Delcour et al., 2012). (Gianibelli et al., 2001; Delcour et al., 2012). LMW-GS and HMW-GS can be linked via intermolecular disulfide bonds forming polymeric networks with molecular weights of several millions (MacRitchie and Lafiandra, 1997).

Glutenin polymeric networks (GPN) are largely studied due to their significant functionality in wheat flour quality, baking quality, and dough properties (Shewry, 2009). GPN can be isolated as unextractable polymeric proteins (UPP) or in a semi-solid form of gluten macro polymer (GMP). UPP are insoluble in 0.5% of SDS, and are solubilized by subsequent sonication (Gupta et al., 1993). GMP is a complex of aggregated glutenins insoluble in 1.5% (w/v) sodium dodecyl sulfate (SDS) and form a gel layer after ultracentrifugation of wheat flour buffered in SDS solution (Don et al., 2003). Elevated content in wheat flour of GMP and UPP leads to an increase of dough firmness, mixing time, and loaf volume (Weegels et al., 1996). Glutenin properties vary between cultivars (Gupta et al., 1996; Johansson et al., 2001; Lindsay and Skerritt 2000; Singh and MacRitchie, 2001).

As described before, gluten is formed during mixing/kneading the dough. During this mixing process a SH-SS interchange occurs between glutenin proteins. Interchange reactions in glutenin proteins under extension leads to a network with polymers aligned in the direction of extension (Delcour et al., 2012).

2.6.4 Lipids.

Lipids by definition are materials soluble in organic solvents (Delcour and Hoseney 2010; Fenema, 2008). Food lipids are commonly categorized as fats (solids) and oils (liquids) depending on their physical state at room temperature. In wheat, lipids are minor constituents representing approximately 3% of total grain mass and they are distributed all over the grain in the form of oil droplets or spherosomes (membrane-bound oil droplets) (Cornell, 2003). The germ contains the highest concentration of lipids as it is approximately 28.5% crude fat (Delcour and Hoseney, 2010). Wheat grain contains 70% non-polar lipids, 20% glycolipids, and 10% phospholipids of the total lipid content (Delcour and Hoseney, 2010). The major non-polar lipids, triacylglycerols, are found in the germ. Glycolipids and phospholipids are present in their majority in bran and endosperm, but concentrations vary. For example, bran contains higher amounts of

phospholipids compared to glycolipids whereas endosperm contains higher amounts of glycolipids compared to phospholipids.

The endosperm is 1 to 2.5% (w/w) lipids (Morrison; Wrigley et al., 2009; Delcour and Hoseney, 2010). Endosperm lipids can be classified depending on their location and/or extraction methods (Chung et al., 2010). In flour, from pure endosperm, lipids are present as either non-starch or starch lipids. Non-starch lipids are about 60% nonpolar, 25% glycolipid, and 15% phospholipid. Non-starch lipids are classified as free, extractable in petroleum or diethyl ether, or bound, extractable only in cold polar solvent mixtures. Free lipids can further be classified as nonpolar (triglycerides, diglycerides, monoglycerides, fatty acids, and steryl esters) and polar (phospholipids, and glycolipids). Bound lipids are phospholipids and glycolipids. Starch lipids are found in an amyloseinclusion complex and are less functional than non-starch lipids. Starch lipids are classified as non-polar and polar. Polar starch lipids are lysophosphatidylcholines, and lysophosphatidylethanolamines (Chong et al., 2009; Delcour and Hoseney, 2010; Tanhehco et al., 2008; Wrigley et al., 2009).

Although lipids are minor constituents of wheat, small variations can affect wheat-quality attributes. For example, volume and texture of a loaf of bread can be affected significantly with a small variation of lipid content in flour (Macritchie 1981; Payret et al., 2011). Lipids can be oxidized. Lipid oxidation has three phases: initiation, the formation of a free radical, propagation, and termination via formation of non-radical products (Maire et al., 2013). Lipid oxidation can occur enzymatically via lipoxygenase

(Maire et al., 2013) or via auto-oxidation. During storage the level of peroxidized lipids within flour increases over time (Richenauer and Goodman, 2003). Lipid peroxidation degrades wheat-flour quality causing rancidity and thus reducing shelf life.

2.7 Viscosity in soft wheat products

Soft wheat applications include both dough-, and batter-based products. Dough and batters are process intermediates made of a mixture of interacting ingredients. Control of viscosity is very important because it impacts final product performance and consumer acceptance through its effects on sensorial characteristics such as texture and consistency. Viscosity (also known as consistency) can be defined as the resistance of a fluid to flow under an applied force (shear stress). Viscosity in a food system depends on several factors such as moisture content, type and concentration of macromolecules and micro molecules, and how these interact. In particular, molecular weight, size, and flexibility of macromolecules are important factors in determining viscosity characteristics of doughs and batters (Fennema 2008; Walstra 2003). Wheat flours contain a wide spectrum of macromolecules and water contents giving a wide variety of final textures ranging from stiff doughs to thin, low viscosity, batters. For example, viscosity of dough-based products such as cookie doughs is affected by water and macromolecules present in the system such as protein, damaged starch, and arabinoxylan concentrations to give a stiff appearance that is crucial for final properties. In contrast, viscosity of batter-based products such as pancake batter is affected by water, fat or oil, sugar concentration, proteins, starch, and AX to produce a flowable viscosity during cooking.

In hard wheat bread doughs sufficient water and mechanical energy are needed to develop gluten to produce a three-dimensional network capable of trapping fermentable gasses produced during fermentation. In contrast, soft wheat applications such as cookies, cakes, pancakes, and donuts, water content is low and viscosity is controlled throughout appropriate solids concentration and the use of chemical leavening systems (Ross and Bettge, 2009). Also oxidative gels of AX and proteins may directly influence batter viscosity and therefore final product characteristics (Bettge and Morris, 2007). In batter applications, development of gluten is minimized, partly because of the high sugar content in the formulations. Therefore, the control of batter viscosity is important to retain gases produced by leavening agents (Bettge and Morris, 2007).

2.8 Solvent Retention Capacity

Another important attribute controlling final characteristics in soft wheat products is moisture content. In soft wheat flours, the major polymers, proteins, starch, and AX all contribute to water absorption properties. For instance, flours with higher damaged starch are able to absorb more water, which is desirable for bread. In contrast, soft wheat applications, such as cakes and cookies, require low water absorption (Slade and Levine, 1994).

Solvent retention capacity (SRC) is a method (AACC International Approved Method 56-11.02) that emphasizes the functionality of the individual major polymers in flour by exploiting their capacity to solvate and entangle, rather than dissolve, in a suitable solvent. These entangled polymeric networks can swell to various degrees and the extent

of swelling can be measured (Kweon et al., 2011). SRC measures flour quality and functionality and predicts quality of products.

The solvents used in the SRC test are water, sucrose, sodium carbonate, and lactic acid. Water SRC is associated with the overall water holding capacity of all four flour polymeric components. Sucrose SRC emphasizes swelling of AX and gliadins. Sodium carbonate emphasizes swelling behavior of damaged starch. Finally, lactic acid exaggerates swelling behavior of glutenin and may predict dough strength (Duyvejonck et al., 2011).

2.9 Oxidative Gelation Capacity

Oxidative gelation (OG) in flour slurry has been studied for almost a century. Durham (1925) indicated that adding hydrogen peroxide to a flour suspension increased its viscosity (Ross et al., 2014; Hoseney and Faubion., 1981; Moore et al., 1990). The OG mechanism includes AX to AX crosslinking through ferulic acid moieties, protein crosslinking with other protein molecules via dityrosine bridges, and AX crosslinking with protein (Morita et al., 1974; Hoseney and Faubion., 1981; Morita et al., 1974; Bettge and Morris, 2007; Carvajal-Millan et al., 2006; Ross et al., 2014).

Recently numerous works have focused on the impact of OG capacity on flour functionality in soft wheat (Ross et al., 2014; Bettge and Morris, 2007; Kweon et al. 2009). OG capacity appears to be an important contributor in viscosity of soft wheat that may affect product quality in batter-based products and dough systems (Kweon et al., 2009; Bettge and Morris, 2007; Ross et al., 2014). Different methods have been developed to measure the OG capacity in flour slurry conditions. Recently a new methodology using the Rapid Visco Analyzer (RVA) measures OG capacity with practical advantages compared to other methods such as Bostwick. These advantages include temperature control of the system as well as characterizing the wide range of viscosities of the flour-water slurry before, during, and after addition of hydrogen peroxide (Ross et al., 2014).

2.10 Batter based applications

Batters are process intermediates that are viscous fluids made of a mixture of interacting components that after heating (baking) produce porous materials (Meza et al., 2011; Delcour and Hoseney, 2010). Batters contain dispersed phases (air bubbles, oil/fat droplets) and vary in viscosity depending on mixing process and concentration of ingredients in the formulation. The main ingredients used in batter formulations are soft wheat flour, eggs, sugar, fat, leaving agents, salt, and water.

The classification of batter-based products is complicated because of the diversity of ingredients used to make these goods. However, the concentration ratios between the main ingredients (water, flour, sugar, eggs, and fat), viscosity of the formulation, and final thermal processing (e.g., baking, cooking, or deep frying) allow classification of batter-based products such as pancakes, cakes or coatings.

2.10.1 Pancakes

Pancakes are food products made from pourable batters with sugar concentrations below 30%, chemically leavened, and made with soft wheat flours as one of the main dry ingredients (Finnie et al., 2006; Kiszonas et al, 2015; Fajardo and Ross, 2015). In appearance, pancakes are round and flat with an average height of 1 to 2 cm and a diameter between 10 to 20 cm (Finnie et al., 2006).

In North America pancakes are commonly made by pouring an aliquot of liquid batter on top of a hot oiled surface and cooking each side for about 1 to 2 min (Finnie et al., 2006; Kiszonas, 2012). In the pancake making process batter is able to flow without being restricted by a baking pan. Pancake making performance is assessed using traits such as diameter, height, and dome after cooking. Pancake batter viscosity is a critical parameter in determining the quality of the final product. For example, if batter viscosity is too high, pancakes will be thicker than desired, will therefore take longer to cook, and may give undesirable characteristics to the product. In contrast, if batter viscosity is too low, pancakes will have larger diameters and reduced height. Therefore, control of pancake batter viscosity is needed to achieve the correct characteristics in the final product (Finnie et al., 2006). In pancakes, flour absorption properties of the main flour polymers have a significant influence in determining the optimal final characteristics of the product (Finnie et al., 2006; Kiszonas, 2013). For example, pancake manufacturers may specify flour protein content for pancake production. Food services, restaurants, and individual customers use pancake premixes. These premixes are a combination of soft wheat flour,

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shortening, salt, emulsifiers, and various saccharides (Finnie et al., 2006). However, the complex interactions between some ingredients are not well understood.

2.10.2 Cakes

Cakes are an important batter-based product where consumer acceptance is based on sweetness of taste, and a light and fluffy structure (Chesterton et al., 2013). No specific definition of cakes is agreed on because of the variety of ingredients used to make these products (Tireki, 2008). However, cakes can be described as food systems consisting of a wide variety of ingredients mixed into a batter with a high level of liquid and a low to medium viscosity that when baked in cake-pans transforms the batter into a cake (Wilderjans et al., 2013).

Cake batters are oil-in-water emulsions with air bubbles and/or fat particles dispersed into a continuous phase containing dissolved or suspended dry ingredients (Sahin, 2008; Sahi, 2008). The ingredients with major relevance in cake batters are soft wheat flours with low protein content, eggs, sugar, fat or oil, and chemical leavening agents.

Cakes can be classified based on the sugar to flour ratio concentration in the formula and mixing process (Wilderjans et al., 2013). High ratio cakes are made with sugar/flour ratio higher than or equal to 1.0 (layer, angel food, and sponge cakes). In contrast, low ratio cakes contain sugar/flour ratios lower than or equal to 1.0 (e.g., pound cakes). Cakes can also be classified by mixing processes, either creaming or foam (sponge) types (Wilderjans et al., 2013; Conforti, 2006). In the creaming process, sugar and fat are first

whipped to form a light and aerated paste before the eggs, flour, and remaining ingredients are added and mixed to form a batter. In the foaming process, two important steps are needed to produce a batter. First, eggs and sugar are whipped to produce a thick foam. Second, sifted flour is folded into the system to form a batter.

The mixing process is an important step for producing a cake batter because it contributes to the final attributes of the finished product. The mixing of a cake batter involves either a single-stage or multi-stage mixing process. In single-stage mixing, all the ingredients are simultaneously blended and a large amount of air bubbles are incorporated into the batter, which is important for cake texture (Delcour and Hoseney, 2010; Conforti, 2006; Wilderjans et al., 2013). Single-stage mixing is commonly used in cake pre-mixes.

Soft wheat flour is one of the most important ingredients in cake production. Its components play important roles in foam production, emulsion stability, and the baking process (Sahi, 2008). For example, proteins and lipids are good sources of surface-active materials that have the capacity to form air-water and oil-water interactions (Sahi, 2008). This is important for the aeration of the batter because air bubbles are trapped into the system. AX are able to bind eight to ten times their own weight in water, thus AX water absorption affects batter viscosity (Sahi, 2008). Starch has no significant influence in foam properties or stability of the emulsion, but the setting of cakes during baking is partially a result of starch gelatinization. As the starch gelatinizes it becomes able to bind 30% of its weight in water.

2.11 Major ingredients in batter-based products.

2.11.1 Eggs

Eggs are multifunctional ingredients. They are made of two principal components; whites and yolks, whites representing about 65% and yolks 35% of the total mass. Water content in whole eggs is about 75% (Cauvain & Young, 2006). Egg whites are approximately 88% water and 11% protein. The composition of the proteins is diverse because there are more than 40 different types. However, ovalbumins are the proteins with the highest concentration (about 54%) (Wilderjans et al., 2013). Egg yolks contain approximately 50% water, 34% lipid, and 16% protein. The lipid composition of yolks is 55% triglyceride, 28% phospholipid, 5% cholesterol, with minor levels of other lipids (Wilderjans et al., 2013). Yolk-lipids are particularly important because during mixing they are able to form and stabilize emulsions. Also yolk-proteins are flexible and capable of adsorbing at oil-water interfaces (Kiosseoglou and Paraskevopoulou, 2006)

In batter-based products eggs contribute to functionality and physicochemical properties during batter mixing and baking. During mixing, egg white proteins contribute to increase the viscosity and increase the incorporation of air bubbles in the system. Egg yolks contribute to emulsification properties in the oil-water interface. During baking the setting of cakes is partially a result of egg protein coagulation. For example, the setting of egg white involves at least three phenomena: denaturation, aggregation, and gelation (Delcour and Hoseney, 2010). Batter formulations can use either whole eggs or egg whites depending on product characteristics. For example, high-ratio angel food cakes use only egg whites because proteins contribute to foaming properties, whereas low-ratio pound cakes use whole eggs. For pound cake both whites and yolks contribute to physicochemical characteristics of the final product.

2.11.2 Sugar

Sucrose is one of the principal ingredients in baked products. Sucrose is a low molecular weight carbohydrate, has high solubility in water, and is able to form highly concentrated solutions with high osmolality (BeMiller and Huber, 2008). Sucrose confers sweetness and color to batter-based products and its optimum concentration depends on the product. For example, pancake batters have sugar content < 30% whereas sugar content in cake batters is > 30%. Consequently, sugar level has an effect on water activity of batters, influences rheological properties in batters by delaying starch gelatinization, and controls the heat-setting temperature of egg proteins during baking (Sahin, 2008; Delcour and Hoseney, 2010). Another effect of sugar in batters is to lower water activity and thus improve shelf-life of final products by impeding growth of microorganisms.

Sugar also participates in Maillard and caramelization reactions during baking and imparts color into the final product. Maillard and caramelization reactions overlap. However, the activation energies are lower for Maillard reactions and therefore they are the first of both reactions to happen.

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2.11.3 Chemical leavening

In baking products, the production of gas is crucial to obtain a light texture and a porous structure. This is an important physical and organoleptic characteristic because it is leads to a good volume, crumb quality, and contributes to the aesthetic of the final product. Leavening gas in baking products is produced during mixing and early stages of baking process (Pyler, 1988; Köksel and Gökmen, 2008). Two types of leavening systems are commonly used in baked products: microbial fermentation and chemical leavening. In doughs, anaerobic microbial fermentation of sugars produces CO₂ gas and ethanol. Batter-based products most commonly use chemical leavening agents: sodium carbonate, ammonium carbonate, and acids (Delcour and Hoseney, 2010; Pyler, 1988). CO₂ gas is produced by sodium carbonate (also known as baking soda) and it is widely used due to its low cost, absence of toxicity, high purity, and lack of aftertaste in the final product (Pyler, 1988). Chemically, sodium carbonate reacts with an acid to produce CO₂ as follows:

 $NaHCO_3 + HA \rightarrow NaA + CO_2 + H_2O$

 CO_2 is produced in three different forms: free carbon dioxide (CO_2), bicarbonate (HCO_3^{-}), or carbonate (CO_2^{-3}). The proportion of each depends on pH and temperature of the solution (Delcour and Hoseney, 2010). Figure 2.6 shows the effect of pH in production of CO_2 .

Figure 2.6. Graphical representation of the percentages of carbon dioxide (CO_2), bicarbonate (HCO_3^{-}), and carbonate (CO_3^{2-}) versus pH



If the pH in the system is above 8 there is production of HCO_3^{-1} and CO_2^{-3} but no production of CO_2 . Furthermore, when acids are not present in the aqueous system, sodium carbonate will release CO_2 when heated above 90°C (Delcour and Hoseney, 2010; Cauvain and Young, 2006; Köksel and Gökmen, 2008). Chemically under these circumstances the reaction is as follows:

 $2NaHCO_3 \rightarrow Na_2CO_3 + CO_2 + H_2O$

In this case residual carbonate flavor is generated and it is not always desirable.

A second way to produce carbon dioxide is with the decomposition of ammonium bicarbonate when heated. However, this is restricted to products with very low moisture content (approximately 5%), for example cookies (Delcour and Hoseney, 2010; Pyler, 1988; Köksel and Gökmen, 2008). Ammonium bicarbonate produces carbon dioxide in a reaction as follows:

 $NH_4HCO_3 \rightarrow NH_3 + CO_2 + H_2O$

Ammonium bicarbonate is also called "volatile salt" due to it reaches a complete dissociation. Therefore, there is no aftertaste residual produced. Ammonium bicarbonate produces CO_2 upon heating above 60°C (Köksel and Gökmen, 2008).

In the baking industry a variety of leavening acids are used. The acids vary in chemical composition, rate of reaction, batter formulation, neutralizing values, and final pH they give to end products (Pyler, 1988; Delcour and Hoseney, 2010; Köksel and Gökmen, 2008). The properties of the most popular leavening acids are listed in table 2.1.

Acid	Formula	Neutralizing value	Final pH of baked product
Monocalcium phosphate			•
monohydrate (MCP)	Ca(H ₂ PO ₄) ₂ · H ₂ O	80	7.1-7.3
Anhydrous monocalcium			
phosphate (AMCP)	$Ca(H_2PO_4)_2$	83.5	7.3–7.5
Sodium acid pyro-			
phosphate (SAPP)	Na ₂ H ₂ P ₂ O ₇	72	7.7-8.3
Sodium aluminum			
phosphate (SALP)	NaH ₁₄ Al ₃ (PO ₄) ₈ · 4H ₂ O	100	7.2-7.4
Sodium aluminum			
sulfate (SAS)	Al ₂ (SO ₄) ₃ · Na ₂ SO ₄	100	7.3–7.6
Monopotassium tartrate			
(cream of tartar)	KHC₄H₄O ₆	45	7.2-7.5
Dicalcium phosphate			
dihydrate (DCP)	CaHPO₄ · 2H₂O	33	-
Glucono-delta-lactone			
(GDL)	C ₆ H ₁₀ O ₆	50	-

Table 2.1. Properties of common leavening acids

The use of baking powders is very popular in the baking industry. Baking powders are a mixture of sodium carbonate, one or more acid agents, and an inert diluent such as cornstarch. This mixture has the objective of creating gas during mixing or baking. In the leavening system the quantitative balance between sodium bicarbonate and acid agent is used in the formula. In this regard the concept of neutralizing value has been developed to determine the amount of acid required in the recipe (Cauvain and Young, 2006; Pyler 1988; Delcour and Hoseney, 2010; Köksel and Gökmen, 2008). The neutralizing value is calculated as the amount of sodium carbonate needed to neutralize 100 g of acid agent and is driven by the following equation:

Neutralizationg value = $\frac{\text{g of NaHCO}_3}{100 \text{ g of acid agent}} \times 100$

Speed rate and reactivity of leavenings system is highly dependent of pH, flour characteristics, and ingredients in the final formulation.

Chapter : 3 Exploring Relationships Between Pancake Quality and Grain and Flour Functionality in Soft Wheats.

Carlos A. Fajardo and Andrew S. Ross

Abstract

The objective of this study was to observe the influence of differences in genotype (variety) and protein concentration on batter flow and pancake making performance of a collection of soft white winter wheats. Wheats were chosen to express contrasting absorption characteristics and oxidative gelation potentials. Pancakes were processed with two formulations, one ("old") with egg, soy, and dairy and one ("new") without. Pancake performance was compared with grain, milling, flour, solvent retention capacity (SRC), pasting, and oxidative gelation characteristics of the flours. Kernel texture, break flour yield, carbonate SRC, and lactic acid SRC were not significantly associated with pancake performance for either formulation ANOVA showed that flour protein concentration had a dominant effect on pancake batter flow and dimensions. Flour protein concentration affected pancakes more than flour protein quality (lactic acid SRC). Water and sucrose SRCs and Rapid Visco Analyzer pasting temperature were negatively correlated with pancake batter flow and dimensions. Pasting temperature was significantly and positively correlated with flour protein, suggesting that correlations with pancake properties might be simply a cross-correlation with protein concentration. Notably, and in contrast to our hypothesis, oxidative gelation potential had no relationship with pancake processing or quality.

3.1 Introduction

Pancakes are produced from fluid batters and are ideally made from soft wheat flours (Kweon et al. 2010). They are also an important domestic end use of soft white winter wheat grown in the U.S. Pacific Northwest (PNW). Soft white winter wheat makes up the vast majority of wheat grown in Oregon, which are, respectively, the wheat type and primary region covered by the Oregon State University wheat-breeding program. Accordingly, we focused on soft winter wheats rather than on soft spring wheats in this study, with the long-term aim of creating information that is specific to improving through breeding the quality of soft white winter wheat for batter-based products. Pancakes can be considered representative of products made from fluid batters with sugar concentrations <30% (Kweon et al. 2011), and so they may contrast with batters with sugar concentrations >30% (e.g., cake batters) (Kweon et al. 2011). There are no reports in the literature about differences in pancake performance for flours milled from collections of soft white winter wheats. Kiszonas (2013) investigated two soft white spring cultivars among five cultivars investigated for the relationship between arabinoxylans and pancake performance, but no soft white winter cultivars were examined. If there are overarching differences between soft white spring and soft white winter wheat performance in batter-based products, we may ascribe these differences to growing conditions (fall versus spring planted) and the different genetic backgrounds of these two sub- classes of soft wheat. Formulation of breeding targets for soft white winter-based fluid batter products made with sugar concentrations <30% would specifically assist soft winter wheat breeders in servicing this segment of the soft wheat market.

Control of batter flow is crucial to the processing and product qualities of batter-based foods. Use of flours unsuitable for pancake making can lead undesired outcomes, such as out-of-specification batter flows. Because pancakes are made with unconfined batters, batter flow characteristics are the main factor determining pancake dimensions. Batter flows outside of specifications lead to pancakes of incorrect dimensions and possible textural deficits. Ross et al. (2014) commented that the current understanding of what constitutes superior quality soft wheat is focused on low absorption characteristics across three of the four solvent retention capacity (SRC) solvents (water, sodium carbonate, and sucrose). However, low-absorption wheat may lead to batter viscosities too low to control spread, to retain bubbles, and to maintain cling of batter-based coatings. Increasing solid concentrations in batters to achieve desired flow characteristics may have functional (e.g., mouthfeel and flavor) and economic implications for processors and end users. The interplay between batter flow, flour solids concentration in the batter, and the absorption capacity of the flour is crucial.

Kweon et al. (2011) reported an SRC profile for pancakes based on soft red winter wheat flour chlorinated to pH 5.1. The SRC profile was water SRC, 55%; carbonate SRC, 73%; sucrose SRC, 109%; and lactic acid SRC, 76%. Kweon et al. (2011) also reported significant correlations between individual SRC results and pancake parameters for unchlorinated flours from 15 wheat cultivars, suggesting that SRC might be a useful tool for assessing flour pancake potential. Kweon et al. (2014) proposed that unchlorinated flours would be preferred for batters with sugar concentrations <30%. This recommendation contrasted with the earlier work of Finnie et al. (2006), who showed that

chlorination contributed to pancakes that had more viscous batters, that were larger and thicker, and that had softer and more cohesive and resilient textures compared with pancakes made from unchlorinated flours.

Pancake manufacturers may specify flour of a fixed protein for pancake production, and it is important to observe how other flour characteristics impact pancake performance when protein content is held constant. However, the justification in this study for looking across locations and across a range of wheat and flour protein concentrations is that this is the reality that millers face when sourcing wheat in the U.S. PNW for milling into pancake flours. Millers need also to know how much influence protein concentration has on pancake performance. This knowledge will allow them to determine how much they can deviate from the flour protein specification and still provide high-quality flours for this application, for example, in harvest years when either high or low protein soft wheat is in short supply (e.g., low protein soft wheat is in short supply in the U.S. PNW from the 2014 harvest) (U.S. Wheat Associates 2014).

The aim of this study was to investigate the pancake making performance of four soft white winter wheat varieties selected to contrast in inherent oxidative gelation potential, SRC absorption characteristics, and protein concentrations. This study was done in part to advance understanding of the physicochemical basis of pancake performance by complementing the studies of Kweon et al. (2011) and Finnie et al. (2006). To accomplish this goal, grain was milled to flour (unchlorinated), and relationships between selected pancake quality attributes and a spectrum of conventional soft wheat quality analyses and oxidative gelation capacity were observed. Our hypotheses were that pancake performance would not be optimized by use of conventionally high quality soft wheat flours with low water, carbonate, and sucrose SRCs, high break flour yield, physically soft kernel texture, and low protein con- centration. We also hypothesized that oxidative gelation capacity and flour pasting characteristics would be associated with pancake performance.

3.2 Materials and Methods

Four soft white winter varieties (Bobtail, Goetze, Skiles, and Tubbs) were obtained from the Oregon State University wheat- breeding program elite winter yield trials. Varieties were chosen to express contrasting varietal qualities. In the 2014 report on preferred wheat varieties for Washington, Oregon, and North Idaho (Washington Association of Wheat Growers 2014), Bobtail is classified as most desirable, Skiles as desirable, and Goetze and Tubbs as acceptable. Grain was grown in Oregon at LaGrande, Moro, Pendleton, and Kaseburg Farm (Sherman County). For analysis, locations were grouped into three categories: high protein (LaGrande), medium protein (Moro), and low protein (Pendleton and Kaseburg Farm). Two field replicates were tested for each variety from each location. Grain was harvested in the summer of 2013. The varieties chosen had divergent oxidative gelation potentials: Tubbs, high; Goetze and Skiles, intermediate; and Bobtail, low (Mattson, 2014). Low-protein grain of the variety Bobtail from Pendleton was replaced by equivalently low-protein Bobtail grain from Kaseburg Farm as a result of an unrecoverable error made when milling the Pendleton samples.

Kernel hardness index (SKHRD) was measured with an SKCS 4100 (Perten Instruments, Hägersten, Sweden; AACCI Approved Method 55-31.01). Prior to milling, grain was tempered to 14% moisture (AACCI Approved Method 26-10.02) and then milled to flour with a modified Brabender Quadrumat Senior experimental flour mill (C. W. Brabender Instruments, South Hackensack, NJ, U.S.A.; Jeffers and Rubenthaler 1977). Break flour and total flour yield were reported. Samples of unchlorinated straight-grade flour were obtained. Wheat protein (WPROT) and straight-grade flour proteins (FPROT) were measured with NIR spectroscopy (Infratec 1241, Foss USA, Eden Prairie, MN, U.S.A.; AACCI Approved Methods 39-10.01 and 39-11.01). Water, carbonate, sucrose, and lactic acid SRCs were measured on straight-grade flours based on AACCI Approved Method 56-11.02. Pasting viscosity of straight- grade flour was measured with a Rapid Visco Analyzer (RVA) (RVA- 4500, Perten Instruments; AACCI Approved Method 76-21.01). RVA pasting temperature and RVA peak viscosity were recorded (Batey 2007). Oxidative gelation was measured following the RVA-based method of Ross et al. (2014). Flour was hydrated for 20 min in 50 mL lidded plastic centrifuge tubes with a Labquake tumbler (Thermo Scientific, Asheville, NC, U.S.A.). After prehydrating, as quickly as possible (within 20 s) and without allowing the suspension to settle by continued gentle hand shaking, 30.0 ± 0.05 g was transferred quantitatively to an RVA canister. The RVA was set at 30 C throughout the entire test sequence. This temperature was chosen for the following reasons: we did not want the revised method to stray too far from the ambient conditions used in the Bostwick method; we wanted to remain in the realm of the conditions used in batter production and deposition, which are below starch gelatinization temperature; and 30 C was sufficiently far from ambient that the temperature controller

was able to maintain a steady temperature. A conventional 10 s high- speed mixing (960 rpm) was applied as in the RVA standard 1 profile (Batey 2007; AACCI Approved Method 76-21.01), and the viscosity of the flour–water suspension was sensed at 160 rpm for 1 min. This element of the test established the flour–water baseline viscosity. The RVA was stopped, and 65 μ L of 3% H₂O₂ was added. The RVA was restarted, the 10 s high-speed agitation repeated, and the viscosity of the suspension sensed at 160 rpm for a further 5 min. Measured parameters were flour–water viscosity (prior to H₂O₂ addition) and oxidative gelation capacity (flour–water suspension viscosity after H₂O₂ addition).

Commercial ultrafine cane sugar was obtained from C&H Sugar (Crockett, CA, U.S.A.). Dextrose was obtained from J. T. Baker (Center Valley, PA, U.S.A.). Sodium carbonate and dried buttermilk were obtained from Bob's Red Mill (Milwaukie, OR, U.S.A.). Sodium pyrophosphate 28 (SAPP 28) was obtained from ICL Performance Products (Lawrence, KS, U.S.A.). Soy flour and dried egg yolk were obtained from Modernist Pantry USA (York, ME, U.S.A.). Canola oil was obtained from Kroger (Virginia Beach, VA, U.S.A.).

Table 3.1 shows the pancake formulations. The old (rich) pancake formulation was based on that of Finnie et al. (2006) and a predecessor to AACCI Approved Method 10-80.01 used by the AACCI Soft Wheat and Flour Technical Committee during development of the approved method (Kiszonas 2013). For the new pancakes the formulation was changed to that of the leaner AACCI Approved Method 10-80.01. However, the old method was retained to observe the effect of the presence or absence of egg, soy, and
dairy. For both formulations, all mixing was done with a Hobart N5 5 qt mixer with the beater attachment (Hobart, Troy, OH, U.S.A.). Enough dry mix for 10 batches was mixed at speed 1 for 2.5 min and stopped; the bowl was scraped down and mixing continued for a further 2.5 min. Dry mix was set aside, and the appropriate quantity was weighed into each batch. Water and canola oil were premixed for 1 min at speed 1 with the same mixer and attachment. The dry ingredients (flour and dry mix) were then added and the batter mixed for a further 40 s at speed 2. The batter was rested for 1 min, after which pancake batter flow (cm) was measured at 30 s flow time with a Bostwick consistometer (CSC Scientific, Fairfax, VA, U.S.A.). At 3 min elapsed time from the incorporation of the wet and dry ingredients, a full number 20 ice-cream scoop was used to pour 40 g of pancake batter onto a 35 cm diameter electric crepe griddle (SAS Krampouz, Pluguffan, France) stabilized at 190 C. Four aliquots of pancake batter each were poured at 10 s intervals from about 8 cm above the griddle surface and cooked for 90 s on each side. Cooked pancakes were cooled for 20 min and assessed. Pancake diameter (PanDi) was measured as the mean of the diameters of two pancakes measured along two perpendicular axes of each pancake. Pancake texture was measured with a Stable Micro Systems TA.TXi texture analyzer (Texture Technologies, Hamilton, MA, U.S.A.). The cooled pancakes were transferred to the texture analyzer for testing. Pancakes were compressed with a 37 mm di- ameter circular probe to 50% strain at 1 mm/s with a trigger force of 50 g. Reported parameters are the modulus of compression (mod- ulus), calculated as the slope of the initial force versus displacement curve in kg/s over the first 2 s of compression, and pancake thickness (mm).

Statistical Analyses. Statistical analyses used the SAS JMP 11 platform (SAS Institute, Cary, NC, U.S.A.). For all traits except SKHRD, a minimum of duplicate analytic replicates was performed. All correlation, regression, and ANOVA analyses were performed with the mean of the analytical replicates as the representative value for each field replicate. Significance for ANOVA was set at $P \le 0.05$ unless otherwise noted. Significance for correlation analyses was set at $P \le 0.01$ to avoid emphasizing weaker correlations that may have been less reliable, unless otherwise noted.

	OLD	NEW		
ingredient	g	g		
Dry ingredients				
Sucrose	9.34	8.50		
Dextrose	2.92	2.75		
Salt (NaCl)	0.59	1.30		
Sodium bicarbonate	1.75	1.58		
Monocalcium phosphate		0.40		
SAPP28 ^a	2.45	1.76		
Soy flour	5.54			
Dried Egg Yolk	1.17			
Dried Butter Milk	1.17			
Other ingredients				
Dry ingredients	24.91	16.28		
Flour	87.50	87.50		
Canola oil	2.92	5.50		
Water (20 °C)	146.50	121.00		

Table 3.1. 1	Pancake	formulations
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^aSAPP28: Sodium Acid Pyrophosphate;

3.3 Results and Discussion

Protein category (high, medium, or low) was used as a categorical main effect in ANOVAs, rather than location as would be normal practice. This choice was made as a response to the exclusion of the low-protein samples of variety Bobtail from Pendleton and their replacement with low-protein samples of Bobtail from Kaseburg Farm. Oneway ANOVA showed that locations LaGrande and Moro had significantly different protein concentrations from each other ($P \le 0.05$), and these both had significantly different protein concentrations compared with locations Pendleton and Kaseburg Farm. The latter two were not significantly different from each other (P > 0.05). Tukey's honest significant difference analysis showed that the locations ranked for protein concentration as follows: LaGrande > Moro > Pendleton = Kaseburg Farm (mean protein concentrations 13.1 > 9.6 > 7.4 = 7.1%, respectively). This analysis allowed us to group locations Pendleton and Kaseburg Farm into a single category for further ANOVA analyses.

Table 3.2 shows mean, range, and standard deviations for all measured grain, flour, and pancake attributes. SKHRD, break flour yield, and water, carbonate, and sucrose SRCs showed that the samples covered a useful range of soft wheat quality. For example, SKHRD ranged from 3 to 46, effectively covering the range of kernel hardness values observed in soft white winter wheat crops over many years. Although mean pancake batter flow was not significantly different between methods (Table 3.2), the range of batter flow was slightly greater for the new formulation, also reflected in a slightly higher range of pancake diameter for the new formulation. This observation suggested but did

not confirm better resolution of pancake performance following the new, lean AACCI Approved Method formulation. RVA pasting temperature and RVA peak viscosity showed a wide range of values despite the absence of partial waxy varieties in this sample set. A wide range of oxidative gelation capacity was observed in these samples. **Table 3.2**. Descriptive statistics of grain, milling, flour, absorption, oxidative gelation, flour pasting characteristics, and pancake performance for 24 soft white winter wheat samples

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WPROT = wheat protein; SKHRD = single-kernel hardness; FY = flour yield; BFY = break flour yield; FPROT = flour protein; SRC = solvent retention capacity; WaterOXI = flour-water viscosity in oxidative gelation test; PeakOXI = peak viscosity in oxidative gelation test; RVA = Rapid Visco Analyzer; temp. = temperature; PV = peak viscosity; and PanDi = pancake diameter.

Table 3.3 shows ANOVA analyses of the measured traits. For WPROT, FPROT,

SKHRD, total flour yield, RVA pasting temperature, and oxidative gelation capacity the

main effects, protein category and variety, were significant and there were no significant interaction terms. For all of these traits except oxidative gelation capacity, the F values indicated that protein category was the dominant influence, suggesting that the magnitudes of these traits are driven primarily by protein concentration. For oxidative gelation capacity, variety had marginally more influence than protein category on this trait. Notably, for the SRCs the patterns were different. For water and carbonate SRCs, variety was the dominant main effect. For sucrose and lactic acid SRCs, protein category was the dominant main effect. This observation potentially reflects the association of the sucrose and lactic acid SRC solvents with protein components in the flour (lactic acid with glutenins, and sucrose with gliadins and arabinoxylans) (Slade and Levine 1994; Gaines 2000). Also, for all four SRCs there were significant protein category \times variety interactions, and for water and carbonate SRCs the magnitude of the interaction effects was of the same order as the main effect variety. For example, the interaction plot (Fig. 1) shows that sucrose SRC for the variety Bobtail has a different response to increasing protein concentration, especially in the jump from the medium to high categories. Bobtail and Skiles showed significant increases in sucrose SRCs from low to medium protein content. In contrast, Goetze and Tubbs varieties showed no significant difference in sucrose SRC from low to medium protein concentration. For the pancake parameters, a three-way ANOVA was also conducted to assess the effect of the old and new formulations. Under these conditions of analysis, variety and protein concentration both influenced the four pancake parameters. Protein concentration dominated pancake batter flow, diameter, and thickness. For example, pancake diameter decreased significantly in all varieties, when using the new pancake formulation, when protein increased from low

to medium concentrations (Figure 2A). However, there was no overall significant change in pancake diameter when protein concentration increased from medium to high (Figure 2A). In contrast, except for Bobtail, pancake diameter continued to decrease as protein concentration increased when using the old formulation (Figure 2B). These data suggest an interaction between the process, the flour, and the additional ingredients in the old formulation. Of note, Bobtail was the most buffered against changes in pancake diameter as protein concentration increased from the medium to high categories. This information also can be of value to millers and processors by highlighting that there is the possibility of sourcing wheat, or specified varieties, at varied protein concentrations with minimal impacts on processing performance. Variety was marginally more influential for modulus.

Formulation (old versus new) was a significant main effect for pancake thickness and modulus. The observation that formulation had no significant effect on pancake batter flow or pancake diameter but had a substantial effect on thickness, was somewhat surprising but well supported by the statistical analysis. One of the two-way interaction terms was significant for each pancake parameter, in each case with low F values, suggesting low impact on the interpretation of the main effects. No three-way interactions were significant.

In the old pancake formulation, Finnie et al. (2006) reported that soy flour affected batter flow, egg yolks affected both batter flow and hardness, and buttermilk affected hardness alone. Beyond that, Bettge (2014) suggested that the number of ingredients in the old formulation may increase variability in the pancake formulation beyond the inherent variability of the flour, and it may mask our ability to assess the functional performance of flours. Bettge (2014) then postulated that flour performance is best differentiated by using a lean formulation. By inference, the simplicity of the new pancake formulation allows us to better assess functionality of flours in pancake making for breeding programs as well as for millers. **Table 3.3.** F-Values from Analysis of Variance of Grain, Milling, Flour, Absorption, RVA, Oxidative Gelation, and Pancake Quality Attributes

Grain

Protein-category *Method

^aProtein-category (high, medium, low) ^bMethod old versus new ^{c*}, **, and ***: significant at P < 0.05, P < 0.01, and P < 0.001, respectively. *

Figure 3.1. Interaction plot of sucrose solvent retention capacity (SRC) versus protein category by variety. Error bars represent \pm SE of sucrose SRC values of each protein category.



Figure 3.2. Interaction plots: **A**, pancake diameter of new formulation versus protein category; and **B**, pancake diameter of old formulation versus protein category. Error bars represent \pm SE of pancake diameter for each protein category



Table 3.4 shows correlation coefficients among the four pancake parameters. Old formulation pancake batter flow was significantly correlated with old formulation pancake diameter, thickness, and modulus and also with new formulation pancake diameter and thickness. New formulation pancake batter flow was also significantly correlated with new formulation pancake diameter and thickness. However, the numerical values of the correlation coefficients among pancake parameters were higher for the new formulation than for the old. This observation suggested that when using the AACCI Approved Method (new) formulation, measuring pancake batter flow would be sufficient to gauge the suitability of a flour sample for pancake making, without cooking the pancakes, saving time and resources, for example in breeding programs. For both formulations, thickness was significantly and negatively correlated with all measures of flow or spread, with higher flow leading to thinner pancakes. Despite the observation that the new formulation pancakes were significantly thicker than the old formulation pancakes (Table 3.4), the thicknesses of pancakes made with each formulation were highly correlated. In the old formulation, modulus was positively correlated with pancake batter flow and pancake diameter and negatively correlated with thickness: that is, as pancakes became thinner they became stiffer. The absence of significant correlations between new formulation modulus and any other pancake parameter is puzzling. However, the results of the correlations match those of the old formulation.

Table 3.5 shows correlation coefficients between pancake batter flow, pancake diameter, thickness, and modulus, on the one hand, and grain, milling, SRC, RVA, and oxidative gelation traits, on the other hand. At $P \le 0.01$ there were no significant correlations

between any pancake attribute and SKHRD or break flour yield. However, there were significant correlations between WPROT, FPROT, and total flour yield and all pancake parameters except modulus for the new formulation. The significant correlations with total flour yield may have resulted from the significant negative correlation between total flour yield and WPROT (r = -0.67; $P \le 0.01$) in this sample set.

Table 3.4. Linear correlation coefficients amongst pancake parameters for pancakes made from 24 soft white winter wheat samples. Correlations are significant at $p \le 0.01$ unless otherwise noted

		Old			New			
		PanDi (cm)	Thickness (mm)	Modulus	Pancake batter flow (cm)	PanDi (cm)	Thickness (mm)	Modulus
				(kg.s-1)				(kg.s-1)
Old	Pancake batter flow (cm)	0.83	-0.75	0.76	0.79	0.69	-0.78	0.34ns
	PanDi (cm)	1.00	-0.79	0.76	0.78	0.71	-0.82	0.51 (p ≤0.05)
	Thickness (mm)		1.00	-0.91	-0.81	-0.76	0.93	-0.37
New	Modulus (kg.s-1)			1.00	0.74	0.74	-0.91	0.41 (p ≤0.05)
	Pancake batter flo		1.00	0.94	-0.91	0.24ns		
	PanDi (cm)					1.00	-0.89	0.15ns
	Thickness (mm)						1.00	-0.35ns

^ans: not significant at $p \le 0.01$.

Abbreviations: PanDi: Pancake diameter

For SRCs there were significant correlations between all pancake parameters and water and sucrose SRCs, except for water SRC with new pancake diameter (although it was significant at $P \le 0.05$, r = -0.51) and sucrose SRC with new modulus (not significant, P > 0.05). Water and sucrose SRCs were significantly correlated (r = 0.75; $P \le 0.01$). Carbonate SRC was significantly associated only with old pancake diameter and new modulus. Notably, carbonate SRC was also significantly correlated with water SRC (r = 0.86; $P \le 0.01$), as was sucrose SRC. However, despite the observation that water and carbonate SRCs were significantly correlated, unlike water and sucrose SRCs, carbonate SRC was not correlated with the majority of pancake traits (Table 3.4). Lactic acid SRC was not significantly associated with any pancake parameter. This observation may be somewhat surprising given the strong correlations between pancake traits and WPROT and FPROT. However, the result may show that pancakes are more affected by the quantity of flour protein and less by its quality as reflected by lactic acid SRC. Overall, the results are in general agreement with the water and sucrose SRC results of Kweon et al. (2011), but with the distinct difference that in this study carbonate and lactic acid SRCs were much less or not at all influential.

For the RVA-based oxidative gelation test (Ross et al. 2014), only the flour–water slurry viscosity (prior to peroxide addition) was correlated with pancake performance. It is of interest that in comparing the correlations between water SRC and pancakes with those between the flour–water viscosity and pancakes, flour–water viscosity had numerically higher correlations. The RVA-based oxidative gelation method (Ross et al. 2014) has a 20 min hydration (according to AACCI Approved Method 56-11.02 on SRC profile) with a two times excess of water, no shear, and no heat prior to viscometric analysis. In one view this prehydrated flour–water slurry bridges a gap between the energetic considerations of the SRC test (Kweon et al. 2011) and the kinetic world of batter processing in which flow (shear), even if only under gravitational force, and heat are important considerations. Comparing batter flow to a viscometric analysis may then account for the numerically higher correlations of the flour–water slurry viscosity with pancake performance compared with water SRC. Notably, oxidative gelation potential

was not correlated with any pancake parameter. There are two possibilities: 1) the RVAbased method was inappropriate for the low-shear processing of pancakes, or 2) oxidative gelation has no influence on pancake batter flow or the resultant pancake performance. These data allow no further speculation.

Table 3.5: Linear correlation coefficients between pancake parameters and grain, milling, flour, absorption, oxidative gelation, and flour pasting characteristics of 24 soft white winter wheat samples. Correlations are significant at $p \le 0.01$ unless otherwise noted.

asting temperature (°C) -0.52

Abbreviations: WPROT: Wheat protein; SKHRD: Single kernel hardness; FY: Flour yield; BFY: Break flour yield; FPROT: Flour protein; SRC: solvent retention capacity; WaterOXI: Flour/water viscosity in oxidative gelation test; PeakOXI: Peak viscosity in oxidative gelation test; RVA: Rapid Visco Analyzer; RVA PV: RVA Peak viscosity; PanDi: Pancake diameter; not significant at $p \le 0.01$.

The cessation of pancake batter flow on heating could also be attributed to the onset of starch swelling and the concomitant increase in viscosity at that stage of processing. It can also be argued that starch may be gelatinized instantly where the batter contacts the hot griddle and, therefore, that starch pasting temperature is irrelevant. However, pancakes have a finite thickness, and observation of pancakes while being cooked shows that much of the batter is still fluid, even at the pancake margins, early in cooking. Therefore, we hypothesized that starch pasting temperature may be a factor in the cessation of flow. Table 3.5 also shows the linear correlation coefficients for RVA parameters versus pancake properties. Pasting temperature was significantly correlated with six of the eight measured pancake properties. There are two elements to consider: 1) pasting temperature in this sample set was significantly and positively correlated with FPROT (r = 0.87; P \leq 0.01), suggesting the correlations with pancake properties might be simply a cross-correlation with protein concentration, and 2) the results are counterintuitive. It is more intuitive to think that pancake diameter would be reduced if the starch began to paste and therefore increase viscosity at a lower temperature. However, the results show that as pasting is delayed (to higher pasting temperatures) pancake diameter became smaller. The idea that this is a cross-correlation with FPROT, and that our hypothesis was flawed, is further supported by the significant negative correlation between pasting temperature and pancake batter flow, because this test is done well below the pasting temperature and there is no obvious causal connection.

3.4 Conclusions

The new, lean pancake formulation gave a wider range of pancake diameter values, suggesting that it had an improved ability to distinguish the performance of different flours compared with the old, rich formulation. Protein concentration was dominant in controlling SKHRD, total flour yield, RVA pasting temperature, and oxidative gelation capacities of the flours. Pancake traits were significantly influenced by both protein concentration and variety.

In this study, water and sucrose SRCs appeared to be useful tools to predict pancake performance, with sucrose SRC being the most effective of the solvents taken individually (Table 3.5). In this study, carbonate and lactic acid SRCs had no significant relationships with any measure of pancake performance except for pancake diameter (old formulation) and modulus (new formulation). This result contrasts with those of Kweon et al. (2011), in which all four solvents were seen to influence pancake quality. Notably, despite the deliberate choice of varieties varying widely in oxidative gelation capacity (Table 3.2), oxidative gelation capacity was not significantly associated with any measure of pancake performance (Table 3.5).

Our hypothesis that pancake performance would not be optimized by use of conventional high-quality soft wheat flours (with low water, carbonate, and sucrose SRCs, high break flour yield, and physically soft kernel texture) was supported by the observations made in this study. Finnie et al. (2006) suggested that the desired quality profile is a batter with higher viscosity that results in larger, thicker, and softer pancakes. Their results also

suggested that this aim was best achieved by chlorination. From our results it would appear, in the unchlorinated flours observed in this study, that at least for thicker pancakes, the most appropriate flour would be one milled from a variety with inherently higher water and sucrose SRCs and grown under management conditions conducive to higher protein.

References

See bibliography

Chapter : 4 Meta-analysis of the USDA Western Wheat Quality Laboratory Genetic and Environmental Study 2001 to 2014: soft white winter wheat kernel and flour traits, Japanese sponge cake volume, and sugar-snap cookie diameter.

Carlos A. Fajardo and Andrew S. Ross.

Abstract

The objective of this study was, via data mining, to identify the genetic and environment (G&E) factors that impacted soft wheat quality in the Pacific Northwest (PNW), and in particular soft white winter (SWW) wheats, across 12 years and to observe possible endquality predictions. Data was collected by the USDA Western Wheat Quality Laboratory between 2001 and 2014. There were 1780 soft wheat samples representing 222 varieties. 1056 samples were white CLUB, 109, soft white springs (SWS), and 615 SWW wheats. Cookie diameter (CODI) and Japanese sponge cake volume (CAVOL) determined by kernel hardness (SKHRD), grain and flour protein contents (WPROT and FPROT), break flour yield (BKFY), total flour yield (FY), and absorption characteristics. Analysis of the overall data set showed that BKFY was strongly and positively associated with CAVOL and CODI. CLUB wheats were associated with superior CODI and CAVOL confirming earlier observations. SWS were clustered between the CLUB and SWW classes, except for one subset of SWS that was associated with higher SRCs. SWW wheats were associated with high grain and flour protein concentrations and lower milling yields and smaller CODI and CAVOL suggesting that SWW tended to be lower quality compared to CLUB wheats. In SWW wheats SKHRD, protein concentrations, ash, sucrose-, and lactic acid SRCs had coefficients of variation (CVs) > 10%. These observations suggested that

these traits were more sensitive to G&E effects compared to post milling and end-product traits. It is unclear why the large variation of SKHRD was not reflected in equally large milling yields. ANOVA showed that G&E had significant effects on SWW traits. For SWW wheats G&E factors showed variability across years particularly 2003 and 2012, which were associated with high absorption and protein concentrations. Across years CODI showed more expected associations with predictive traits than CAVOL. Meta-analysis showed that BKFY was the most useful single trait in selecting for CODI and CAVOL.

4.1 Introduction

Wheat (*Triticum aestivum*) is a one of the most important food crops. Wheat is consumed worldwide and is made into a wide range of products. In the U.S. Pacific Northwest (PNW) climatic conditions favor production of soft wheat. Three soft wheat types are planted in the PNW, soft white winter (SWW), soft white spring (SWS), and club (CLUB). Soft wheat in the PNW is important for export markets such as South Korea and Japan where one of the important applications of soft wheat is in sponge cake manufacture.

Wheat quality is defined in terms of suitability for specific end-uses. This is important for breeders, farmers, flour millers, and food producers and consumers. Breeders assess quality to identify cultivars that meet agronomic demands. Millers assess grain quality to obtain better flour yields and other desired flour characteristics (Souza et al., 2012). Food

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producers assess grain and flour quality to ensure processing performance that suits themselves and end-product quality that satisfies their customers.

Important quality traits in soft wheat include kernel hardness, protein concentrations in grain and flour, milling yields, and absorption characteristics of flour. Kernel texture is a primary determinant of end-uses: soft kernel textures mill into flour suitable for cookies and cakes, and hard kernel textures mill into flour suitable for bread products. Kernel hardness is a factor contributing to starch damage during milling and hence absorption properties that affect end-products (Bettge and Morris, 2007). Protein concentration also determines the uses of the grain and the resultant flour. Optimum bread is associated with higher flour protein concentrations and optimum cakes and cookies are associated with low flour protein concentrations (Delcour and Hoseney, 2010). Milling yields are important because they are associated with mill profitability, and from a functionality perspective, flour particle size and damaged starch levels, both which influence the viscosities of doughs and batters. Absorption properties of flours are measured using tests that predict final characteristics of products. These tests include solvent retention capacity (SRC), pasting viscosity in the Rapid Visco Analyzer (RVA), Mixograph, and others. Despite some of these traits being heritable, yearly variations in crop and climate conditions affect all quality parameters.

Soft wheats are bred to have soft kernel texture, low absorption of aqueous liquids, and low starch damage. These are related, and heritable traits (Souza et al., 2012). Low grain and flour protein contents are also desirable in soft wheats, but are largely determined by growth environment. For soft wheat applications, gluten network development is generally undesirable and soft wheats are often bred to have weaker dough characteristics than hard wheats. However, the gluten forming proteins are not inert in soft wheat applications. Flour proteins may contribute to appropriate geometry and texture in cookies and to gas-cell structure in cakes (Pareyt et al., 2010; Wilderjans et al., 2008).

Soft wheat applications can be classified as dough-based or batter-based. Dough-based products include cookies and crackers. Batter-based products include pancakes and waffles, cakes, and coatings. Soft wheat products contain primarily flour, sugar, fat, and water. Formulations vary in contents and ratios of sugar, water, and fat, and these affect rheology of process intermediates and texture of end-products. Soft wheat product process intermediates range from from stiff dry doughs to a thin flowable batters. The ratios of ingredients to flour also determines final product characteristics. For example, cookies contain a maximum water concentration of 5% in the total mass. In contrast, cakes contain water more than 30% in the total mass.

Sugar snap cookies and Japanese sponge cakes (SC) are used to assess flour functionality in soft wheats. The SC test is based on the procedure of Nagao et al. (1976) and cake volume is the primary trait of interest. However, this test requires a high level of technical skill to reduce random variability in cake volume. The sugar snap cookie test is arguably the most important quality test used in soft wheat breeding programs and is based on AACC International Approved Method 10-52.02. However, this test can suffer technical variation due to difficulty in replicating cookie diameters with the same flour on different days (Souza et al., 2012).

Assessments of soft wheat quality and functionality currently require that breeding programs define environmental and genetic factors that affect the suitability of wheat or flour for final products. The USDA Western Wheat Quality Lab (WWQL; Pullman, WA) has conducted quality testing for wheat breeding programs for over 20 years. Beginning in 1997 they began to accumulate data in what they termed the G&E (genetics and environment) study. The value of this dataset was that all analyses were done in a single reference laboratory and so issues of inter-laboratory variation were avoided. For some time, it has been a goal of the OSU Cereal Quality Program to mine this dataset to see if the information contained therein would help to validate or refute current selection strategies. These strategies are based on typical soft wheat quality measurements: kernel hardness, protein concentrations in grain and flour, milling yields, and absorption characteristics of flour. In particular, we were curious to observe the capability of the grain and flour analyses to predict the quality of selected end-products: sugar snap cookies and Japanese sponge cakes. Elements of the dataset make it problematic to analyze, particularly with what could be termed "conventional" statistics: e.g. multifactor ANOVA. The issue is the radically unbalanced nature of the data structure. As a matter of necessity, the spectrum of varieties and experimental lines examined in each year changed as unpromising experimental lines and older varieties were discarded and new varieties began to dominate. Another matter of necessity was that locations changed from year to year based on climatic and other stress factors that led some locations to be

excluded, or others included, in different years. The grain submitted to the WWQL also came from at least 6 public and 3 private breeding companies.

The objective of this study was to identify the environmental and genetic factors that impacted SWW functionality across a twelve-year period of the G&E study and to observe if possible the value of the grain and flour analyses on end-product quality predictions. To achieve this aim we used the pre-existing data and applied conventional and multivariate analyses to identify trends and associations between traits. Because the OSU breeding program breeds > 90% SWW wheat, this analysis focused primarily, but not wholly, on the SWW samples from the G&E study. This study was done in part to advance understanding of the differences in soft wheat quality traits that affect of sugar snap cookie diameter and Japanese sponge cake volume in an effort to improve breeding outcomes for the OSU SWW wheat breeding program. The hypothesis was that generic and environmental factor had the same variability across years and we also hypothesized that current screening selection of SWW wheat quality traits would predict both sugarsnap cookie diameter and Japanese sponge cake volume

4.2 Materials and Methods

Data from a total of 1780 soft wheat samples representing 222 varieties and three wheat types were obtained from the G&E study run by the WWQL. 1056 samples were white CLUB, 109 SWS, and 615 SWW. Grain was submitted to the WWQL by the OSU (1), Washington State/USDA (3), and Univ. of Idaho (2) wheat breeding programs and from Syngenta, Westbred, and Limagrain Cereal Seeds (1 program each). Samples were

harvested in the PNW in 61 locations across Oregon, Washington, and Idaho from 2001 to 2014. Quality analysis was done at the WWQL.

Test weight (TWT) was measuring using AACCI Approved Method 55-10.0.1. Kernel hardness index (SKHRD) was measured with an SKCS 4100 (Perten Instruments, Hägersten, Sweden; AACCI Approved Method 55-31.01). Wheat protein (WPROT) and flour protein (FPROT) concentrations were measured using NIR spectroscopy (Infratec 1241, Foss USA, Eden Prairie, MN, U.S.A.; AACCI Approved Methods 39-10.01 and 39-11.01). Water, carbonate, and sucrose SRCs were measured using AACCI Approved Method 56-11.02. Grain was milled on a modified Brabender Quadrumat Senior (Bradender GmbH & Co., Germany) using the method of Jeffers and Rubenthaler (1977). Break flour (BKFY) and straight grade flour yield (FY) were calculated as the proportion of break flour and total flour to total mill products. The percentage of ash (FASH) was determined using the AACCI Approved Method 08-01.01.

Baking quality parameters were sugar snap cookie diameter (CODI) and Japanese sponge cake volume (CAVOL). Sugar snap cookies were made using the AACC Approved Method 10-52.02. CAVOL was determined based on the Japanese sponge cake procedure of Nagao et al. (1976). The formula for making sponge cakes was flour (100 g, as-is moisture), sugar (100 g), fresh whole egg (100 g, without shell), and water (40 mL). To prepare egg foam for sponge cakes in the Nagao et al. (1976) procedure, fresh whole eggs (700 g) and sugar (700 g) were hand-mixed with a whisk in the 12 L stainless steel bowl of a Hobart mixer (Hobart, Troy, OH) to obtain a homogenous blend. The mixture was warmed to 41°C in a 50°C water bath to completely dissolve the sugar in the whole eggs. The egg-sugar mixture was continuously whipped for 0.5 min on low speed, for 5.75 min on high speed, and for 0.5 min on low speed using the Hobart mixer equipped with a wire whisk. Deionized water (280 mL at 50°C) was added in two parts (140 mL each), at 3.75 min and 4.75 minutes respectively. After a total whipping time of 6.75 min, the specific gravity was measured using a 100 mL specific gravity cup (Magnuson Engineers, San Jose, CA). The desired specific gravity was 25 ± 0.7 g/100 mL. If the egg foam density was out of specification, whipping time was adjusted in a new batch of egg and sugar to achieve the desired density. Specific gravity measurement is important to compensate for the variability in egg quality and hence foam density. The whipped egg foam was divided into four portions of 240 g. 100 g flour was sprinkled over each portion of the whipped egg foam and then incorporated by gently folding 80 times using a wooden rice scoop. The batter was poured into paper-lined round cake pans (15.2 cm diameter, 5.7 cm high) and baked at 190°C for 35 min. After baking the cake was promptly removed from the pan, cooled at room temperature, and placed in a cabinet with limited air circulation for 24 hrs. Sponge Japanese cake volume (CAVOL) was measured by rapeseed displacement. Sponge cakes were cut in half for visual examination of crumb fineness and uniformity.

Statistical analyses were performed using SAS JMP Pro 12.0.1 (SAS Institute Inc. Cary NC, USA). One-way ANOVAs and correlations were performed using the Fit X x Y platform. A p-value of \leq 0.01 was used to indicate significance unless otherwise noted. Mean-centered and auto-scaled principal component analyses (PCA) were performed

using the Multivariate: Principal Component platform. Partial Least Squares (PLS) regressions were performed on the Multivariate: PLS platform. PLS was performed using the Nonlinear Iterative Partial Least Squares (NIPALS). For PLS models missing data were imputed using the mean of the non-missing values for each model effect or response column. To create training and calibration sets, data was split randomly. Standard error of calibration (SEC) and standard error of validation (SEV) were calculated on the training and validation set respectively using the formula:

SEP or SEC = $\sqrt{((x-x_{pred})/n)^2}$

Where x = the observed value, $x_{pred} =$ the predicted value, and n = the number of observations (Bellon-Maurel et al, 2010). RPD (Relative prediction deviation, or the ratio of performance to deviation): was calculated as the standard deviation of all observations in the set to the SEP or SEC (Bellon-Maurel et al, 2010; Chang et al 2001).

4.3 **Results and discussions**

4.3.1 Total data set.

The overall data set included CLUB, SWS, and SWW varieties. The data set had 1788 samples. There were 4, 13, 13, 1, 85, and 92 missing data points for SKHRD, FY, BKFY, FASH, carbonate-, and sucrose SRC respectively. To summarize this data PCA was used (figure 4.1). The first two principal components (PCs) explained 50.4% of the variability in the overall data set. This analysis was used to show the strong clustering of each class particularly across PC1. The PC1 dimension was characterized by high to low CODI, FY,

and BKFY. Also the vectors of the water, carbonate, and sucrose SRCs, WPROT, and FPROT in the factor space partially explained data variability in both PC1 and PC2. The vector of CAVOL suggested it was more influenced by the variability across PC2. The vector of CAVOL was opposite to the vectors of WPROT and FPROT indicating the dataset showed the generally recognized negative relationship between CAVOL and FPROT.

The wheat types were clustered across PC1 but not PC2. SWW had a PC1 cluster that extended with little overlap to the positive side of PC1 suggesting an overall tendency of the SWW samples to have higher WPROT and SRC values, and lower CODI, FY and BKFY. In contrast, CLUB wheats were clustered towards negative values of PC1 and tended towards higher FY, BKFY, CODI, and CAVOL. SWS in the majority were clustered in the middle between CLUB and SWW. There was one SWS cluster associated in PC2 with both high SRCs and high CAVOL. These samples all came from the same location, Pullman WA, in the same year. This analysis confirmed certain rules of thumb used in breeding soft wheat, for example the association between high BKFY and larger CODI and the overall better quality of CLUB wheats compared to the other two soft wheat types. The clustering of the SWWs towards the "lower quality" end of PC1 led us to further investigate this wheat type, both from the viewpoint that overall it needed more attention for quality improvement, and for the needs of the Oregon State University (OSU) wheat breeding program that is focused on the SWW type (about 70% of the total breeding effort).

Figure 4.1. Principal Component Analysis of the full data set including all CLUB (A), SWS (B), and SWW (C) wheat samples.



TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume.

4.3.2 SWW subset only: ANOVA

Table 4.1 shows the summary statistics for the 615 SWW samples only. There were a number of missing values for FY, BKFY, carbonate-, sucrose- and lactic acid SRCs, and CODI. The large number of missing values for LA SRC is because this parameter only became available in 2013. In terms of the spread of the distributions the most variable parameters in order were SKHRD, FPROT, WPROT, lactic acid SRC, and sucrose SRC.

CODI, TWT, and FY had notably lower coefficients of variance (CVs) over the period of the study suggesting lower sensitivity to changes in G&E factors. Mean values for all parameters were appropriate for SWW (Soft Wheat Quality Targets: APPENDIX 1) except for mean WPROT, FPROT, FASH which were slightly higher than the targets, and mean FY, BKFY, CODI, and CAVOL which were slightly lower than the targets.

	Ν	Mean	SD	Min	Max	CV
TWT	615	60.6	2.1	50.9	64.6	3.4
SKHRD	615	30.7	9.5	-1.8	53.4	31.1
WPROT	615	10.8	1.8	5.3	17.6	16.9
FY	613	66.7	3.3	52.9	73.6	4.9
BKFY	613	45.7	4.5	31.6	56.5	9.9
FPROT	615	9.0	1.7	4.6	16.2	19.2
FASH	615	0.4	0.05	0.27	0.66	11.4
Wa SRC	615	56.9	3.4	49.6	70.4	6.0
Ca SRC	596	72.8	5.9	59.5	95.9	8.1
Su SRC	564	93.6	10.8	73.9	143.5	11.6
LASRC	164	98.0	16.4	67.0	138.2	16.7
CODI	614	9.2	0.3	8.3	9.9	3.2
CAVOL	615	1222	84	940	1425	6.9

Table 4.1. SWW subset summary statistics for grain, flour, cookie diameter, and baked product parameters.

TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume.

Source	TWT	SKHRD	WPROT	FY	BKFY	FPROT	FASH	Wa SRC	Ca SRC	Su SRC	La SRC	CODI	CAVOL
Year													
\mathbf{R}^2	0.12	0.17	0.16	0.22	0.44	0.11	0.22	0.23	0.41	0.53	0.01	0.11	0.38
F value	6.87*	10.68*	9.53*	14.88*	40.68*	6.31	14.06*	15.30*	34.11*	51.66*	0.18 NS	6.28*	31.32*
Variety													
\mathbf{R}^2	0.32	0.63	0.23	0.35	0.58	0.25	0.40	0.39	0.53	0.60	0.52	0.40	0.48
F value	1.47*	5.35*	0.93 NS	1.66*	4.24*	1.02 NS	2.03*	1.99*	3.38*	4.47*	1.25 NS	2.10*	2.86*
Location													
R^2	0.51	0.17	0.48	0.63	0.51	0.47	0.42	0.49	0.59	0.42	0.13	0.35	0.30
F value	16.45*	3.32*	14.65*	27.23*	16.71*	14.42*	11.79*	15.44*	23.63*	11.33*	11.93*	8.71*	6.78*

Table 4.2. F-values from ANOVA for grain, milling, and flour traits, and end-products for the SWW subset.

TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume. Significance at $p \le 0.01$; NS = no significant at p > 0.01

Table 4.2 shows the results of one-way ANOVA for each response variable for the factors, year, variety, and location for the SWW subset. Year and location, both environment (E), significantly affected all parameters. Variety significantly affected all parameters except WPROT and FPROT. Given the strong influence of E on WPROT and FPROT (Triboï et al., 2003; Meizan et al., 1977; Pierre et al., 2008) it might be expected that variety ("G") would have little or no influence on these traits. As a result of the unbalanced nature of the dataset it was not possible to run multi-factor (2- or 3-way) ANOVAs.

4.3.3 SWW samples only: Principal Component Analysis

Figure 4.2 shows PCA (score and loadings plots) of the SWW subset. PC1 and PC2 accounted for about 58% of the variability in the data. In general, the patterns of association were similar to those seen for the complete dataset (figure 4.1). For example, BKFY, CODI, and CAVOL were all positively associated with each other in the top left

quadrant of the loadings plots. Similarly, all of the SRCs were clustered in the upper right hand quadrant.

CODI was strongly and negatively associated with both WPROT and FPROT. Higher CODI was also associated with higher BKFY, and lower SRC values (in PC1), but not as strongly as the negative association of CODI with protein content.

CAVOL was associated somewhat differently than CODI with the grain, milling and flour traits. CAVOL showed little variability across PC1, differences in CAVOL largely ascribed to the PC2 dimension. Across PC2, CAVOL appeared to be associated with higher BKFY, higher carbonate- and sucrose SRCs, and lower WPROT and FPROT. It is our view that it is important to note that the vector of BKFY in the factor space allowed it to be associated positively with both larger CODI (in PC1), and larger CAVOL (in PC2). This is also consistent with the vector of BKFY between CODI and CAVOL in the factor space for the complete dataset (figure 4.1).

When observing the scores plot and the clustering of the years a large number of samples from 2003 and 2014 were associated, in PC1, with high absorption and protein concentrations (figure 4.2: C and M). In contrast, 2012 (figure 4.2: K) was more strongly associated with high TWT and FY, also across PC1. Unfortunately, the unbalanced nature of the dataset meant that there were samples from 2003 that covered almost the entire range of PC1. On further observation the 2003 samples that extended towards the positive side of PC1 all came from locations in Oregon, and in particular locations on the Columbia River Plateau. This led to a further subdivision of the data: subdivided for each

year of the study (Section 4.3.4).

Figure 4.2. PCA plots for the SWW subset with years shown on the score plot and traits on the loading plot



TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume; Letters represent harvesting year.

4.3.4 SWW samples only: Principal Component Analysis by Year

SWW samples were analyzed by year using PCA to observe whether patterns of associations were consistent across time. PCA loadings plots for each year where data was available are shown in figure 4.3. The analysis of locations showed some different patterns of association within the factor space from year to year, suggesting that both environment and genetic factors had significant influences on SWW traits. PC1 and PC2 accounted for between 46 % (2013) and 72% (2014) of the variability in the data.

PCA showed some associations that were expected from experience. These associations were supported by the relative vectors of the traits in the factor spaces both for the full dataset (figure 4.1), and for the SWW subset (figure 4.2). CODI was positively associated with FY and BKFY in 8 of 10 years. CAVOL was positively associated with BKFY and FY in 7 of 10 years. The exceptions were: in 2001 and 2009, CAVOL was orthogonal to BKFY and FY; and in 2002, CAVOL was either orthogonal (PC1) to, or negatively associated (PC2) with BKFY and FY.

CODI showed the expected negative associations with water-, carbonate-, and sucrose-SRCs in 7 of 10 years. Unexpected results were observed in 3 of 10 years. In 2001 and 2013 CODI was positively associated with water- and carbonate-SRCs. In 2012 CODI was orthogonal to carbonate- and sucrose SRCs. In 2013 CODI was also positively associated sucrose SRC.

CAVOL showed variable association with SRCs. For example, focusing on sucrose-SRC, because cakes are high sucrose systems, CAVOL was sometimes positively associated with sucrose-SRC (2002), sometimes orthogonal to sucrose-SRC (2001, 2003, 2009, 2011, 2012, 2013, 2014), and sometimes negatively associated (2004, 2010). This suggests a tenuous link between sucrose-SRC, CAVOL, and by inference between AX

and CAVOL. CAVOL's relationship to the other absorption traits was also similarly variable.



Figure 4.3. PCA loading plots for grain, milling, and flour traits and end-products for each year of available data

TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume.

4.3.5 Partial Least Squares Regressions to predict CODI and CAVOL

PLS analysis was used to see if the standard grain and flour analyses could be used to model the outcomes for CODI and CAVOL. The following variables were chosen as the x-factors for the program to select amongst for prediction: SKHRD, WPROT, BKFY, FY, FASH, water-, and carbonate-SRCs. Second iterations of the CAVOL PLS analyses were performed with CODI as an additional x-factor. For the 2013/2014 analyses lactic acid-SRC was added a further potential x-factor.

4.3.5.1 Partial Least Squares Regressions on SWW wheats.

For CODI, PLS selected 6 factors: BKFY, FPROT, WPROT, FY, water-SRC, and sucrose-SRC. These are listed in order of the variable importance coefficient (high to low). For CAVOL, PLS selected 7 factors: BKFY, WPROT, sucrose-SRC, FPROT, carbonate-SRC, FASH, and FY. For CAVOL and adding CODI as an x-factor, PLS again selected 7 factors that included CODI: BKFY, CODI, WPROT, sucrose-SRC, FPROT, carbonate-SRC, and FY.

Table 4.3 shows the results of the PLS regression for the full SWW subset. R-squared values were not strong. The highest variability accounted for in any prediction was 62%, for the training population predicting CODI. All RPD values were low, with CAVOL having higher RPDs than CODI. Bellon-Maurel et al (2010) indicated these categories for RPD:

1) RPD >2: excellent models

2) 1.4 < RPD < 2; fair models
3) RPD <1.4: non-reliable models.

Others, notable Williams and Norris (1987) have indicated *much* higher values of RPD to indicate reliability.

However, even using the less stringent criteria of Bellon-Maurel et al (2010) the CODI PRD values indicated barely "fair" models and the RPDs for CAVOL, with or without CODI, as "excellent". This suggests some degree of reliability in predicting CAVOL at least using the underlying traits, BKFY, WPROT, sucrose-SRC, FPROT, carbonate-SRC, FASH, and FY, with or without CODI. Of note is the almost constant association of high BKFY with larger CODIs and CAVOLs (Figures 4.1, 4.2) with only a few infrequent exceptions (Figure 4.3, section 4.3.4).

	n	R-squared	SEC	SEV	RPD
CODI					
Training set	316	0.62	0.19		1.5
Validation set	299	0.59		0.2	1.5
CAVOL					
Training set	316	0.53	37.5		2.3
Validation set	299	0.52		34.9	2.4
CAVOL with CODI					
Training set	316	0.55	36		2.2
Validation set	299	0.51		34.6	2.3

Table 4.3. Results of PLS regression analyses of the full SWW subset to predict CODI and CAVOL. Entries were randomly assigned to training and validation sets.

CODI = Cookie diameter; CAVOL = Japanese sponge cake volume; SEC = standard error of calibration; SEV = standard error of validation; RPD = Relative prediction deviation

4.3.5.2 SWW wheat samples from 2013 and 2014

Table 4.4 shows results of PLS analysis for SWW wheat samples from 2013 and 2014.

For CODI PLS selected 6 factors: FY, BKFY, SKHRD, WPROT, FPROT, and water-

SRC. Again, these are listed in order of the variable importance plot coefficient (high to

low). For CAVOL PLS selected 4 factors: SKHRD, BKFY, water-SRC, and carbonate-

SRC. For CAVOL with CODI as a potential x-factor, PLS again selected the same 7

factors with the first 4 factors in the in the same order as the analysis in the absence of

CODI: SKHRD, BKFY, water-SRC, carbonate-SRC, FY, CODI, and FPROT.

Table 4.4: Results of PLS regression analyses to predict CODI and CAVOL of SWW wheats from 2013 and 2014 only. Entries were randomly assigned to training and validation sets.

	n	R-squared	SEC	SEV	RPD
CODI:					
Training set	84	0.6	0.15		1.2
Validation set	80	0.61		0.14	1.2
CAVOL:					
Training set	84	0.62	46		1.5
Validation set	80	0.59		40	1.7
CAVOL with CODI:					
Training set	84	0.66	51		1.4
Validation set	80	0.55		42	1.7

CODI = Cookie diameter; CAVOL = Japanese sponge cake volume; SEC = standard error of calibration; SEV = standard error of validation; RPD = Relative prediction deviation

4.3.5.3 Virtual selections of SWW wheats based on kernel hardness and break flour yield

Table 4.5 shows one-way ANOVA of a virtual selection on the SWW samples. The selections were based on SKHRD and BKFY. BKFY was the most important factor in PLS regressions, based on the variable importance coefficient. SKHRD emerged as an important factor in PLS for both CODI and CAVOL when only the 2013-14 data was observed. In addition, SKHRD and BKFY are used in soft-wheat breeding programs as the major selection traits all over the world. Arguably SKHRD is the fundamental underlying trait that controls flour particle size, damaged starch, BKFY, and absorption characteristics (Souza et al 2012). To observe the effectiveness of SKHRD and BKFY as stand-alone selection traits we sorted samples from 2013 and 2014 samples based on these two traits. Then the softest and hardest 10% based on SKHRD were identified, similarly the highest and lowest 10% based on highest and lowest BKFY.

Selection trait	CODI	CAVOL
SKHRD		
n= 16		
higher quality: low SKHRD	9.0	1249
low quality: high SKHRD	8.8	1067
BFY		
n=16		
higher quality: high BKFY	9.3	1239
low quality: low SKHRD	8.8	1072

Table 4.5. One-way ANOVA of virtual selection based on kernel hardness and break flour yield for samples from 2013 and 2014.

CODI = Cookie diameter; CAVOL = Japanese sponge cake volume; SKHRD = Kernel hardness; BFY = Break flour yield.

Using SKHRD as the selection trait there was an increase of 0.2 cm in CODI and 182 mL in CAVOL (Table 4.5). Using BFY there was an increase of 0.5 cm in CODI and 167 mL in CAVOL. The increase in CODI was slightly bigger when using BKFY than when using SKHRD as the sole selection trait. However, the increase in CAVOL was slightly bigger when using SKHRD than when using BKFY as the sole selection trait. In both cases there were significant increases in the quality (diameter, volume) of the end-products. This suggests that the selection strategies used by soft wheat breeding programs that focus on SKHRD and BKFY are appropriate.

4.4 Conclusions

Figure 4.1 showed PC analysis of overall associations among traits in this data set from 2001 to 2014. BKFY, CODI, and CAVOL were strongly positively associated with each other, supporting similar observations of associations among these traits (Souza et al., 2012; Gaines, 1985; Kaldy et al., 1991). SKHRD is arguably the most fundamental trait for selecting soft wheats (Souza et al., 2012; Pauly et al., 2013; Kaldy et al., 1991, Pomeranz and Williams, 1990). However, in this study for the overall samples SKHRD showed low leverage and weak associations with CAVOL and CODI (Figure 4.1). Grain and flour protein showed negative associations with BKFY, CAVOL, and CODI confirming the generally recognized negative relationship between CAVOL, CODI, BKFY and FPROT (Gaines, 1985; Bettge and Morris, 2007; Payret et al., 2008). SRCs showed stronger negative associations with CODI compared to CAVOL.

Wheat classes clustered in PC analysis (Figure 4.1). CLUB wheats tended to have higher FY, BKFY, CAVOL, and CODI confirming observations of Nagao et al. (1977) of CLUB wheat's superior performance for CODI and CAVOL. SWS were clustered between the CLUB and SWW classes, except for one subset of SWS that was associated with higher SRCs. SWW wheats tended to have higher WPROT, FPROT, and SRCs, and lower CODI, FY and BKFY. SWW tended to have lower quality compared to CLUB wheats, and overlapped with the SWS wheats.

Analysis of SWW wheat samples showed that SKHRD, FPROT, WPROT, lactic acid-, and sucrose SRC had CVs higher than 10% (table 4.1). These CVs suggested that these traits were more sensitive to G&E effects compared to other traits. In contrast TWT, BKFY, FY, water- and carbonate-SRCs, CAVOL and particular CODI had CVs lower than 10%. It is unclear why the large variation of SKHRD was not reflected in similarly large variations in milling yields.

ANOVA showed that Environment (Year and location) had a significant effect (F-value) on all measured traits for SWW wheats (table 4.2). Genetics (variety) had a significant effect on all traits except WPROT and FPROT. This is expected as genetics has a little or no effect on these traits (table 4.2: Souza et al., 2012).

Similar to the overall data, PCA for SWW wheats showed strong and positive significant associations between BKFY, CAVOL, and CODI suggesting that BKFY was an important trait related to larger CODIs and CAVOLs (figure 4.2). CODI was associated

with low values for all four SRCs. In contrast, CAVOL was either weak or not significantly associated with all four SRCs, but associated with *higher* carbonate- and sucrose SRCs.

SWW wheat traits showed were clustered by years (figure 4.2). For example, 2003 and 2014 were associated with high absorption and high protein concentrations. The 2003 and 2014 samples were all grown in the Columbia River plateau region of Oregon. This suggested that location affected absorption and protein levels. Souza et al (2012) also observed variations in traits across locations for soft wheats in the eastern U.S.A.

SWW trait associations were studied across years (figure 4.3). CODI and CAVOL showed the expected positive associations with FY and BKFY in 8 of 10 years suggesting that targeting milling yields to improve cookie and cake quality is a useful strategy. CODI showed expected negative associations with SRCs in 7 of 10 years suggesting that targeting SRCs is a useful strategy, at least for CODI. In contrast, CAVOL showed variable associations with SRCs. An example, focused on sucrose SRC because cakes contain high amounts of sugar, CAVOL was sometimes associated positively with sucrose SRC (2002), sometimes orthogonal (2001, 2003, 2009, 2011, 2012, 2013, and 2014) and sometime negatively (2004 and 2010) with sucrose SRC, suggesting a tenuous ling between these traits (figure 4.3). Souza et al. (2012) suggested that sucrose SRC may be a good predictor for BKFY and hence end product quality. This study contrasted with Souza's observations because in this study sucrose SRC had variable associations with CAVOL.

PLS regression showed for CODI that 6 factors including BKFY, FPROT, WPROT, FY, water- and sucrose SRC were important. However, the PLS model based on the criteria of Bellon-Maurel (2010: Section 4.3.5.1.) was only "fair", i.e. not that good (table 4.3). PLS regression for CAVOL showed that 7 factors including BKFY, WPROT, sucrose-SRC, FPROT, carbonate-SRC, FASH, and FY, with or without CODI were the most significant. According to the criteria of Bellon-Maurel (2010) the model was considered "excellent" for prediction. When analyzed alone, samples from 2013 and 2014 highlighted a similar set of traits, but the models were considered to be poor. Again BKFY was the single most important trait in predicting CAVOL and CODI for SWW wheats. Virtual selection using samples from 2013 and 2014 based either on SKHRD or BKFY showed significant gains in CAVOL and CODI suggesting these as primary effects both in predicting both end product quality and selecting the best genotypes.

This study suggested that breeding selection for SWW wheats should be focus on BKFY and SKHRD in the PNW.

References

See Bibliography

Chapter : 5 Oxidative gelation capacity and particle size distributions of soft wheat flour in relation to Japanese sponge cake and sugar snap cookie quality. Carlos A. Fajardo and Andrew S. Ross.

Abstract

Japanese sponge cake volume (CAVOL) and sugar-snap cookie diameter (CODI) were compared with grain and milling traits, solvent retention capacity (SRC), oxidative gelation capacity (PeakOXI), and median particle size (d(0.5)) of soft wheat flours. There were 615 soft wheat samples representing 174 varieties. Across all samples tested kernel hardness (SKHRD), protein concentrations, ash, and lactic acid SRC had coefficients of variation (CVs) > 10%. The variability of lactic acid SRC was similar to the variability of WPROT and FPROT suggesting that lactic acid SRC was associated with protein concentration. End-products had lower CVs than many other traits suggesting that the other ingredients in the formulations may have compensated for variability in soft wheat properties. PeakOXI was significantly correlated with cookie diameter (CODI) but not with Japanese sponge cake volume (CAVOL). This contrasted with our hypothesis that PeakOXI would affect both products similarly. Notably 13 soft wheat samples had PeakOXI values higher than 800 cP. PeakOXI values this high have never been observed in soft wheats prior to this study. d(0.5) had a significant positive relationship with SKHRD and negative with BKFY. d(0.5) was significantly and negatively correlated with CAVOL and CODI suggesting that lower particle sizes produced larger CAVOLs and CODIs. SRCs were all positively correlated with PeakOXI, and negatively correlated with CODI. SRC correlations with CAVOL were either negative and less strong than

those with CODI, or not significant. ANOVA of multiple linear regression analysis for CODI, and based on effect size (F-value), showed that in this sample set d(0.5), PeakOXI, and WPROT were the most important traits predicting CODI. ANOVA of the multiple linear regression and correlation analyses for CAVOL, showed that BKFY was the important trait in predicting CAVOL. Selections based on BKFY or SKHRD were the effective selecting lines with higher CODI and CAVOL.

5.1 Introduction

Wheat quality is defined in terms of suitability for specific end-uses. In soft wheats, the important quality traits include kernel hardness, protein concentrations in grain and flour, milling yields, and absorption characteristics of flour. Kernel hardness is the most fundamental quality factor and produces flours with finer particles and low damaged starch during the milling process (Souza et al., 2012; Pauly et al., 2013). Protein levels in grain and flour tend to be low in soft wheats. The formation of a viscoelastic gluten network is not desirable for soft wheat end products such as cookies and cakes and low inherent gluten strength and low protein assist in avoiding this outcome (Pareyt and Delcour, 2008). Soft wheat applications include a wide range of water content in their formulations, going from doughs to a viscous or semi-viscous batters (Kiszonas et al., 2015; Bettge et al., 2007; Choi et al 2013; Pareyt et al., 2008). Typically sugar-snap cookies and Japanese sponge cakes serve as key parameters to measure the quality of soft wheats.

Flour particle size is a milling effect that has an effect on quality of cookies and cakes. For example, Choi and Baik (2013) reported that small particle sizes (lower than 60 μ m) produce larger sponge cake volumes. Particle size distribution indicates what particle sizes are present in what proportions. For example, median particle size (d (0.5)) is the distribution of the particle below 50% of the population. The effect of the median particle size on soft wheat traits and end-product quality is still understood.

Flour absorption characteristics are crucial parameters and have a significant influence on viscosities of doughs and batters. Many factors in soft wheat flours influence water absorption properties including damaged starch, flour particle size, protein content and composition, and non-starch polysaccharides such as arabinoxylans (AX) (Souza et al., 2012). Flour absorption properties are measured using the solvent retention capacity (SRC) test (AACC International Approved 56-11.02). The SRC test is based on the thermodynamics polymer-solvent compatibility and measures the swelling behavior of a specific polymer in a specific solvent. Water measures the overall swelling behavior of flour polymers. Sodium carbonate (5% w/w) enhances swelling properties damaged starch. Sucrose (50% w/w) enhances swelling properties of AX and gliadins. Lactic acid (5% w/w) enhances swelling properties of glutenins. Soft wheat flours are expected to have low absorption characteristics and hence low SRC values (Kweon et al., 2011).

Soft wheat applications contain high levels of sugar and/or fat (Pareyt et al., 2008; Choi and Bail, 2013; Wilderjans et al., 2013). Nonetheless, absorption properties of flours have a significant impact on end-product quality. For example, flour proteins are able to bind

water affecting viscosities of doughs and batters. In sugar-snap cookies, flour proteins affect spread, geometry, and texture of cookies (Pareyt et al., 2008). In cakes, flour proteins serve as water binders, increasing batter viscosity and affecting migration, coalescence and hence loss of gas cells (Wilderjans et al., 2008).

AX make up 85% of all non-starch polysaccharides in wheat (Saulnier et al., 2007). AX have a big influence on end-use quality (Kiszonas et al., 2015; Bettge and Morris, 2007). The heavy influence of AX is a result of their high water absorption because AX are able to bind 10 times their weight in water. In formulations, AX could compete for water with other ingredients or flour constituents. AX also have the property to form weak gels under oxidizing conditions such as in the presence of H_2O_2 (Hoseney and Faubion, 1981). Crosslinking of AX is reported to affect batter viscosity in soft wheat products (Bettge and Morris, 2007). Increased viscosity due to the oxidative crosslinking of AX has been associated with decreased sugar-snap cookie spread and lower Japanese sponge cake volume (Kiszonas et al., 2015; Bettge and Morris, 2007)

The oxidative gelation (OG) capacity of AX has been extensively studied (Hoseney and Faubion, 1982; Bettge and Morris, 2007; Izydorczyk and Biliaderis, 1995). However, a method to measure the OG capacity has not been agreed upon. For example, Bettge and Morris (2007) reported a method using the Boskwick but it has limitations in poor resolutions at high viscosities that could lead to poor repeatability. Most recently, Ross et al. (2014) developed a new methodology to measure the OG viscosity using the Rapid Visco Analyzer (RVA). This method has several advantages compared to previous tests.

These advantages are temperature control during the test, continuous viscosity measurements in the RVA that enable observation of thixotropic viscosity reduction at constant shear rate, and high sensitivity in measuring a wide range of relevant viscosities.

Understanding the soft wheat traits that best describe functionality in end-products is important for wheat breeders in the US Pacific Northwest (PNW), and for the millers and food producers who use PNW soft wheats. OG capacity of AX may have a significant impact in soft wheat quality and limited research has been done using the method described by Ross et al. (2014). The aim of this study was to measure the relationships between OG capacity, d (0.5), other soft wheat quality traits, and end-product quality. Another aim was to investigate what were the traits that most affect sugar-snap cookie diameter and Japanese sponge cake volume. The hypothesis was that OG capacity had the same effect in both sugar-snap cookies and Japanese sponge cake volume.

5.2 Materials and Methods

A total of 615 soft wheat samples representing 174 varieties were obtained from a genomic and environmental (G&E) study conducted by USDA Western Wheat Quality Lab (Pullman, WA: WWQL). Two wheat types were included; 152 samples were white CLUB wheats and 463 were soft white winter (SWW) wheats. Samples were harvested in the PNW in 25 locations in 2011. Quality analysis was done at the WWQL. OG capacity and particle size d(0.5) analyzes were done by the Oregon State University cereal quality program.

Test weight (TWT) was measuring using the AACCI Approved Method 55-10.0.1. Kernel hardness index (SKHRD) was measured with an SKCS 4100 (Perten Instruments, Hägersten, Sweden; AACCI Approved Method 55-31.01). Wheat protein (WPROT) and flour proteins (FPROT) were measured with NIR spectroscopy (Infratec 1241, Foss USA, Eden Prairie, MN, U.S.A.; AACCI Approved Methods 39-10.01 and 39-11.01. Water, carbonate, sucrose, and lactic acid SRCs were measured using AACCI Approved Method 56-11.02. Grain was milled on a modified Quadrumat Sr. using the method of Jeffers and Rubenthaler (1977). Break flour (BKFY) was calculated as the portion of break flour to total products and total flour yield (FY) were reported. The percentage of ash (FASH) from 4 g of flour was determined using the AACCI Approved Method 08-01.01.

OG capacity was measured using the RVA-based method of Ross et al. (2014). Flour was hydrated for 20 min in 50 mL lidded plastic centrifuge tubes with a Labquake tumbler (Thermo Scientific, Asheville, NC, U.S.A.). After prehydrating, as quickly as possible (within 20 s) and without allowing the suspension to settle by continued gentle hand shaking, 30.0 ± 0.05 g was transferred quantitatively to an RVA canister. The RVA was set at 30°C throughout the entire test sequence. A 10 s high-speed mixing (960 rpm) was applied as in the RVA standard 1 profile (Batey, 2007; AACCI Approved Method 76-21.01), and the viscosity of the flour–water suspension was sensed at 160 rpm for 1 min. This established the flour–water baseline viscosity. The RVA was stopped, and 65 µL of 3% H₂O₂ was added. The RVA was restarted, the 10 sec high-speed agitation repeated, and the viscosity of the suspension sensed at 160 rpm for a further 5 min. Measured parameter was the peak OG capacity viscosity (PeakOXI). The particle size distribution of 40 mg flour suspended in 150 mL isopropanol alcohol (IPA) was measured on a Mastersizer & Hydro 2000s (Malvern Instruments, Ltd., Malvern, Worcestershire, UK). IPA was used to prevent flour particle swelling. Particle refractive parameter and absorption indices were set to 1.52 and 0.1 respectively (Hu et al., 2007). Obscuration varied from 7% to 12% indicated optimal sample concentrations. Laser diffraction was conducted based on the assumption that flour particles conform to a hard, spherical particle model (Rawle, 1993). Particle size analysis (PSA) provides a number of calculated values (e.g., diameter mean, surface mean, weight mean, moment mean) and in this study the parameter d (0.5) indicating the median particle size was reported. This parameter describes the center of gravity of the corresponding distributions (Rawle, 1993).

Sugar snap cookies were made using the AACC Approved Method 10-52.02 and cookie diameter (CODI) was measured. The Japanese sponge cake procedure of Nagao et al. (1976) was used and volume (CAVOL) was measured. The formula for making sponge cakes was flour (100 g, as-is moisture), sugar (100 g), fresh whole egg (100 g, without shell), and water (40 mL). To prepare egg foam for sponge cakes in the Nagao et al. (1976) procedure, fresh whole eggs (700 g) and sugar (700 g) were hand-mixed with a whisk in the 12 L stainless steel bowl of a Hobart mixer (Hobart, Troy, OH) to obtain a homogenous blend. The mixture was warmed to 41°C in a 50°C water bath to completely dissolve the sugar in the whole eggs. The egg-sugar mixture was continuously whipped for 0.5 min at low speed, for 5.75 min at high speed, and for 0.5 min on low speed using the Hobart mixer equipped with a wire whisk. Deionized water (280 mL at 50°C) was

added in two parts (140 mL each), at 3.75 min and 4.75 minutes respectively. After a total whipping time of 6.75 min, the specific gravity was measured using a 100 mL specific gravity cup (Magnuson Engineers, San Jose, CA). The desired specific gravity was 25 ± 0.7 g/100 mL. If the egg foam density was out of specification, whipping time was adjusted in a new batch of egg and sugar to achieve the desired density. Specific gravity measurement is important to compensate for the variability in egg quality and hence foam density. The whipped egg foam was divided into four portions of 240 g. 100 g flour was sprinkled over each portion of the whipped egg foam and then incorporated by gently folding 80 times using a wooden rice scoop. The batter was poured into paperlined round cake pans (15.2 cm diameter, 5.7 cm high) and baked at 190°C for 35 min. After baking the cake was promptly removed from the pan, cooled at room temperature, and placed in a cabinet with limited air circulation for 24 hrs. Sponge Japanese cake volume (CAVOL) was measured by rapeseed displacement. Sponge cakes were cut in half for visual examination of crumb fineness and uniformity.

Statistical analyses were performed using SAS JMP Pro 12.0.1 (SAS Institute Inc. Cary NC, USA). One-way ANOVAs and correlations were performed using the Fit X x Y platform. A p-value of ≤ 0.01 was used to indicate significance unless otherwise noted. Stepwise analyses were performed using the Fit a model: Stepwise using the default minimum Bayesian information criterion (BIC) on forward direction method.

5.3 Results and discussions

Table 5.1 shows mean, range, and standard deviations for all measured grain, flour, and end-product traits. The number of observations was highly unbalanced for SRC traits. There were 493, 377, 493, and 493 missing values for water-, sodium carbonate-, sucrose-, and lactic acid SRC. However, these were important traits in describing quality of soft wheats. For example, the variability of lactic acid SRC was similar to the variability of WPROT and FPROT suggesting that lactic acid SRC could be affected by protein concentration. Souza et al. (2012) also reported a wide range of lactic acid SRCs values in soft wheats. PeakOXI showed a wide range of values from 75.5 to 4005 cP. Interestingly, PeakOXI values higher than 800 cP are not previously reported in the literature using the RVA method (Fajardo and Ross, 2015; Ross et al., 2014). The samples were 13 SWW wheats samples and the varieties were WA8155, VCF0914, CFSP0061, OR2080518, Tubbs06, and 5J040150-1. Notably, the variety Tubbs showed high PeakOXI previously in the OSU wheat breeding program (Fajardo and Ross, 2015). Table 5.2 shows the summary of statistics of these samples for grain, milling, and flour traits. Similar to the full dataset SKHRD, WPROT, and FPROT showed the high variability in the data (CV higher than 10%). However, only four samples had carbonate SRC values, and the rest of SRCs were not available.

Table 5.3 shows linear correlation coefficients between PeakOXI, d (0.5), grain and flour traits, SRCs, CODI, and CAVOL. PeakOXI was significantly and negatively correlated with FY and BKFY. Milling yields are important traits for soft wheat quality and these results suggested that high PeakOXI could be related to a poor flour quality. PeakOXI

was significantly and positively correlated with all four SRC parameters and the strongest coefficient was with sucrose SRC (r = 0.73, $p \le 0.01$). Kweon et al. (2011) reported that sucrose SRC is a good predictor of OGC and these results suggested that the RVA method from Ross et al. (2014) is suitable to measure this property. d (0.5) was significantly and positively correlated with SKHRD, WPROT, FPROT, water SRC, carbonate SRC, and sucrose SRC.

Table 5.1. Summary statistics of grain, flour, absorption, oxidative gelation capacity, median particle size distributions, sugar-snap cookie diameter, and Japanese sponge cake volume

	Ν	Mean	SD	Minimum	Maximum	CV
TWT	615	61.8	1.7	56.4	65.5	2.7
WPROT	615	9.1	1.4	5.3	13.6	15.7
SKHRD	615	29.1	10.6	-11.4	53.4	36.5
FY	615	70.3	1.8	61.4	76.1	2.6
BKFY	615	47.1	2.9	34.0	55.7	6.2
FPROT	615	7.7	1.4	4.2	11.6	17.8
FASH	615	0.39	0.04	0.27	0.54	10
Wa SRC	120	53.5	1.9	49.3	59	3.5
Ca SRC	237	71.0	3.6	62.7	88.6	5.1
Su SRC	120	79.8	3.9	74.4	90.7	4.9
La SRC	120	83.3	14.7	63.9	143	17.6
PeakOXI	615	269.1	366.6	75.5	4005	136.2
d(0.5)	615	56.8	9.0	31.5	94.2	15.8
CODI	615	9.4	0.2	8.1	10.1	2.6
CAVOL	615	1249	59	985	1415	5

TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = Flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = Median particle size distribution; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume.

	Mean	SD	Minimum	Maximum	CV
TWT	61.1	2.1	56.8	64.5	3.5
WPROT	8.5	1.3	6.1	10.4	15.2
SKHRD	28.9	8.0	9.8	40.3	27.6
FY	68.5	1.6	65.3	70.4	2.3
BKFY	44.8	2.8	40.2	49.6	6.2
FPROT	7.1	1.2	4.4	8.8	17.3
FASH	0.38	0.03	0.35	0.44	7.3
PeakOXI	2323	1066	857	4005	45.9
d (0.5)	53.5	7.5	42.0	66.1	14.1
CODI	9.2	0.1	9.0	9.5	1.5
CAVOL	1255	32	1215	1315	2.6

Table 5.2 Descriptive statistics of grain, flour, sugar-snap cookie diameter, and Japanese sponge cake volume for samples with oxidative gelation viscosity higher than 800 cP

TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = Flour protein; FASH = flour ash; PeakOXI = Oxidative gelation capacity; d(0.5) = Median particle size distribution; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume.

	PeakOXI	d(0.5)	CODI	CAVOL
TWT	ns	0.42	-0.19	-0.21
WPROT	ns	0.22	-0.50	-0.46
SKHRD	ns	0.80	-0.57	-0.50
FY	-0.23	ns	0.36	ns
BKFY	-0.24	-0.50	0.63	0.51
FPROT	ns	0.20	-0.44	-0.45
Wa SRC	0.56	0.47	-0.45	-0.25
Ca SRC	0.42	0.20	-0.58	-0.18
Su SRC	0.73	0.20	-0.36	ns
La SRC	0.54	-0.29	-0.40	ns
FASH	ns	-0.20	ns	0.14
PeakOXI	1.00	ns	-0.19	ns
d(0.5)		1.00	-0.46	-0.46
CODI			1.00	0.49

Table 5.3. Linear correlation coefficients (r-values) between oxidative gelation capacity, median particle size distributions, and baking products and soft wheat quality traits.

TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = Median particle size; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume. Significance at $p \le 0.01$. d(0.5) was also significantly and negatively correlated with BKFY and lactic acid-SRC. Gaines (1985) also reported significantly and negatively correlations between particle size and BKFY. d (0.5) was not significantly correlated with FY. These results confirm previous studies indicating that soft kernel texture is associated with flour with finer particles and low damaged starch due to the milling process (Choi and Baik, 2013).

Among the two end-products, CODI showed larger r-values. CODI was significantly and negatively correlated with SKHRD, WPROT, FPROT, SRCs, PeakOXI, and d(0.5). CODI was also significantly and positively correlated with FY, BKFY, and CAVOL. Similar relationships are reported in the literature (Kaldy and Rubenthaler, 1987; Gaines, 1985; Bettge and Morris, 2007). However, in this study the magnitude BKFY had the largest correlation coefficient among traits. These relationships confirm that low particle size and low damaged starch content produced during milling had a significant impact on spread of cookies. Furthermore, it is reported that flour proteins do not form a viscoelastic network, but rather act as water binders that affect texture and shape of cookies (Pareyt et al., 2008). These results of this study suggest that the solvation of flour proteins decreased the spread of cookies more than network formation, given the larger r-values associated with WPROT and FPROT in comparison to lactic acid SRC. However, gluten network formation may still be a contributing factor. Furthermore, OG capacity of AX may to reduce cookie diameter through enhanced sugar-syrup sequestration as suggested by Bettge and Morris (2007).

CAVOL was significantly and negatively correlated with SKHRD, WPROT, FPROT, water-, and carbonate SRCs, and d(0.5). Similar relationships were reported in the literature (Kaldy and Rubenthaler, 1987; Gaines, 1985). However, in this study SRCs did not show large or significant relationships with CAVOL as they were for CODI. Interestingly CAVOL was significantly and negatively correlated with FASH. These results contrasted with previous studies indicating a negative or not significant associations between CAVOL and FASH (Kaldy and Rubenthaler, 1987; Gómez et al., 2010). CAVOL was significantly and positively correlated with BKFY. CAVOL had similar relationship compared to CODI in terms of flour particle size and flour protein functionally. Also, the order of magnitude of the three largest correlations were BKFY, SKHRD, and d(0.5) suggesting that these traits influenced greater volume of cakes. Important to the aims of this study, the PeakOXI was not significantly correlated with CAVOL and this result was supported by lack of a significant correlation between CAVOL and sucrose-SRC. These correlations contrasted with the results of Bettge and Morris (2007) that indicated that PeakOXI was more likely to have influence in batterbased products with low shear conditions.

Figure 5.1 shows a scatterplot matrix between PeakOXI, d(0.5) CODI, and CAVOL. PeakOXI showed a negative trend with CODI. In contrast, PeakOXI did not show a trend with d(0.5) and CAVOL. d(0.5) showed a negative and similar trend with both CAVOL and CODI. CAVOL and CODI showed a positive trend. Figure 5.1 Scatterplot matrix between oxidative gelation capacity, median particle size distribution, sugar-snap cookie diameter, and Japanese sponge cake volume.



PeakOXI = Oxidative gelation capacity; d(0.5) = Median particle size; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume

The stepwise regression analysis was used as a tool to understand the predictors that best described functionality of traits in end-products and minimized variability and multicollinearity among traits. CODI and CAVOL were used as predictors. For CODI selection, the stepwise regression eliminated SKHRD, BKFY, and sucrose SRC based on forward selection on the minimum BIC model. For CAVOL selection, the stepwise regression eliminated SKHRD, FY, all SRCs and d(0.5) based on forward selection on the minimum BIC model. After the stepwise analysis, one-way ANOVA was used to describe the main effects predicting CODI and CAVOL.

Table 5.4 shows ANOVA with CODI as the response variable. All F-values were statistically significant and the variation in the model was well explained with an R^2 of 0.70. In order of effect size, d (0.5), PeakOXI, WPROT and FY had the largest

magnitude effects on CODI. Among absorption characteristics, carbonate SRC had the largest magnitude effect. These results suggest that the OG capacity had a significant impact in the spread of cookies. Bettge and Morris (2007) also reported that OG reduced CODI. Protein concentration appeared to have an important influence on water binding capacity that affected the spread of cookies, but the effect of lactic acid-SRC indicated that the viscoelastic properties of these proteins still had an effect.

Source	CODI
Whole model R ²	0.70
F values	
Whole model	27.2**
WPROT	28.83**
FY F value	28.65**
FPROT F value	17.36**
Wa SRC F value	5.77*
Ca SRC F value	19.60**
La SRC F value	7.63**
PeakOXI	29.94**
d (0.5) F value	36.53**

Table 5.4. F-values of ANOVA analysis of the best model predicting CODI

WPROT = wheat protein; FY = flour yield; FPROT = flour protein; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; PeakOXI = Oxidative gelation capacity; d (0.5) = median particle size distribution; *, ** indicate $p \le 0.01$ and p < 0.05 respectively.

Table 5.5 shows ANOVA with CAVOL as the response variable. The whole model only explained 37% of the variation in CAVOL. For this model, BKFY had the largest significant effect on CAVOL. PeakOXI had a smaller but still significant effect. In contrast WPROT did not have a significant effect on CAVOL in this model. However, results of WPROT suggest that the quality of protein may not have a significant effect on

CAVOL but may be an indicator of soft wheat quality as it is reported that higher grain protein concentrations produce smaller volume in cakes (Gaines, 1985; Gómez et al., 2013; Nakamura et al., 2012).

Source	CAVOI
Whole model R ²	0.37
F values	
Whole model	88.69**
WPROT	1.38 ns
BKFY	150.92**
PeakOXI	5.29*

Table 5.5. F values of ANOVA analysis of the best model predicting CAVOL

WPROT = wheat protein; BKFY = Break flour yield; PeakOXI = Oxidative gelation capacity; *, ** indicate $p \le 0.01$ and p < 0.05 respectively; ns = no significant.

5.3.1 Virtual selections of SWW wheats based on OGC and two typical screening traits at OSU

Table 5.6 shows one-way ANOVA of a virtual selection placed on the SWW samples from the 2011 dataset. The strategy was to place selection pressure on the SWW samples by either or both removing the lowest quality 10% (46 of 463 entries) or selecting the highest quality 10% of the SWW samples based on the use of a single trait for selection.

Tables 5.4 and 5.5 showed that PeakOXI had significant effects in the prediction models for CODI and CAVOL. Given that part of our aim was to understand the value of OG on SWW end-products we decided to use this as a selection criterion. Using PeakOXI we gained reductions of 20.1 in SKHRD, 1.1% in WPROT, 1.0% in FPROT, and 11.1 μ m in

d(0.5). All of these reductions could be considered quality improvements. There were also increases: 2.7% in FY, 6.6% in BKFY, 0.2% in FASH, 0.45 cm in CODI, and 53 mL in CAVOL. All these, except the increase in FASH, are also quality improvements. Whether the changes observed in the PeakOXI parameters are good or bad is unclear.

From a more practical viewpoint, the workflow in the lab during the summer screening of early generation lines is the primary use of SKHRD, followed by BKFY for screening and selection. Using only SKHRD we gained reductions of 39.1 in SKHRD (expected), 2.4% in WPROT, 1.9% in FPROT, and 28.4 μ m in d(0.5). All of these reductions could be considered quality improvements and were of greater magnitude than observed using PeakOXI as the screening trait. There were also increases: 1.1% in FY, 7.5% in BKFY, 0.2% in FASH, 0.6 cm in CODI, and 134 mL in CAVOL. All these, except the increase in FASH, are also quality improvements and were again greater than those gained by using PeakOXI, except for FY.

Using BKFY as the primary criterion we gained reductions of 26.3 in SKHRD (expected), 1.6% in WPROT, 1.5% in FPROT, and 18.2 µm in d(0.5). All of these reductions could again be considered quality improvements and were also of greater magnitude than observed using PeakOXI as the screening trait, except for the decrease in median particle size. There were also increases: 3.5% in FY, 10.7% in BKFY, 0.2% in FASH, 0.58 cm in CODI, and 122 mL in CAVOL. The gains in quality, except for poorer FASH quality, were of similar magnitude to those seen for selection based on SKHRD. In all cases higher PeakOXI was associated with the nominally poorer quality selection.

Considering that our typical workflow uses SKHRD to primarily screen the 4th generation material and then BKFY to screen 5th and subsequent generations, we considered it to be of value to make further section on the SKHRD selections based on BKFY. We resorted the softest and hardest 46 samples based on BKFY and selected the 23 softer samples with highest BKFYs, and the 23 harder samples with the lowest BKFYs (Table 5.6).

It seemed that selection first on SKHRD and then on BKFY (Table 5.6) yielded no obvious gains in the quality of the best lines compared to selecting just on SKHRD or on BKFY alone. Clearly the BKFY data is of value as this can be collected while creating flour for subsequent SRC analyses. It is unfortunate that SRC data, nor sufficient flour to do the missing SRCs, was not available for all samples in this set.

This data is supportive of the outcomes of the full data analysis for this sample set in BKFY and PeakOXI. BKFY was the most significant quality parameter in predicting CAVOL. PeakOXI was one of the main effects for both CODI and CAVOL. Furthermore, PeakOXI could be associated with poor soft wheat quality effect.

This data contrast with the outcomes of the full data set in BKFY, d(0.5), and SKHRD. BKFY as a primary selection contrasted to the full data set in an increase in CODI, whereas in the full data set was not a significant effect in predicting CODI. d(0.5)showed reduction in selection of CODI when selecting for BKFY and contrasted with the full data because it was the largest effect in magnitude predicting CODI. SKHRD was not a significant effect in predicting for both CODI and CAVOL in the data set.

Selection trait: PeakOXI n=92	SKHRD	WPROT	FY	BKFY	FPROT	FASH	d(0.5)	PeakOXI	CODI	CAVOL
higher quality: low PeakOXI	12.6b	8.0b	70.9a	50.2a	6.6b	0.40a	46.2b	92.8b	9.64a	1292a
lower quality: high PeakOXI	32.7a	9.1a	68.2b	43.6b	7.6a	0.38b	57.3a	1082a	9.19b	1239b
Selection trait: SKHRD n = 92	SKHRD	WPROT	FY	BKFY	FPROT	FASH	d(0.5)	PeakOXI	CODI	CAVOL
higher quality: low SKHRD	5.9b	7.5b	70.7a	51.4a	6.5b	0.41a	39.4b	176b	9.68a	1325a
lower quality: high SKHRD	45.0a	9.9a	69.6b	43.9b	8.4a	0.39b	67.8a	325a	9.08b	1191b
Selection trait: BKFY n = 92	SKHRD	WPROT	FY	BKFY	FPROT	FASH	d(0.5)	PeakOXI	CODI	CAVOL
higher quality: high BKFY	10.2b	8.1b	71.2a	52.0a	6.7b	0.41a	44.2b	114b	9.66a	1324a
lower quality: low BKFY	36.5a	9.7a	67.7b	41.3b	8.2a	0.39b	62.4a	441a	9.08b	1202b
Selection trait: SKHRD then BKFY n = 46	SKHRD	WPROT	FY	BKFY	FPROT	FASH	d(0.5)	PeakOXI	CODI	CAVOL
higher quality: high BKFY	8.6b	7.6b	70.2a	50.2a	6.3b	0.41a	42.1b	244a	9.61a	1311a
lower quality: low BKFY	43.8a	10.1a	70.4a	45.6b	8.6a	0.39b	65.7a	282a	9.17b	1207b

Table 5.6. One-way ANOVA of virtual selection based on oxidative gelation capacity, kernel hardness, and break flour yield

TWT = Test weight; SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = Flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = Median particle size distribution; CODI = Cookie diameter; CAVOL = Japanese sponge cake volume.

5.4 Conclusions

Table 5.1 showed that there was a range of variability in the traits measured. Some traits had high CVs" e.g. WPROT, FPROT, SKHRD, and lactic acid SRC, while others had quite low CVs e.g. FY, BKFY, water-, carbonate-, and sucrose-SRCs, and CODI and CAVOL. This suggest that, for example, SKHRD is much more sensitive to G&E effects than the downstream flour and end-product traits except for PeakOXI, d(0.5), and lactic acid SRC. It is not clear why this large variability in, say, SKHRD is not translated into equally large variability in milling properties. Similar could be asked for PeakOXI.

Significant relationships between PeakOXI and protein contents, and SRCs were observed (Table 5.3). The largest significant relationship was between PeakOXI and sucrose SRC in agreement with the results of Kweon et al. (2011). Notably 13 soft wheat samples had PeakOXI values higher than 800 cP. PeakOXI values this high have never been observed in soft wheats prior to this study. Interestingly the variety Tubbs was one of the 13 samples with high PeakOXI and was previously reported as high PeakOXI variety (Fajardo and Ross, 2015; Mattson, 2014). However, the implications of the observations are unclear. Further research is needed, and the varieties that make up these 13 samples are a valuable genetic resource for that future work.

In this study d(0.5) was positively associated with SKHRD and negatively with BKFY (Table 5.3). This was in agreement the findings of Gaines (1985) using soft red winter

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wheats. The lack of significant relationships between d(0.5) and PeakOXI suggested that flour particle size did not influence the OG capacity of flour.

End-product quality showed low variability suggesting that the other ingredients in the formulations (e.g. eggs and sugar) buffered the end-products against the large variability in SKHRD, protein contents, and PeakOXI. CODI is a common predictor of overall soft wheat quality. In this study CODI had similar significant relationships with other soft wheat traits when comparted to those reported previously (Gaines, 1985; Kaldy and Rubenthaler, 1987). CODI also had significant negative relationships with PeakOXI supporting previous results (Finnie et al., 2006; Bettge and Morris, 2007) saying that OG capacity increased the batter viscosity and was correlated with reduction of the reduction of CODI. In contrast to CODI, CAVOL showed lower magnitudes in r-values indicating weaker relationships with the other traits. CAVOL showed similar significant relationships with SKHRD, WPROT, and BKFY when compared to those reported previously (Gaines 1985; Kaldy and Rubenthaler, 1987). Among SRC values CAVOL only showed significant and weak correlations with water- and carbonate SRCs suggesting that the increased damaged starch in flour decreases CAVOL. Similar to Nakamura et al (2010), CAVOL was not significant correlated with lactic acid SRC suggesting that lactic acid SRC has no influence on Japanese sponge cake quality. In contrast to Nakamura et al (2010), in this study CAVOL did not show significant correlations with sucrose SRC. These results suggested that AX and their oxidative gels did not have an effect on CAVOL, but one could speculate that AX and their oxidative gels have an effect on batter viscosity (see Chapter 6). Interestingly CAVOL was

positively correlated with FASH in contrast to reports of either no significant or negative correlations (Gaines, 1985; Kaldy and Rubenthaler, 1987).

Selection of SWW for suitability for specific products is crucial for the OSU SWW wheat breeding program. This study showed that soft wheat traits had stronger relationships with sugar-snap cookies compared to their relationships with Japanese sponge cakes. When selecting for sugar-snap cookies, ANOVA showed that d(0.5), PeakOXI, WPROT, and FY were the most significant traits predicting CODI (Table 5.4). However, correlation analysis (Table 5.3) showed that BKFY, carbonate SRC, SKHRD, and d(0.5) were the most important traits. When selecting for Japanese sponge cakes, both ANOVA and correlation analysis showed that BKFY was the single most significant trait for predicting CAVOL. However, in correlation analysis (Table 5.3) SKHRD, and protein contents also had significant effects on CAVOL with similar r-values to BKFY. Table 5.6 showed that the best gains for CODI using a single trait for selection was with SKHRD. The best gains for CAVOL were made using BKFY. The latter agreed with both ANOVA and correlation analyses that showed BKFY to be the most influential on CAVOL (Tables 5.3, 5.5).

Overall, in this study BKFY and SKHRD were related to higher soft wheat quality, confirming previous reports indicating that these are fundamental traits in breeding selection (Souza et al., 2012). In contrast to our hypothesis OG capacity had different effects for CODI and CAVOL. OG capacity was related to poor flour quality. However, OG capacity might have an influential effect on batter viscosity that was not measured in this study. Because of the unbalanced SRC data, further research is needed to use SRC test as a selection tool for end-product quality.

References

See Bibliography

Chapter : 6 Measuring batter properties to predict cake making quality.

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Abstract

The objective of this study was to investigate the relationships between wheat quality traits, and cake batters and cake making quality in three cake types: Japanese sponge cake (SC), layer cake (LC), and pound cake (PoC). A parallel aim was to use the Rapid Visco Analyzer (RVA) to develop methodology to rapidly predict cake quality performance for screening or selection in wheat breeding. Five soft white winter (SWW) wheat samples with different inherent oxidative gelation capacity (PeakOXI) and absorption characteristics were selected. In SC, the varieties did not show significant differences in cake quality traits. SC volume had a strong negative association with oxidative gelation capacity (PeakOXI). Batter viscosity in this study was significantly and negatively correlated with SC volume. For layer cake (LC), the variety Tubbs, with the harder kernel and higher absorption characteristics, had the largest LC volume. In contrast to SC, LC volume was significantly and positively associated to PeakOXI. However, in agreement with SC, batter viscosity was significantly and negatively correlated with LC volume. Therefore, the test to measure batter viscosity in this study was fast enough (8 min) and suitable for both SC and LC. In pound cake (PoC), the variety Kaseburg, with high protein content, had the largest cake volume. PoC was significantly and positively associated with protein concentration suggesting that flour proteins were important for larger volumes and confirming other observations in the literature. PoC volume was not associated with PeakOXI refuting our hypothesis that PeakOXI would be associated with

all three cake types. For PoC batter viscosity did not predict volume, thus our hypothesis that a single method would be able to predict all three cake types was wrong. The varieties Skiles and Kaseburg performed better for SC volumes. Kaseburg performed better for PoC volume. Tubbs performed better for LC volume. Rosalyn showed a similar response for all cakes.

6.1 Introduction

Cakes are made of a variety of interacting ingredients. A highly aerated tender structure is a desired outcome in the finished product. Physicochemical properties and quality of cakes are governed not only by the ingredients *per se*, but also by their ratios and concentrations in the formulation. Soft wheat flour is one of the most important ingredients and because its components provide viscosity to the batter and promote the retention and expansion of air bubbles as well as ensuring uniform dispersion of the ingredients for optimum performance (Wilderjans et al., 2008; Wilderjans et al., 2010).

Kernel hardness is one of the most important traits in common wheat. In particular, soft kernel textures produce flour with low particle sizes and low starch damage (Choi and Baik, 2013). Flour particle size is an important factor and influences both cake volume and cake crumb structure. Particle size is reported to be positively correlated with batter viscosity (Moiraghi et al., 2011; Nakamura et al., 2010). Batter viscosity was also reported to be negatively correlated with sponge cake volume (Moiraghi et al., 2011; Choi and Bail, 2013). Hence small particles produce an optimum batter viscosity and

greater cake volumes with finer crumb texture (Gomez et al., 2010; Choi and Baik, 2013; Moiraghi et al., 2011).

The development of a gluten network in cake batters is restricted because of the choice of soft wheat flour with low intrinsic gluten strength and low protein concentration as the raw material, and high levels of sugar and water in the formulation. (Wilderjans et al., 2008; Delcour et al., 2012). However, it has been reported that flour proteins provide gascell walls in cakes with higher resistance to collapse after baking, giving a more uniform gas-cell distribution and larger cake volumes (Wilderjans et al., 2008).

The solvent retention capacity (SRC) test was developed to predict soft wheat quality of baked products by examining the individual contributions to flour absorption characteristics of the major polymer components (arabinoxylans, starch, gliadins, and glutenins), predicting the influence of polymers during processing and in end-products. The SRC test uses four different solvents (water, 50% w/w sucrose, 5% w/w sodium carbonate, and 5% w/w lactic acid: Kweon et al., 2011). Water-SRC is associated with the overall swelling properties of the polymers. Sucrose-SRC is associated with swelling properties of arabinoxylans and gliadins. Sodium carbonate-SRC is associated with swelling properties of damaged starch. Lactic acid-SRC is associated with swelling properties of glutenins (Kweon et al., 2011). Nakamura et al. (2010) reported significant ($p \le 0.01$) negative correlations between Japanese sponge cake volume and water and sucrose-SRCs (r = -0.54, and -0.67 respectively). However, extensive reports about predicting suitability of unchlorinated flours for different cake-types performance using SRCs are not available.

During batter mixing the ingredients in the formulation are incorporated to form a stable batter. Baking converts the batter into a semisolid material. Viscosity in cake batters is important because the viscous batter traps the gas bubbles that are produced during mixing as a consequence of whipping (Chesterton et al., 2013) and/or chemical reactions of leavening agents. These processes affect attributes such as volume and tenderness (Wilderjans et al., 2010).

Cake batters have Non-Newtonian rheology. This makes it complicated to measure viscosity and to predict behavior of cake batters during baking (Sahin, 2008). The structural change from flowable batter to semi-solid material properties of cakes is driven primarily by changes in starch, such as gelatinization, and in proteins, such as crosslinking and heat setting. Upon heating, batter viscosity decreases to a value approximately 10% of that at room temperature, but the viscosity is still high enough to minimize gas cell coalescence, disproportionation, and bubble rise, and to keep starch granules suspended before gelatinization (Wilderjans et al., 2010). With continued heating, starch swells and some proteins denature and coagulate simultaneously and as a result there is a tremendous increase in viscosity, giving the solid-like appearance (Wilderjans et al., 2013; Sahin, 2008).

Cakes can be classified using two categories: the ingredients used in the formulation and the mixing process. Based on the ingredients, cakes can be classified as high and low ratio. High ratio is based on the amount or ratio of sugar being higher than the amount or ratio of flour in the formulation. Examples of high ratio cakes are Japanese sponge cakes and layer cakes. Based on mixing process, cakes can be classified as a creaming or foaming (meringue). This classification is based on how the sugar is dissolved to produce the batter (Wilderjans et al., 2010). For example, sponge cakes are examples of high ratio cakes made of batters using a foaming mixing method. Layer cakes are high ratio cakes made of batters using a creaming mixing method. And pound cakes are low ratio cakes made of batters using a creaming mixing method.

Sponge cakes are a popular soft wheat application in Asian countries. These are highratio cakes that use a foaming method to produce the batter. In particular, Japanese sponge cake (Nagao et al., 1976) can be used as to assess for soft wheat quality, as soft wheat flours are commonly used for sponge cake making (Choi and Baik, 2013). The Japanese sponge cake method originally reported in the Western literature by Nagao et al., (1976) uses soft wheat flour, whole eggs, and sugar. However, because of the high level of technical skill required by operators, the method is not a AACC-I Approved Method and is not used worldwide.

Layer cakes are high-ratio cakes rich in fat. Gas cells are incorporated into the fat phase during the mixing of the batter at room temperature. Layer cakes can be used for assessment of cake flour quality (AACC-I Approved Method 10-90.01).

Pound cakes are low-ratio cakes. The main ingredients, flour, sugar, eggs, and butter (or other shortening) are all at the same concentration (1:1:1:1) and the cake batter is initiated by creaming the butter with the sugar.
Different methods and techniques to measure batter viscosity properties during processing have been reported (Wilderjans et al., 2008; Nakamura et al., 2012). However, Nakamura et al (2012) reported a method that was not reproducible in our lab. Wilderjans et al. (2008) reported a method to measure batter viscosity using the RVA but the method required 30 min to run, in addition to sample preparation time, and only predicted functionality of one type of cake batter: pound cake batters. Rheological properties of batters are difficult to measure because batters contain multiple dispersed phases and, as a partial consequence, exhibit Non-Newtonian behavior.

Understanding flour functionality and cake batter viscosity is important in determining the optimal functionality of flour in cake making. However, as a result of the complexity of the interactions, variations in the relative concentrations of ingredients, and the diversity of cake-making methods, it is a challenge to predict cake making performance from the physicochemical characteristics of the raw materials. Therefore, it is of interest to understand how wheat quality parameters affect the functionality of cake batters and cake performance. Chapter 4 showed that the standard soft wheat quality tests were only able to predict about 60% of the variability in Japanese sponge cake volume. Chapter 5 showed that the addition of other quality traits: d(0.5) and PeakOXI were not able to improve prediction to any practical extent.

The aim of this study was to investigate the relationships between wheat quality parameters, cake batters, and cake making quality in three cake types: Japanese sponge cake, layer cake, and pound cake by using a discrete sample set, and the application of novel methods for predicting cake performance across three cake types, rather than the previous focus on just Japanese sponge cakes. Five soft white winter wheat samples were selected. These contrasted in inherent oxidative gelation capacity and SRC absorption characteristics. A parallel aim was to use the Rapid Visco Analyzer (RVA) to develop methodology to rapidly predict cake quality performance for screening or selection in wheat breeding. Our hypotheses were: cake batter and cake making quality for Japanese sponge cake, layer cake, and pound cake could be predicted using a single test; and batter viscosity and oxidative gelation capacity would be associated with cake making quality.

6.2 Materials and Methods

6.2.1 Materials

Five soft white winter wheat samples (Bobtail, Rosalyn, Skiles, Tubbs, and Kaseburg) were obtained from the Oregon State University wheat breeding program elite winter yield trails. Grain was grown in Oregon at the Hyslop Crop Science Research Farm, Corvallis, Oregon and harvested in 2014. The samples were chosen to express contrasting varietal qualities, particularly oxidative gelation capacity and SRC absorption characteristics. In the 2015 Preferred Varieties Lists for Washington, Oregon, and Idaho (Washington Association of Wheat Growers, 2016), Bobtail and Kaseburg were classified as most desirable, Rosalyn, Skiles, as desirable, and Tubbs as least desirable. The Hyslop Farm site was chosen as it was known to produce grain of low protein content. Prior to milling, the samples were tempered to 14%. Wheat was milled at the Wheat Marketing Center (Portland, OR) on a pilot-scale, Miag-Multomat mill (Buhler-Miag Co Switzerland). Break flour and total flour yields were reported.

Kernel hardness index (SKHRD) was measured with an SKCS 4100 (Perten Instruments, Hägersten, Sweden; AACCI Approved Method 55-31.01). Wheat protein (WPROT) and flour proteins (FPROT) were measured with NIR spectroscopy (Infratec 1241, Foss USA, Eden Prairie, MN, U.S.A.; AACCI Approved Methods 39-10.01 and 39-11.01). Water, carbonate, sucrose, and lactic acid SRCs were measured using AACCI Approved Method 56-11.02. Pasting viscosity was measured with an RVA-4500 (Perten Instruments Hägersten, Sweden; AACCI Approved Method 76-21.01).

Oxidative gelation was measured using the RVA-based method of Ross et al. (2014). Flour was hydrated for 20 min in 50 mL lidded plastic centrifuge tubes with a Labquake tumbler (Thermo Scientific, Asheville, NC, U.S.A.). After pre-hydrating, as quickly as possible (within 20 s) and without allowing the suspension to settle by continued gentle hand shaking, 30.0 ± 0.05 g was transferred quantitatively to an RVA canister. The RVA was set at 30°C throughout the entire test sequence. A 10 s high-speed mixing (960 rpm) was applied as in the RVA standard 1 profile (Batey 2007; AACCI Approved Method 76-21.01), and the viscosity of the flour–water suspension was sensed at 160 rpm for 1 min. This established the flour–water baseline viscosity. The RVA was stopped, and 65 µL of 3% H₂O₂ was added. The RVA was restarted, the 10 s high-speed agitation repeated, and the viscosity of the suspension sensed at 160 rpm for a further 5 min. Measured parameter was the oxidative gelation capacity (PeakOXI).

The particle size distribution of 40 mg flour suspended in 150 mL isopropanol alcohol (IPA) was measured on a Mastersizer & Hydro 2000s (Malvern Instruments, Ltd.,

Malvern, Worcestershire, UK). IPA was used to prevent flour particle swelling. Particle refractive parameter and absorption indices were set to 1.52 and 0.1 respectively (Hu et al., 2007). Obscuration varied from 7% to 12% indicated optimal sample concentrations. Laser diffraction was conducted based on the assumption that flour particles conform to a hard, spherical particle model (Rawle, 1993). Particle size analysis provides a number of calculated values (e.g., diameter mean, surface mean, volume mean) and these investigate the size distribution and properties of samples. In this study the parameter median particle size, d (0.5), represented 50% of particles below these diameters was reported.

Commercial ultrafine cane sugar was obtained from C&H Sugar (Crockett, CA, USA). Pasteurized AA grade eggs were obtained from local a supermarket. Dried egg whites were obtained from Modernist Pantry USA (York, ME, USA). Nonfat dried milk, unsalted butter, and salt were obtained from Kroger (Virginia Beach, VA, USA). Vegetable shortening was obtained from Crisco (Orrville, OH, USA). Baking powder was obtained from ACH Food Co. Inc. (Memphis, TN, USA).

6.2.2 Cake batter and baking procedures.

Table 6.1 shows the cake formulations. Japanese sponge cakes (SC) were made at the Wheat Marketing Center (Portland, OR) based on the procedure of Nagao et al. (1976). To prepare egg foam, fresh whole eggs (700 g) and sugar (700 g) were hand-mixed with a whisk in the 12 L stainless steel bowl of a Hobart mixer (Hobart, Troy, OH) to obtain a homogenous blend, and then the mixture was warmed to 41°C in a 50°C water bath to completely dissolve the sugar in the whole eggs. The egg-sugar mixture was continuously

whipped for 0.5 min on low speed, for 5.75 min on high speed, and for 0.5 min on low speed using the Hobart mixer equipped with a wire whisk. During mixing, the application of heat to the mixing bowl prevents the temperature of the mix from dropping below 30°C. Deionized water (280 mL at 50°C) was added in two parts (140 mL each), at 3.75 min and 4.75 minutes respectively. After a total whipping time of 6.75 min, the specific gravity was measured using a 100 g specific gravity cup (Magnuson Engineers, San Jose, CA). The desired specific gravity was 22.5 ± 0.7 g/100 mL based on the Wheat Marketing Center's in-house procedures. If the egg foam density was out of specification, whipping time was adjusted in a new batch of egg and sugar to achieve the desired density. Specific gravity measurement is important to compensate for the variability in eggs quality and hence foam density. The whipped egg foam was divided into four portions of 240 g. 100 g flour was sprinkled over each portion of the whipped egg foam and then incorporated by gently folding using a wooden paddle 80 times. The batter was poured into rounded lined cake pans of 15.2 cm diameter and 5.7 cm height and baked at 190°C for 30 min.

Layer cakes (LC) were manufactured based on AACC-I approved method 10-90.01. Cake batter density was determined using a 100 g specific gravity cup (Magnuson Engineers, San Jose, CA). 200 g of cake batter was pour into a cake pans 15.2 cm diameter and 5.7 cm height. Cakes were baked in a Baxter Mini Rotating Rack Oven (Baxter Corp. Orting, WA, USA) at 150 °C for 22 min.

Pound cakes (PoC) were manufactured based on the procedure of Wilderjans et al. (2008). Butter and sugar were mixed in an N50 5-Quart Hobart mixer (Hobart, Troy, OH)

using the beater attachment at speed 1 for 0.5 min and at speed 2 for 3 min. After, eggs and flour were poured into the mixing bowl and mixed at speed 1 for 30 sec. After this time salt ant leavening agent were added to the mix for a final mix at speed to for 4 min. 250 g of batter was place into baking pan (rectangular baking pan 14.6 cm x 8.3 cm x 5.7 cm). Batter density was determined using a 100 g specific gravity cup (Magnuson Engineers, San Jose, CA). Cakes were baked in a Baxter Mini rotating rack oven (Baxter Corp. Orting, WA, USA) at 150 °C for 30 min

Table 6.1. – Cake formulations

Ingredients	Japanese sponge cake (g)	Layer cake (g)	Pound cake (g)
Flour	100	200	150
Sugar	155	280	150
Whole eggs	155		150
Dried egg whites		18	
Butter			150
Shortening		100	
Nonfat dry milk		24	
Baking powder		15	6
Salt		6	1.5
Water deonized	31	290	

6.2.3 Cake assessment

After baking cakes were promptly removed from their pans, cooled at room temperature, and placed in a cabinet with limited air circulation for 24 hrs. Cakes were weighed and the weight reported. Cake volume was measured using a scanning laser bread volume meter (Perten Instruments, Hägersten, Sweden; AACC-I Approved Method 10-14.01). Sponge cakes were cut in half with a knife for a visual examination of interior appearance (maximum score of 20) and crumb firmness (maximum score of 30) (in-house methods, Wheat Marketing Center, Portland, OR). Crumb structure parameters of cake were measured using the C-Cell food structure image analysis instrument (Caliber Control International Ltd, United Kingdom). A one-inch thick slice of each cake was placed into the C-Cell for image analysis. Reported parameters were cell number, diameter, area, and volume.

6.2.4 Cake batter viscosity

The RVA was used to measure cake batter viscosity. The RVA was set to 30°C for 2 min. Temperature was then increased from 30°C to 90°C at 15°C per minute. These temperatures were chosen to mimic the mixing and early baking stages of cake production. A 10 s high speed mixing (960 rpm) at the beginning was applied as in the RVA standard profile (AACC International Approved Method 76-21.01). Viscosity of the suspension was sensed at 160 rpm for the rest of the test. Initial batter peak viscosity (BPV) and batter minimum viscosity (BMV) were recorded (FIG 6.1). Determinations were done at least in duplicate.

Figure 6.1. RVA plot for cake batter viscosity.



The ratio of ingredients in this test was based on the formulation used to prepare Japanese sponge cake (Table 6.1). Egg-sugar solution was made of 100 g of fresh eggs, 100 g of sugar and 40 mL of deionized water. These were mixed vigorously and warmed up to 40°C using a Thermolyne heat and stir plate (Barnstead International, OH). This temperature is achieved during the making of Japanese sponge cake (Nagao et al., 1976). 30 g of egg-sugar solution and 12.5 of flour were poured into a lidded 50mL plastic conical-bottom centrifuge tube and shaken in a Shakematic (Perten instruments, Hägersten, Sweden) to homogenize the mixture and incorporate air bubbles. After shaking, as quickly as possible and with continued gentle hand shaking to prevent the suspension to settling, 30 ± 0.05 g was quantitatively transferred to an RVA canister.

Layer cake and pound cake batters were prepared as described above (Section 6.2.2.). 30 \pm 0.05 g of batter was transferred into an RVA canister and measured as described in this section.

Viscosity of the main ingredients (flour, eggs, and sugar) in cake batters was measured in a reverse stepwise removal procedure to see how lean the flour suspension could be and still predict cake performance. Three different systems were tested in the RVA using the same RVA temperature profile as used for the cake batters (Figure 6.1); Flour-watersugar-eggs (FWSE), flour-water-sugar (FWS), and flour-water (FW). The FWSE system was prepared mixing 10 g of flour and 25 g of water-sugar-eggs solution. The watersugar-eggs solution was prepared using 50 g of eggs hand beaten and mixed with 100 g of a 50% w/w sucrose solution prepared based on AACC International Approved Method 56-11.02. For the FWS system, 12 g of flour and 24 g of 50% sucrose solution was used. For the FW system, 12 g of flour and 18 g of deionized water were used. Each system was poured into a lidded 50mL plastic conical-bottom centrifuge tube and shaken in a Shakematic (Perten instruments, Hägersten, Sweden). After shaking, as quickly as possible and with continued gentle hand shaking prevent the suspension from settling, 30 ± 0.05 g was quantitatively transferred to an RVA canister and into to the RVA and the test commenced.

6.2.5 Statistical analysis.

Statistical analyses were performed using SAS JMP Pro 12.0.1 (SAS Institute Inc. Cary NC, USA). A p-value of ≤ 0.01 was used to indicate significance unless otherwise noted.

Mean-centered and auto-scaled principal component analyses (PCA) were performed using the Multivariate: Principal Component platform. Linear correlation coefficients were performed using the Multivariate: Multivariate platform.

6.3 Results and discussion

Table 6.2 shows mean, range, and standard deviations of all measured grain, flour, and cake traits, and ingredient interactions systems. The samples in this study showed a useable range of soft wheat quality. For example, WPROT ranged from 7.7 to 9.6%. However, as expected in this study there were contrasting results in quality traits among the samples. For example, the variety Tubbs had the highest PeakOXI value (677 cP). Bobtail, in contrast, had the lowest PeakOXI value (176.5 cP). Among quality traits SKHRD, WPROT, FPROT, FASH, lactic acid SRC, Peak OXI, and d (0.5) had CVs higher than 10%. Lactic acid SRC was similar to the variability of WPROT and FPROT suggesting that lactic acid SRC could also be affected by protein concentration. Among cake traits, SC BPV, SC BMV, LC volume, LC cell diameter, LC cell volume, LC BPV, PC BMV, and viscosity measurements of the ingredient interaction systems had CVs higher than 10%. These results suggest that soft wheat quality traits had an impact on different properties among cakes. Interestingly SC volume showed low variation (CV =2.5%). However, even maximum SC volume was lower than the target (1280 mL) for quality traits for the Pacific Northwest (Pacific Northwest Wheat Quality Council 2015, Appendix I).

The three cake types were different to each other. Although the correlations were nonsignificant, SC volume was negatively associated with LC and positively associated with PoC volume (Section 6.3.5).

Table 6.3 shows one-way ANOVA of soft wheat quality traits and cake quality traits measured in this study. FY and BKFY were single values because there was no replication during milling of samples. For soft wheat quality traits R-squared explained a significant variability of all the models ranging from 0.89 to 1.00. Varieties showed significant differences with soft wheat quality traits. PeakOXI, SKHRD, WPROT, and sucrose SRC showed the largest effect in magnitude (F-values) suggesting that these traits had higher contrasting characteristics amongst varieties in this study. For example, Tubbs showed the highest PeakOXI, SKHRD, and sucrose SRC. In contrast, Bobtail and Kaseburg showed the highest WPROT and FPROT.

For SC traits there were no significant differences among varieties (p > 0.05: Table 6.3). These observations suggested that the contrasting characteristics of the varieties did not affect sponge cakes probably because of the mixing process used to prepare the batter of this cake and other ingredients such as sugar and eggs may have masked the contrasting characteristics of flours.

For LC traits there were significant differences between LC volume, LC BMV amongst varieties (Table 6.3). The significant difference was for the variety Tubbs, which is considered as least desirable in 2015 (Washington Association of Wheat Growers, 2016)

and in this study showed the largest LC volume and the minimum LC BMV compared to the other varieties suggesting that the quality characteristics of Tubbs such as PeakOXI, sucrose SRC and SKHRD had a positive effect on LC volume.

For PoC traits there were significant differences between PoC volume, PoC number of cells, PoC area of cells, PoC BPV, and PoC BMV amongst varieties (Table 6.3). The significant largest effect in magnitude was for PoC BMV suggesting that the differences of quality traits in varieties had a significant impact in batter viscosity during early stages of baking process. For example, Kaseburg showed the largest PoC volume, PoC number of cells, and PoC BMV and also had the highest amount of FPROT content suggesting that protein concentration had a positive effect on formations of cells and hence higher cake volume. Wilderjans el al. (2008) observed that gluten forming proteins had an effect on setting the structure of pound cakes.

For ingredient interaction systems there were significant differences among varieties suggesting that measurement of viscosity was able to predict cake batter viscosity requires the amount and all ingredients in the cake formulation (Table 6.3).

Figure 6.2 shows a PCA loading plot identifying patterns among soft wheat quality traits. The first two principal components (PCs) explained 82.6% of the variability of the data. PC1 was dominated by SKHRD, water SRC, PeakOXI, FY, lactic acid SRC, and carbonate SRC. PC2 was dominated by WPROT, FPROT, sucrose SRC, FASH, d(0.5), and BKFY. Of concern was the positive association of d(0.5) and higher BKFY. This is opposite to the association observed in Chapter 5 (Table 5.3) and contrary to normal expectations of smaller particle sizes with higher BKFYs. More conventionally, high BKFY was associated with low-sucrose-SRC and low FASH. Surprisingly BKFY was orthogonal to SKHRD, showing no association between BKFY and kernel texture for this sample set. Notably, PeakOXI was orthogonal to sucrose-SRC. This suggested no association between PeakOXI and sucrose-SRC. This contrasted with results of Kweon et al. (2011) that indicated that sucrose-SRC was a good predictor of PeakOXI.

	Moon	SD	Min	Max	CV
<u> </u>	Mean	50	NIII	Iviax	
Grain ana flour	14.0	12.0		24.4	05.6
SKHKD	14.0	12.0	4.6	34.4	85.6
WPROI	8.5	0.9	/./	9.6	11.0
BKFY	43.6	2.6	40.1	46.9	5.9
FI	69.5	2.0	66.7	/1.4	2.9
FPROI	6.9	0.7	6.2	/.9	10.2
FASH W- CD C	0.44	0.05	0.40	0.50	12.4
wa SKC	55.3	1.9	53.9	58.5	3.4
Ca SRC	//.1	4.1	/2.8	83.3	5.3
Suc SRC	94.7	6./	88.4	102.2	/.1
Lac SKC	96.2	10.8	85.0	110.7	11.2
PeakOXI	364.1	204.4	1/6.5	6/7.0	56.1
d (0.5)	31.5	3.3	26.1	34.6	10.6
Sponge cake	1000	21	1105	1056	0.5
SC volume	1220	31	1185	1256	2.5
SC Number of cells	5238	386	4596	5602	7.4
SC Cell diameter	3.1	0.2	2.9	3.4	6.5
SC Area of cells	54.9	0.1	54.8	55.0	0.2
SC Cell volume	9.8	0.8	9.1	10.8	7.6
Batter viscosity		10.4.1	(17.5	0.60.0	14.0
SC BPV	795.1	134.1	617.5	969.0	16.9
SC BMV	402.6	151.0	228.5	401.0	12.9
Layer cake	100.0	50.0	112.5	57 0 0	10.6
LC volume	498.2	52.8	443.5	578.0	10.6
LC Number of cells	3143	117	2957	3267	3.7
LC Cell diameter	2.5	0.5	1.9	3.3	21.9
LC Area of cells	52.7	0.9	51.2	53.6	1.7
LC Cell volume	6.7	1.5	4.7	8.3	22.9
Batter viscosity					
LC BPV	3378	362	2929	3830	10.7
LC BMV	710.9	31.1	676.0	746.0	4.4
Pound cake					
PoC volume	387.9	23.9	361.5	425.0	6.2
PoC Number of cells	3122	173	2880	3337	5.5
PoC Cell diameter	1.6	0.1	1.5	1.7	4.6
PoC Area of cells	49.4	0.7	48.4	50.2	1.3
PoC Cell volume	4.1	0.3	3.8	4.5	6.2
Batter viscosity					
PC BPV	3760	425	3047	4131	11.3
PC BMV	788.2	62.9	714.5	842.5	8.0
Ingredient interaction system	1				
FW peak viscosity	243.8	58.2	191.5	327.5	23.9
FW minimum viscosity	158.9	31.5	131.5	205.0	19.8
FWS peak viscosity	902.7	194.4	722.5	1176.5	21.5
FWS minimum viscosity	387.8	79.7	314.5	508.0	20.6
FWSE peak viscosity	649.0	83.7	507.5	725.0	12.9
FWSE minimum viscosity	207.3	44.2	145.0	258.0	21.3

Table 6.2. Summary of statistics of grain, flour, cake quality, and ingredient interaction viscosity.

SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = median particle size; SC = Japanese sponge cake; BPV = Batter peak viscosity; BMV = Batter minimum viscosity; LC = Layer cake; PoC Pound cake; FW = Flour-water; FWS = Flour-water-sucrose; FWSE = Flourwater-sucrose-eggs.

FLOUR TRAITS	SKHRD	WPROT	BKFY	FY	FPROT	FASH	Wa SRC	Na SRC	Su SRC	La SRC	PeakOXI	d(0.5)
Whole model												
R-squared	0.99	0.99	no ANOVA	no ANOVA	0.98	0.89	0.97	0.99	0.98	0.96	1.00	0.96
F-value	222*	541*			90*	10*	37*	137*	107*	34*	1307*	27*
Effect means												
Bobtail	9.48c	9.4a	46.9	71.2	7.3b	0.40b	54.2b	72.8d	90.8b	110.7a	186d	32.6ab
Kaseburg	7.0dc	9.6a	40.1	68.6	7.9a	0.50ab	53.8b	78.6b	101.8a	100.8ab	177d	26.1c
Rosalyn	14.4b	7.7c	44.3	71.4	6.2c	0.40b	55.3b	76.2c	90.2b	85.0c	373c	33.5ab
Skiles	4.6d	7.7c	42.1	68.5	6.3c	0.40b	54.b	74.5cd	88.4b	98.4b	409b	30.8b
Tubbs	34.4a	8.2b	44.4	66.7	6.9b	0.55a	58.5a	83.3a	102.2a	86.2c	677a	34.6a

Table 6.3. one-way ANOVA of grain, flour, cake quality, and ingredient interaction viscosity between varieties.

		SC Number of	SC Cell	SC Area of	SC Cell		
SPONGE CAKE	SC volume	cells	diameter	cells	volume	SC BPV	SC BMV
Whole model							
R-squared	0.79	0.78	0.42	0.11	0.46	0.5	0.68
F-value	NS	NS	NS	NS	NS	NS	NS
Effect means							
Bobtail	1199a	4596a	3.39a	54.8a	10.8a	863a	378a
Kaseburg	1249a	5207a	3.10a	55.0a	10.3a	618a	229a
Rosalyn	1213a	5602a	2.87a	54.9a	9.0a	805a	333a
Skiles	1256a	5371a	3.12a	55.0a	9.7a	722a	292a
Tubbs	1185a	5416a	3.00a	54.8a	9.2a	969a	401a

		LC Number of	LC Cell	LC Area of	LC Cell		
LAYER CAKE	LC volume	cells	diameter	cells	volume	LC BPV	LC BMV
Whole model							
R-squared	0.89	0.41	0.66	0.57	0.79	0.78	0.87
F-value	10*	NS	NS	NS	NS	NS	8.48*
Effect means							
Bobtail	458b	2956a	2.3a	52.8a	6.1a	3612a	741a
Kaseburg	443.5b	3266a	1.9a	51.1a	4.7a	3830a	746a
Rosalyn	512ab	3116a	2.8a	52.8a	8.2a	3126a	700ab
Skiles	499.5ab	3183a	2.3a	53.0a	6.0a	3393a	691ab
Tubbs	578a	3194a	3.3a	53.6a	8.3a	2928a	676b
Rosalyn Skiles Tubbs	512ab 499.5ab 578a	3116a 3183a 3194a	2.8a 2.3a 3.3a	52.8a 53.0a 53.6a	8.2a 6.0a 8.3a	3126a 3393a 2928a	700a 691a 676t

		PoC Number	PoC Cell	PoC Area	PoC Cell		
POUND CAKE	PoC volume	of cells	diameter	of cells	volume	PoC BPV	PoC BMV
Whole model							
R-squared	0.88	0.89	0.68	0.92	0.74	0.88	0.98
F-value	8.8*	10.3*	NS	14*	NS	8.9*	49.5*
Effect means							
Bobtail	361.5b	2880b	1.6a	49.6a	4.1a	3730ab	714.5b
Kaseburg	425a	3336.5a	1.7a	50.1a	4.5a	3047b	834.5a
Rosalyn	394.5ab	3196a	1.5a	49.5a	4.1a	4008a	725b
Skiles	376b	3161ab	1.5a	48.3b	3.8a	3884a	824.5a
Tubbs	382.5b	3035ab	1.5a	49.2ab	4.0a	4131b	842.5a

INGREDIENT	FW peak	FW low	FWS peak	FWS low	FWSE peak	FWSE low
INTERACTION	viscosity	viscosity	viscosity	viscosity	viscosity	viscosity
Whole model						
R-squared	0.92	0.92	0.96	0.98	0.98	0.97
F-value	14.4*	14.6*	29.0*	55.78	69.4	47.32
Effect means						
Bobtail	191.5c	131.5b	738c	358c	650.5b	208.5bc
Kaseburg	327.5a	205a	1176.5a	508a	671.5ab	258a
Rosalyn	222.5bc	143b	854.5bc	331.5c	725a	187c
Skiles	198bc	137b	722.5c	314.5c	507.5c	145d
Tubbs	279.5ab	178ab	1022ab	427b	690.5ab	238ab

SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = median particle size; SC = Japanese sponge cake; BPV = Batter peak viscosity; BMV = Batter minimum viscosity; LC = Layer cake; PoC Pound cake; FW = Flour-water; FWS = Flour-water-sucrose; FWSE = Flour-water-sucrose-eggs. Significance at $p \le 0.05$.



Figure 6.2. Principal component analysis among soft wheat traits.

SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = median particle size

6.3.1 Japanese sponge cake

Figure 6.3 shows PCA of soft wheat and SC quality traits. The first two PCs accounted for 70.5% of the variability of the data. PC1 was dominated in the positive direction by SKHRD, water-SRC, PeakOXI, SC BPV, SC Number of cells d(0.5), and on the negative side by SC volume, SC cell volume, SC Area of cells, SC cell diameter. PC2 was dominated in the positive dimension by sucrose-SRC, FASH, and in the negative dimension by BKFY, FY, and SC BMV. WPROT and FPROT were vectored along both PCs. SC volume was strongly negatively

associated with PeakOXI, which supports the findings of Chapter 5. SC volume was not associated with BKFY, but was associated with lower SKHRD, as expected.



Figure 6.3. Principal component analysis between Japanese sponge cake volume and soft wheat traits.

SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = median particle size; SC = Japanese sponge cake; BPV = Batter peak viscosity; BMV = Batter minimum viscosity;

Interestingly lactic acid-SRC was in the same direction than SC volume along PC1. This relationship suggested that glutenins had a contribution to the batter viscosity and possible effects on crumb setting, beyond the simple effects of protein concentration, which was not as

strongly associated with SC volume. Wilderjans et al. (2008) reported that gluten forming proteins had an effect on cell wall structure in pound cakes. These associations suggest a similar effect in Japanese sponge cakes and were supported by positive associations of lactic acid-SRC and area, volume, and diameter of cells in Japanese sponge cake.

PeakOXI, d(0.5), SC BPV, SC number of cells, and water-, and sucrose-SRCs were associated with each other and were vectored opposite to SC volume. These results suggested that PeakOXI had a significant effect on viscosity of SC cake batter and produced a larger number of cells and lower SC volume. Similar to the analysis of soft wheat quality traits, in the presence of SC traits in the analysis, there was still minimal association between sucrose-SRC and PeakOXI.

Carbonate SRC was orthogonal to SC BMV and hence no associations between these parameters. Carbonate SRC was associated with SC BPV but the SC BPV has small leverage. These results suggest that damaged starch affected during the reduction in batter viscosity at early stages of baking process.

Table 6.4 shows linear correlation coefficients between SC cake volume and sponge cake cell and batter viscosity traits. At p < 0.05 SC volume was significantly and negatively correlated with both SC BPV and SC BMV. These results are very important for breeding programs because these significant relationships will lead to an analysis that can to predict Japanese sponge cake volume in less than 8 min.

Parameter	SC volume
Batter viscosity	
SC BPV	-0.92
SC BMV	-0.95
Cake cells	
SC Number of cells	ns
SC Cell diameter	ns
SC Area of cells	0.90
SC Cell volume	ns

Table 6.4. Linear correlation coefficients between Japanese sponge cake volume and cake quality traits.

SC = Japanese sponge cake; BPV = Batter peak viscosity; BMV = Batter minimum viscosity; significance at level p < 0.05

6.3.2 Layer cake

Figure 6.4 shows PCA of soft wheat and layer cake quality traits. The first two PCs accounted for 84.4% of the variability of the data. PC1 was dominated in the positive direction by LC volume, SKHRD, water SRC PeakOXI, LC Cell diameter, and LC Cell volume, LC area of cells, d(0.5), and on the negative direction by FPROT, WPROT, LC BPV, LCBMV, and lactic acid SRC. PC2 was dominated in the positive dimension by sucrose SRC, FASH, LC Number of cells, sodium carbonate SRC, FY, and BKFY. LC volume was associated with low WPROT, FPROT, and low lactic acid SRC as expected.



Figure 6.4. Principal component analysis between layer cake volume and soft wheat traits.

SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = median particle size; BPV = Batter peak viscosity; BMV = Batter minimum viscosity; LC = Layer cake.

In contrast to SC volume, LC volume was positively associated with PeakOXI (in PC1). These results suggest that absorption capacity of AX had a positive impact on LC batter viscosity affecting the volume of LCs. These results contrasted to Japanese sponge cake findings. It is plausible that the divergence in association between SC volume and LC volume with PeakOXI is a function of differences in the mixing method (creaming versus foaming) as they were both high-ratio cakes.

Interestingly LC volume was positively associated with SKHRD and d(0.5). These results contrasted with a report indicating that softer kernels produce flours with lower particle sizes, and produce larger LC volumes (Gómez et al., 2010). However, the samples used in the study from Gómez et al. (2010) were from Spain and characteristics of that wheat may be inherently different. Therefore, the quality attributes of the samples in this study may have different genetic characteristics that influenced layer cake batter viscosity and as a consequence the volume.

LC cake volume showed negative associations with WPROT, FPROT, lactic acid-SRC, LC BPV, and LC BMV. These results indicated that absorption properties of glutenins may compete for water with other ingredients increasing the viscosity of the cake batter leading to lower layer cake volumes. Furthermore, these results suggest that lactic acid-SRC could be a predictor (negative) for layer cake volume. Notably, LC BPV and LC BMV were also negatively associated with LC volume indicating the batter viscosity measured in the RVA could also be a good predictor of layer cake volume.

Carbonate SRC was orthogonal to both LC BPV and LC BMV indicating that damaged starch was not associated with batter viscosity in layer cakes in this sample set.

Table 6.5 shows linear correlation coefficients between LC volume and layer cake cell and batter viscosity traits. At P < 0.05 LC volume was significantly and negatively correlated with both LC BPV and LC BMV. These results are very important for breeding programs because that batter viscosity measured in the RVA could be a good predictor of volume in both LC and SC.

Parameter	LC Cake volume
Batter viscosity	
LC BPV	-0.96
LC BMV	-0.93
Cake cells	5
LC Number of cells	ns
LC Cell diameter	0.95
LC Area of cells	ns
LC Cell volume	ns

Table 6.5. Linear correlation coefficients between layer cake volume and cake quality traits.

BPV = Batter peak viscosity; BMV = Batter minimum viscosity; LC = Layer cake. Significance at level p < 0.05

6.3.3 Pound cake

Figure 6.5 shows PCA of soft wheat and PoC quality traits. The first two PCs accounted for 75.5% of the variability of the data. PC1 was dominated in the positive direction by PoC volume, PoC number of cells, PoC cell volume, PoC area of cells, PoC cell diameter, PoC BMV, WPROT, FPROT, and in negative direction by PeakOXI, d(0.5), PoC BPV, and BKFY. PC2 was dominated in the positive dimension by water-, carbonate-, and sucrose SRCs, FASH, SKHRD, and in the negative dimension by FY.

PoC volume was strongly and positively associated with both WPROT, FPROT. However, lactic acid-SRC was vectored between PC1 and PC2. These results suggest that, in contrast to LCs, the concentration of protein had a larger effect on setting the structure of cakes than the absorption or network behavior of the glutenins *per se*. Wilderjans et al. (2008) also reported that the protein concentration improved pound cake volume.



Figure 6.5. Principal component analysis between pound cake volume and soft wheat traits.

SKHRD = single-kernel hardness; WPROT = wheat protein; FY = flour yield; BKFY = break flour yield; FPROT = flour protein; FASH = flour ash; Wa SRC = Water solvent retention capacity; Ca SRC = Sodium carbonate solvent retention capacity; La SRC = Lactic acid solvent retention capacity; d(0.5) = median particle size; BPV = Batter peak viscosity; BMV = Batter minimum viscosity; PoC Pound cake.

PoC volume was associated with low d(0.5) and BKFY. These results confirm previous studies indicating that particle size has a significant effect on cake volume (Choi et al., 2013). Furthermore, PoC volume was associated with low PoC BPV and high PoC BMV. PoC volume was associated with all PoC cell traits indicating that great volumes had high volume, area, and diameter of cells. Interestingly PeakOXI, water-SRC, and SKHRD were oriented to PC2 and orthogonal to PoC volume indicating that these traits had a minimum effect on PoC volume.

PoC volume was not significantly correlated with any cell and batter viscosity traits measured in this study, although PoC BPV appeared to be negatively associated in the PCA (Figure 6.4). These results suggested that the method for measuring batter viscosity using the RVA is not a good predictor of PoC volume and requires improvements according to relationships founded in PCA (Figure 6.4).

6.3.4 Ingredient interaction systems.

Figure 6.6 shows PCA of volumes of the different cakes and the ingredient interaction viscosity systems measured using the RVA. The first two PCs accounted for 85.6% of the variability of the data. PC1 was dominated by all the ingredient interaction viscosity parameters and PoC volume. PC2 was dominated by LC volume and SC volume in opposite directions. SC volume was orthogonal to the ingredient interactions systems and hence there were no associations. Similarly, LC volume was orthogonal to the ingredient interaction systems. Only FWSE peak viscosity showed a low association with LC volume along PC2 indicating that increasing the viscosity of all major ingredients increased LC volume. However, the magnitude was low and no further interpretation could be drawn. Interestingly along PC2, SC volume and LC volume had opposite directions indicating that they are affected by different traits.

No significant correlations were found between ingredient interaction systems and volume of any cake type. These results suggest that in order to measure and predict cake volume is necessary to use all the ingredients and their specific concentrations in the formulation, including salt and leavening chemicals, and the same mixing process used to produce the cake batter.

Figure 6.6. Principal component analysis between cake volumes and ingredient interaction systems measured in the RVA.



SC = Japanese sponge cake; BPV = Batter peak viscosity; BMV = Batter minimum viscosity; LC = Layer cake; PoC Pound cake; FW = Flour-water; FWS = Flour-water-sucrose; FWSE = Flour-water-sucrose; FW

6.3.5 Associations between varieties and cake volume.

Figure 6.7 shows PCA of cake volumes (vectors) and varieties (letters). The first two PCs accounted for 86.4% of the variability of the data. The varieties used in this study showed different performance among the three cake-types. SC volume was vectored along PC1. Skiles and Kaseburg performed better SC volumes. PoC volume was vectored along positive PC1 and PC2. Kaseburg performed better for PoC volume. LC volume was vectored along negative PC1 and positive PC2. Tubbs was performed better for LC volume. Rosalyn was in the center of the PCA and showed similar response among the three cake-types. Bobtail showed that this variety had no good performance in any cake of any type.



Figure 6.7. Principal component analysis between varieties and cake volumes.

SC = Japanese sponge cake; LC = Layer cake; PoC = Pound cake.

6.4 Conclusions

In this study soft wheat quality parameters showed different associations amongst the three cakes assessed. In SC, the varieties did not show significant differences in cake quality traits despite the contrasting characteristics of the selected samples (Table 6.3). For example, SC volume ranged from 1185 to 1256 mL indicating that the contrasting grain and flour characteristics of Tubbs and Bobtail had no effect on SC volume (Table 6.3). Conversely to this study, Kaldy and Rubenthaler. (1987) reported a significant difference between SC volume and five SWW wheats from eastern Canada. SC volume was negatively associated with PeakOXI, SKHRD, water-, carbonate-, and sucrose-SRCs, d(0.5), and batter viscosities confirming previous observations (Kaldy and Rubenthaler, 1987; Gaines, 1985; Choi and Baik, 2013; Kweon et al., 2011). SC volume was positively associated with lactic acid SRC and grain and flour protein concentrations contrasting with other observations in the literature (Kweon et al., 2011). The reason for these

positive associations is unclear. SC volume was not associated with BKFY contrasting with other observations where it was associated (Kaldy and Rubenthaler, 1987; Gaines, 1985). It seems that this lack of association was a result of the unusually low flour yields of Kaseburg. However, the G&E study (see chapter 4) also showed a similar lack of association between SC volume and BKFY in some years, and the reason why it happened in that dataset is unclear. C-cell parameters were overlapped with either SC volume or SC BPV indicating that this test did not offer any extra value to assess cake quality for breeding purposes (Figure 6.3). Batter viscosity (SC BPV, and SC BMV) was significantly and negatively correlated with SC volume confirming previous observations (Nakamura et al., 2010; Moiraghi et al., 2011; Kaldy and Rubenthaler; 1987). The significance of the correlations in this study is that this method to measure batter viscosity requires only 8 min, shorter than the method of Nakamura et al. 2010, and is able to predict cake quality. Therefore, the novelty of the method is a promising test for breeding purposes.

There were significant differences between varieties for LC volume (Table 6.3). Similar to SC there was no difference between varieties for c-cell traits indicating that this test did not show any extra value to assess LC quality for breeding purposes. Interestingly for LC the variety Tubbs had the largest volume (Table 6.3). These results contrasted with the general criteria that softer kernels, and flours with low water-, carbonate-, and sucrose-SRCs, and small particle sizes would produce larger cake volumes. Also, the variety Tubbs is considered as a "least desirable" variety (Washington Association of Wheat Growers, 2016) and in this study, it showed a potential use for LC making. The strong positive association between LC volume and PeakOXI is relevant because it was not seen with the other cake-types suggesting that this trait is a relevant

parameter for LC volume. Similar to the SC, batter viscosity measurements (BPV, and BMV) were significantly and negatively correlated with LC volume (Table 6.5) indicating that the method has also potential in selecting for breeding programs for LC as well as SC.

Pound cakes showed significant differences among varieties (Table 6.3). In this study PoC were associated with high protein content (Figure 6.5) confirming previous observations by Wilderjans et al. (2008) indicating that increased flour protein produced higher PoC volumes. They showed that this was because the formation of a mixed network between flour proteins and egg proteins. Pound cakes were negatively associated with d(0.5) and BKFY. This may be an artifact. Kaseburg had the largest PoC volume and this sample but had had the lowest milling yields in the study (Table 6.3). Based on previous experience these flour yields were unusually low. Batter viscosity measurements were not significantly correlated with pound cakes contrasting our hypothesis in the sense that a single test would be able to predict the three cake types in this study.

In this study using batters made with:

-flour and water,

-flour, water, and sugar,

-or flour, water, sugar, and egg

did not predict cake volume. Therefore, in order to measure batter viscosity our results showed that it was crucial to use all ingredients (including baking powder, salt, butter, shortening) and their concentrations in the relevant formulation. Overall, the varieties in this study showed differences in cake performance (Figure 6.7). Skiles and Kaseburg gave larger SC volumes. Kaseburg gave the largest PoC volume, possibly as as result of it higher FPROT. Tubbs gave the largest for LC volume despite its ranking as a poor quality soft wheat variety. Rosalyn showed a similar response for all cakes. Contrary to our hypothesis oxidative gelation was negatively associated with SC, positively associated with LC, and not associated with PoC.

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See Bibliography

Chapter: 7 General Conclusions.

Pancake quality (Chapter 3)

Influences of genotype and protein concentration on batter flow and pancake making performance were observed. This was the first study in the literature exclusively assessing soft white winter (SWW) wheats in pancakes (Kiszonas et al., 2015). Pancake batters were produced using single-step mixing and formulations had sugar concentrations < 30% (Kweon et al., 2011). Two formulations were used in the study: one based on Finnie et al (2006) called "old" and one another based on the AACC-I Approved Method 10-80.01 called "new". In this study the "new" formulation had an improved ability to distinguish the performance of different flours compared with the "old" as a result of wider range of pancake diameter values. ANOVA showed that protein concentration had a dominant effect on pancake batter flow, pancake dimensions, kernel hardness (SKHRD), and total flour yield (FY). The result for SKHRD was surprising because of the wide variation in inherent SKRD among the varieties. Pancake making performance for either formulation was not significantly correlated with SKHRD, break flour yield (BKFY), carbonate SRC, or lactic acid SRC. Based on comparing lactic acid SRC and flour protein concentration, flour protein quantity appeared to affect pancake performance more than flour protein quality. Among the SRC solvents, sucrose SRC appeared to be a useful tool to predict pancake performance. In this study oxidative gelation capacity (PeakOXI) did not have an effect on pancake traits despite using samples that were specifically selected to have contrasting Peak(OXI) properties.

This study showed that pancake making performance would not be optimized by conventional superior high-quality soft wheat flours with soft kernel texture, high break flour yield, and low water-, carbonate-, and sucrose SRCs. Also this study contrasted with observations made by Finnie et al. (2006). In the Finnie et al. (2006) study they indicated that batters with higher viscosities resulted in larger, thicker, and softer pancakes. In this study higher viscosities resulted in smaller, thicker, and harder pancakes. The contrast may have occurred as a result of the use of unchlorinated flour in this study and chlorinated flours in Finnie et al. (2006).

From our results it would appear with unchlorinated flours, at least for thicker pancakes, the most appropriate flour would have higher water and sucrose SRCs and be grown under management conditions conductive to higher protein.

The results in this study lead to these testable research questions:

- How does flour particle size affect pancake making performance? It has been reported that particle size has an effect on other batter-based products, particularly cakes (Choi and Baik, 2013) and the observations in that study were supported by the results presented in Chapter 5.
- What is the difference in pancake making performance between chlorinated and unchlorinated SWW flours?

G&E study (*Chapter 4 and 5*)

Meta-analysis of "conventional" soft wheat quality traits versus Japanese sponge cake (SC), and sugar-snap cookies across 12 years of data were reported (Chapter 4). In addition, samples of small amounts SWW wheat flour, accompanied by conventional soft wheat quality data, were obtained from 2011. To extend our understanding we tested these flours for two additional, non-conventional, quality parameters: oxidative gelation capacity (PeakOXI) and median particle size (d(0.5)) (Chapter 5).

As a result of the large unbalanced nature of the dataset in Chapter 4 the use of MANOVA or linear correlation coefficients did not give much in the way of practical information. Principal component analysis (PCA) and partial least square (PLS) regression models were used to obtain useful actionable information from the data. PCA and PLS also proved to valuable in assessing the data in Chapter 5. For both chapters the overall data showed that break flour yield (BKFY) was the single most important trait positively associated with both SC volume (CAVOL) and sugar-snap cookie diameter (CODI). CLUB wheats were associated with superior CODI and CAVOL confirming earlier observations (Nagao et al., 1977). Soft white spring (SWS) wheat characteristics were intermediate between the CLUB and soft white winter (SWW) wheats. In Chapter 4, where the comparison was made, SWW wheats were associated with higher grain and flour protein concentrations, lower milling yields, high SRCs, and smaller CODI and CAVOL. This suggested that SWW wheats tended to be lower in quality compared to CLUB and most SWS wheats. The association between SWW wheats and higher grain and flour protein concentrations may have been a result of growing locations which tended to be skewed towards low rainfall environments.

SWW wheats are of particular interest for the Oregon State University (OSU) wheat breeding program. In both studies, SWW wheats showed CVs > 10% for kernel hardness (SKHRD), grain and flour protein concentrations, ash, sucrose-, and lactic acid SRCs. The variability of lactic acid SRC was similar to the variabilities of WPROT and FPROT suggesting that lactic acid SRC was associated with protein concentration as well as protein quality. These observations suggested that hardness, protein, ash, and two SRCs were more sensitive to G&E effects than were the end-product traits that had CVs < 10%. *It is unclear why the large variation of SKHRD was not reflected in equally large variation in milling yields*. ANOVA showed that both G&E had significant effects on SWW traits. In chapter 4 *across years, CODI had more years where it showed the expected associations with predictive traits than did CAVOL.*

In both studies a virtual selection based on high BKFY and low SKHRD were effective in selecting lines with higher CODI and CAVOL suggesting the OSU breeding program should be focus on these two traits as main factors for SWW wheats.

In chapter 5, PeakOXI was significantly correlated with CODI but not with CAVOL. This contrasted with our hypothesis that PeakOXI would affect both products similarly. *Notably 13 SWW samples had PeakOXI values higher than 800 cP. PeakOXI values this high have never been observed in soft wheats prior to this study*. This is a valuable genetic resource for further studies that may lead to ways to better exploit oxidative gelation. For example, in Chapter 6 PeakOXI was positively associated with layer cake volume.

Data mining used in these studies gave us an important knowledge about quality traits across years and important information for a better selecting in the OSU wheat breeding program.

The observations from these studies lead to these next steps:

- What are the genetic factors that produced a large PeakOXI in the 13 SWW varieties?
- What are the changes in SWW wheat quality traits across years when using the same locations and varieties in Oregon?, and how do these changes affect the selection for end-product quality?
- Is it possible to use other statistical tools to make future prediction models for selecting varieties based on end-product quality?. For example, use of time series analysis and/or empirical dynamic modeling.

Cake quality (Chapter 6)

The cakes studied in Chapter 6 were batter-based products where the batter was produced using two mixing steps and containing sugar concentrations > 30% in the formulations. This study investigated the relationships between wheat quality traits, cake batters, and cake making quality in three cake types: SC, layer cake (LC), and pound cake (PoC). *In this study we also used the Rapid Visco Analyzer (RVA) and developed a method able to predict SC and LC quality in 8 minutes. This could be useful for screening or selection for cake quality in soft wheat breeding programs.*

PCA was again an important statistical tool for observing associations between traits. In SC, there were significant differences in cake quality traits between varieties. *SC volume had a*
strong negative association with PeakOXI. Batter viscosity in this study was significantly and negatively correlated with SC volume confirming observations by Nakamura et al. (2010).

For LC, the variety Tubbs, with the harder kernel and higher absorption characteristics, had the largest LC volume. *In contrast to SC, LC volume was significantly and positively associated with PeakOXI*.

In PoC, Kaseburg, with the highest protein content, had the largest cake volume. PoC was significantly and positively associated with flour protein concentration suggesting that flour proteins were important for larger volumes and confirming other observations in the literature (Wilderjans et al., 2008). PoC volume was not significantly associated with PeakOXI.

Overall, Skiles and Kaseburg performed better for SC. Kaseburg performed better for PoC. Tubbs performed better for LC. Rosalyn showed a similar response for all cakes.

The observations from this study lead to next steps:

- To observe relationships between SWW wheat quality traits and cake making performance using a larger set of samples.
- To investigate batter viscosity of SC and LC in a larger sample set to confirm results from this study.
- To analyze cake batters using other techniques such as FT-IR to observe specific interactions among ingredients in cake formulations.

The overall impact of the studies reported is:

- For pancakes, the most important soft wheat trait is flour protein concentration. Water-, and sucrose SRCs were potentially useful parameters for predicting pancake quality.
- For SC and sugar-snap cookies break flour yield was the most important single trait in predicting higher SC volumes and larger cookie diameters. Therefore, selection in soft wheat breeding should be focused on kernel hardness and break flour yields as primary factors.

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Appendices

Appendix 1: Quality targets for soft white wheat

Quality Targets

PNW WQC 2015 Crop Year

QUALITY TARGETS FOR SOFT WHITE WHEAT Quality Targets Steering Committee			WWQL Data N=1249 Quad Milled G&E Data		PNW WQC Data N=78 Miag Milled Released Varieties*		SRW WQC N=83 Miag Milled	
		Varieties	representin	q				
Grain Quality Parameter		80% of PNW Crop						
Test Weight (lb/bu)	>60	61.4	1.6	62.0	1.5	1		
Kernel Hardness (SKCS 4100)	≤ 45 ?<35?	31.2	10.5	28.2	10.3	1		
Kernel Diameter (mm) (SKCS 4100)	>2.5	2.67	0.27	2.73	0.1823]		
Kernel Weight (mg) (SKCS 4100)	>35	37.93	5.7	39.07	5.4551	1		
Falling Number (seconds) (in absence of sprout)	≥ 300							
Protein (%, 12% mb)	10.5	10.6	1.7	11.0	1.4]		
Ash (%, 12% mb)	≤1.30	1.37	0.16	1.34	0.1249]		
		•						
Flour Quality Parameter								
Protein (%, 14% mb) ¹	<8.71	9.0	1.6	9.3	1.3	8.6	1.2	
Ash (%, 14% mb) at 67% extraction ²	< 0.38	0.39	0.05	0.45	0.05	0.40	0.40	
Flour Yield (%) ²	>68.15	68.2	2.6	77.2	1.0			
Break flour yield (%) ²	>46.75	48.6	3.3	26.9	2.9			
Milling Score ²	>83.47	83.0	3.9					
Wet Gluten (%, 14% mb)	<27				•			
Farinograph Absorption (%, 14% mb) @ 8.7% protein ³	<55]						
Farinograph Stability (minutes) ³	<7.0							
Mixograph Absorption (%) @ 8.7% protein	<53.97	55.1	2.5	54.8	2.0	54.5	1.5	
Color / Polyphenol Oxidase (L-DOPA A ₄₇₅)	<0.5	0.840	0.290	0.870	0.370			
Solvent Retention Capacity: Water (%)	<58	55.6	2.8	55.6	2.8	53.0	2.8	
Solvent Retention Capacity: Carbonate (%)	<75	72.0	4.2	72.6	3.5	72.6	5.2	
Solvent Retention Capacity: Sucrose (%)	<95	95.4	7.1	97.2	6.5	93.2	11.4	
Solvent Retention Capacity: Lactic acid (%)	60-170	107.8	23.4	96.0	16.8	104.3	14.4	
SDS Sedimentation Volume (mL/g) @ 8.7% protein	7.0-14.0	11.2	4.1	11.0	3.4	9.1	2.5	
Sugar-Snap Cookie Diameter (cm) @ 8.7% protein	9.3	9.34	0.27	9.25	0.26	9.20	0.35	
Sponge Cake Volume (cc)	1280	1274	69	1249	67	1314	62	
¹ "Protein Differential"the difference between wheat and flour is greate	r on the Western Wheat]		* Varieties w	th over 10K acres			

² Western Wheat Quality Lab Quadrumat Milling System.
³ 50-gram mixing bowl.

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