Impact of controlled sprouting of wheat kernels on bread baking performance

by

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Abstract

A laboratory-scale method for wheat germination was developed and used to compare hard red winter wheat varieties for sprout related attributes, activity, and whole wheat bread baking performance. WB 4458, WB Grainfield, LCS Mint, LCS Wizard, SY Monument, and T158 wheat varieties grown in three Kansas locations were germinated with the developed small-scale germination method and falling number values were compared. Byrd, Tam 204, and T158 were germinated with a scaled-up germination method aimed at generating samples in three falling number ranges of less than 120 seconds (low falling number and highly sprouted), 250±40 seconds (medium falling number), and 350±40 seconds (high falling number and low sprouting). Controls were un-germinated, sound (>400 seconds falling number), samples of each variety. The control whole grain and sprouted wheat was ground into flour. A mixograph was used to determine dough water absorption and mixing time. Whole wheat bread was made to determine bread volume, crumb characteristics, and bread texture. Overall there were few significant differences within each wheat variety for the different levels of germination. The only significant difference observed in all three varieties was that each highly sprouted grain (<120 seconds falling number) produced bread with significantly lower elasticity than the control within each variety, indicating that this level of germination produced a gummier bread. Elasticity was positively correlated with falling number (r=+0.71). A focused analysis on the Byrd variety compared the germinated samples to samples generated with added malted barley to the same falling number ranges. RVA analysis showed the gelatinization profiles for germinated and malted samples were similar within each falling number range. The highly and medium sprouted grain had significantly lower dough water absorption than the malted counterparts for those levels and the medium sprouted grain also had a lower mix time then the malted sample.

There were no significant differences in bread volume, crumb characteristics, or bread texture except the highly sprouted grain had significantly lower elasticity than the control and the malted counterpart was not significantly different. In general, this experiment demonstrated that variety and germination conditions are important considerations in sprouting wheat and that whole wheat flour made from a wide range of germination levels produced quality bread that was not different from the control for most of the parameters investigated.

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Dedication

To the memory of Titus K. Tsimonjela. He was convinced graduate school was an important next step in my life and pushed for me to work towards that goal when I was nervous about returning to college after eight years had passed since my undergraduate degree. Titus even spent time working on GRE test words with me as I prepared to take that exam before applying to graduate school. I think he would have been very pleased with how everything worked out and his presence is missed.

Chapter 1 - Introduction

Sprouted grains are made through a process of controlled germination. The process begins with steeping or soaking the grain in excess water followed by germination in a warm and moist environment. Germination is halted when the grain is dried to stop the reactions occurring. During controlled germination, enzyme activity is increased as the grain prepares to become a plant. In general, germination can increase antioxidants, vitamins, and fiber in grain in addition to increased bioavailability of minerals, as phytic acid is degraded. Germination conditions, including steeping and germination times, can greatly influence the profile of the subsequent germinated grain.

Sprouting grains is a current and growing market trend. In the United States, the market share of sprouted grains was worth $30 million in 2015 and is estimated to reach $250 million in 2018 by nutrition expert, Julian Mellentin (Crawford 2017). During preliminary studies, commercially available sprouted wheat flours were reviewed for baking characteristics while making whole wheat bread (Appendix D). This demonstrated that these "sprouted wheat" flours were incredibly varied.

Hard red winter wheat is the major class of wheat used in breadmaking. In the past this field was dominated by refined white flour products in the United States. Currently, consumers are looking for ways to increase whole grain consumption. Sprouted whole wheat bread may be one answer to fulfilling demand with new and interesting products. It is important to understand how different germination conditions and enzyme activity levels in wheat effects the finished whole wheat bread produced.

This study first started with the development of a small-scale germination method. Different hard red winter wheat varieties which were grown in three Kansas locations were

compared using the generated method. Then, the germination method was scaled up to produce larger samples of hard red winter wheat varieties sprouted to different levels. The whole wheat flour germinated to different levels was baked into bread and compared.

Objectives

1. Develop a small-scale laboratory method for wheat germination.
2. Analyze different varieties of HRW wheat for sprout related attributes, activity and bread baking characteristics.

Chapter 2 - Review of Literature

Sprouting History

Sprouting of grains and legumes has experienced a recent trendiness in Western culture, but the practice has documented historical roots. In China, sprouting is referenced in written documents as far back as 3000 BC. The Emperor of China noted that sprouted legumes could be used to alleviate ailments. The therapeutic uses included treatment for bloating, muscular cramps, digestive disorders, and lung weakness (Wigmore 1986). The Chinese Emperor Shen-Nung wrote about his expertise in growing mung beans in 1282 BC (Evans 2012). The *Pen Ts'ao Kang Mu*, written in the 1500s, detailed the use of Chinese pharmaceuticals and listed that sprouts could be used medicinally for reducing inflammation, treating rheumatism, toning the body or as a laxative (Wigmore 1986). Eastern cultures have a deep history of using sprouted grains and legumes as well as reporting their use as a medicinal food in the diet.

The first documented use of sprouts in Western culture was by Captain James Cook in the 1770s. Scurvy, caused by a lack of vitamin C in the diet, was a major problem on the long sea voyages where fresh fruits and vegetables were not available to the crew. Captain Cook generated a sprouted legume concoction that was used as a diet supplement to combat scurvy until it lost favor to bringing citrus fruits instead of the sprouts (Wigmore 1986; Evans 2012). Another example of sprouts being used to combat scurvy occurred during WWI. British Dr. John Wiltshire treated sixty soldiers in the hospital for scurvy with four ounces of lemon juice or four ounces of sprouted haricot beans daily. The patients receiving the sprouted bean treatment improved at a greater rate than the group consuming lemon juice. In addition to the faster healing time, the sprouts were cheaper and easier to transport to the troops in the field than lemons, so Dr. Wiltshire concluded that sprouts were the better option and provided it as his

recommendation at the end of his experiment (Wigmore 1986). Another example of sprouts as a treatment for scurvy occurred in India during a food shortage that began in 1938. To counter the death rate due to scurvy, the government implemented a program providing sprouted grain or chickpeas two times a week to over 200,000 people. When the incidence of scurvy reduced to nothing in just four months, the government stopped the rationing program. The result of its stoppage was another rise in death rates, five months after which the program was re-implemented (Wigmore 1986).

The use of sprouts was not popular or well-known in Western culture until the 1970s. In the United States, this decade saw an increased interest in healthy food options as the effects of convenient, refined, and processed foods on increasing obesity rates were becoming linked. Sprouting became one trend for a healthier diet during this time as people were more interested in making healthier food at home. Alfalfa sprouts and bean sprouts were two common products making their first appearances in the produce section as mainstream items. Kits became available on the market with easy-to-use and specialty equipment for sprouting at home (Wigmore 1986; Evans 2012).

In 2008, the American Association of Cereal Chemists, International (AACCI) defined sprouted grains as: "Malted or sprouted grains containing all of the original bran, germ and endosperm shall be considered whole grains as long as sprout growth does not exceed kernel length and nutrient values have not diminished. These grains should be labeled as malted or sprouted whole grain." However, there are no current FDA guidelines for labeling sprouted grain products. Today, there is renewed interest in sprouted grains and legumes as many recognize them as a functional food. A food can be classified as a nutraceutical or functional food when it improves health or provides some benefit to the consumer beyond the basic

nutritional content of the food (Nelson et al. 2013). Research is being conducted to investigate the vitamins, minerals and enzymes generated during sprouting, determining how this leads to better health and disease prevention and demonstrating how to use sprouted ingredients in commercially accepted products.

Wheat Kernel

Triticum aestivum L., commonly known as wheat, is a hexaploid cereal grain that is a member of the grass family. The dry single seed contained in the caryopsis is called the kernel. The wheat caryopsis is encompassed by a floral envelope, or hull, that is easily detached during threshing, leaving an uncovered kernel that is rounded on the side of the seed which contains the germ and exhibits a crease along the length of the opposite side (Delcour and Hoseney 2010a). Wheat can be classified as hard or soft, depending on the force needed to pulverize the kernel. Red or white classification of wheat is based on pigment. Planting season determines an additional spring or winter classification for wheat.

The protective outer covering of the wheat kernel is comprised of several layers that make up the pericarp and accounts for about 12% of the kernel by weight (Sluimer 2005b). The pericarp is securely attached to the three layers that constitute the seed coat. The middle layer of the seed coat contains pigment, and the inner layer connects the seed coat to the nucellar epidermis. The nucellar epidermis, or hyaline layer, connects to the single cell thick aleurone layer. The aleurone is the final inner layer that completely encompasses the wheat kernel and overlays the starchy endosperm and the germ. The aleurone is notable for containing high enzyme activity as well as high vitamin concentrations (Delcour and Hoseney 2010a).

The starchy endosperm cells become more irregular in shape towards the center of the wheat kernel with cell walls containing arabinoxylans, β-glucans, and hemicellulose (Delcour

and Hoseney 2010a). The endosperm makes up about 85% of the kernel weight (Sluimer 2005b). The composition of the starchy endosperm is large-lenticular and small-spherical starch granules contained in a protein matrix. Glutenin and gliadin, which form gluten, the wheat storage protein, are the main constituents of the protein matrix (Delcour and Hoseney 2010c).

The embryo, or germ, is the final part of the wheat kernel and accounts for 2.5-3.5% of the kernel. One part of the germ is the embryonic axis which contains the radicle (embryonic root), covered by the coleorhiza and coleoptile sheathed shoot meristem. The second vital component of the germ is the scutellum which acts as the storage portion of the germ (Delcour and Hoseney 2010a; Rodriguez et al. 2015). The germ consists of protein, sugar (sucrose and raffinose), lipids, vitamins and enzymes, and it is the site of formation for the growing plant (Delcour and Hoseney 2010a).

Germination

Defining germination is a complicated task with no exact means of measurement. In general, germination is the time between the transformation of a quiescent, resting seed with low moisture, through the processes that occur until plant growth. Germination is initiated with water uptake (imbibition) and is terminated with the protrusion of the embryonic axis, generally the radicle (root), through the seed coat (Mayer and Poljakoff-Mayber 1989a; Bewley and Black 1994a).

Environmental Factors Affecting Germination

Germination is affected by water, oxygen, temperature, and light. During imbibition, the seed will accumulate two to three times its mass in water (Bewley and Black 1994c). Seed composition, seed coat permeability, and water availability dictate imbibition rate and extent (Mayer and Poljakoff-Mayber 1989b). Water is imbibed during three phases; the first phase is

characterized by a rapid uptake in water, followed by a plateau in the second phase and concluding with a second rise in water accumulation as the third phase. The first phase commences metabolic activity in the kernel as well as initially experiencing a loss of low molecular weight solutes until selective permeability is reestablished within a few hours (Bewley and Black 1994c; Srivastava 2002). During phase two, little water is absorbed by the kernel as enzymes are reactivated and synthesized, protein and hormones are synthesized in preparation of radicle emergence. Phase two is completed as the radicle elongates by cell expansion and penetrates the seed coat which also signals the end of germination. Phase three marks the beginning of plant growth (Bewley and Black 1994c; Srivastava 2002).

Seed imbibition initiates three main respiratory pathways: glycolysis, the pentose phosphate pathway and the citric acid cycle. These pathways generate ATP and NADPH to provide energy and power biosynthesis mechanisms. Glycolysis can produce more ATP in aerobic conditions compared to anaerobic (Bewley and Black 1994c). Seeds can perform aerobic respiration during initial water uptake, but the increase in hydration is matched by an inflation of the respiration rate and oxygen consumption (Srivastava 2002). Oxygen use by the germinating seed also occurs in three distinct phases. The first phase is characterized by an initial large increase in oxygen consumption as respiration increases with water uptake but then slowly increases further during phase two as all enzymes have been reactivated and the number of mitochondria is relatively stable. The radicle emerges at the end of phase two, which signals the end of germination and the beginning of plant growth (phase three); this requires another increase in oxygen consumption for respiration (Bewley and Black 1994c).

The temperature at which a seed may germinate is flexible and was reported as 15-31°C for wheat (Mayer and Poljakoff-Mayber 1989b). The temperature range is affected by genetic

differences in varieties of a species, age of seeds, and seed source. Temperature works in conjunction with the other factors of water, oxygen, and light. Therefore, it is not exact but a flexible range with the ideal temperature for germination depending on multiple factors. These factors along with temperature affect the rate of germination and the capacity of germination (Bewley and Black 1994d).

The effect of light on germination is another complicated variable. Continuous white light may inhibit germination in many species (Bewley and Black 1994d). Grass family varieties demonstrate a negative response to the blue part of the light spectrum during germination. This is due to the fact that grain on the surface of the soil would encounter more blue light than those buried in the soil and germination would be inhibited (Rodriguez et al. 2015). It seems that the period of light exposure as well as the time during imbibition can be variables affecting germination (Mayer and Poljakoff-Mayber 1989b).

Germination Promoters and Inhibitors

Hormones in the plant are important in regulating germination and growth through stimulation mechanisms or inhibition. Gibberellins (GA) are a group of more than 80 identified hormones that promote seed germination while abscisic acid (ABA) is a hormone inhibiting germination (Bewley and Black 1994f). Germination is regulated by the amount of GA and ABA present in the seed and how the other seed tissues respond to it, although the mechanism is still unclear (Srivastava 2002).

It is presumed that GA_3 is released by the embryo and travels to the aleurone layer where α-amylase and other hydrolytic enzymes are stimulated. Hydrolytic enzymes may also be generated in the scutellum in the embryo. The α-amylase moves to the starchy endosperm and begins to hydrolyze starch reserves such that if the availability of monosaccharide sugars

reaching the embryo is too great, the production of more α-amylase is halted (Bewley and Black 1994f). It has been proposed that ABA reaches the highest levels in the seed during development, declines as the mature seed dries, and is responsible for blocking the synthesis of certain proteins and transcription of nucleic acid (Mayer and Poljakoff-Mayber 1989d; Bewley and Black 1994f). Another hormone, cytokinin, is also involved in the regulation and promotion of germination and plant growth. Once again, the mechanism is still under investigation, but an increase in cytokinin seems linked to radicle elongation (Mayer and Poljakoff-Mayber 1989d). Ethylene also promotes seed germination and is produced in small amounts by the seed shortly after imbibition. Ethylene can also be present in the soil or applied externally to stimulate growth. While the pathway is not clear, it seems that ethylene may act as a hormone or act in a way that promotes the hormone activity that encourages germination (Mayer and Poljakoff-Mayber 1989d). An inhibitor of germination is coumarin, which blocks metabolic pathways initiated by germination and interferes with proteinase action to break down protein (Mayer and Poljakoff-Mayber 1989d; Srivastava 2002). Other phenolic compounds may also contribute to inhibiting germination (Srivastava 2002; Rodriguez et al. 2015).

Mobilization of Food Reserves During Germination

A major goal during germination is to mobilize the food reserves stored in the grain for use by the developing plant. During the first few days of imbibition, sucrose and raffinose in the embryo provide energy for early growth (Bewley and Black 1994e). Cell-wall hydrolyzing enzymes, glucanases and arabinoxylanases, are synthesized in the scutellum and released into the endosperm. At the same time, or slightly delayed, α-amylase is released from the scutellum to begin starch hydrolyzation in the endosperm. In the aleurone, α-amylase, α-glucosidase, debranching enzymes and cell-wall hydrolyzing enzymes are synthesized and released into the

endosperm. Concurrently, β-amylase, present in the sound grain starchy endosperm is activated (Bewley and Black 1994e). Gibberellins (GA) aid in maintaining an acidic environment around pH 4.5 that is the optimum for the hydrolytic enzymes by promoting citric acid and phosphoric acid secretion by the aleurone (Srivastava 2002).

Amylose and amylopectin are the major polymers of starch present in wheat. On average, wheat starch is made of 25% amylose and 75% amylopectin (Sluimer 2005b). Amylose is linear with α-1,4 linkages of α-D-glucose and amylopectin is highly branched with α-1,4 and α-1,6 linkages (Delcour and Hoseney 2010b). Amylose and amylopectin are hydrolyzed by enzymes to generate sugars. Amylose is broken down into glucose and maltose by α-amylase and into maltose by β-amylase. Amylopectin is hydrolyzed into glucose, maltose and limit dextrin by α-amylase and into maltose and limit dextrin by β-amylase. Limit dextrin contains the α-1,6 branched linkages which cannot be hydrolyzed by the α- and β-amylase. Maltose is broken down into glucose by the action of α-glucosidase. Glucose generated by the starch catabolism reactions is transported to the scutellum and converted to sucrose that is sent to the growing root and shoot (Bewley and Black 1994e).

Triacylglycerols, the storage lipids in seeds, are hydrolyzed by lipases to make glycerol and free fatty acids. The glycerol and free fatty acids are mainly converted to hexose and then sucrose, which is sent to the scutellum for use by the root and shoot (Bewley and Black 1994e). Storage protein is hydrolyzed by proteinases which can be classified as endopeptidases, or exopeptidases (aminopeptidases and carboxypeptidases). In the aleurone, proteinase releases individual amino acids used to synthesize enzymes such as α-amylase. In the starchy endosperm, the proteinase aids in protein mobilization of β-amylase as well as cell wall hydrolysis. There is also proteinase activity in the scutellum and axis (Bewley and Black

1994e). Free amino acids, especially glutamine, are important for protein synthesis for embryo growth and protein synthesis during germination (Mayer and Poljakoff-Mayber 1989c). Phytic acid contains phosphate and is called phytin when it is bound to potassium, magnesium or calcium; it is stored in the aleurone. Phytin is hydrolyzed by phytase to free the phosphate and cations for use in germination (Bewley and Black 1994f).

Pre-Harvest Sprouting

Wheat generally experiences a period of rest after seed development and germination, referred to as dormancy, and is thought to be controlled in part by sensitivity to ABA (Bewley and Black 1994d). However, if the wheat is exposed to wet conditions in the field before harvest, pre-harvest sprouting (PHS) may occur. Uncontrolled and uneven PHS in the field results in wheat with high α-amylase activity that is not desirable to grain elevators or flour milling operations, so the farmer is paid less for the grain. PHS in wheat is responsible for an estimated loss of $1 billion dollars (US) annually in the world (Rodriguez et al. 2015).

One method to counteract PHS has been a focus on dormancy in wheat breeding programs. Dormancy is a complicated trait that involves many genes as well as various chromosomal locations. In addition, seed coat pigment has been linked to ABA metabolism causing white wheat varieties to be more susceptible to PHS than red wheat varieties (Rodriguez et al. 2015). Wheat varieties bred to express deeper dormancy may cause problems with proper germination when planted in the field or a desired controlled germination or malting process (Bewley and Black 1994d; Rodriguez et al. 2015).

Malting

Malting is the process of controlled grain germination that involves steeping, germination and kilning. Kernels which are plump, sound, uniform in size, non-broken, and readily

germinable are ideal for malting (Fox et al. 2003; Delcour and Hoseney 2010d). Steeping is the first step in the process where the cereal grain is hydrated to a moisture content of 42-44% at low temperatures around 15°C to deter microorganism activity (Noots et al. 1999; Delcour and Hoseney 2010d). Aeration or agitation may be applied during the steeping phase of malting. Next, the imbibed grain is removed from the water and placed on germination beds that create a moist environment with airflow for around four to six days. The relative humidity may reach 100% at this stage and the temperature remains around 15°C to continue to discourage microbial activity. Germination is halted through controlled drying before the acrospire (root) becomes as long as the kernel (Delcour and Hoseney 2010d).

Kilning is the final step in the malting process where the grain is dried to stop germination without disrupting the enzyme activity. At this point, the kernels have about 45% moisture content. Depending on the desired characteristics of the final product, the kilning temperature may remain low to preserve enzyme activity or the temperature may be raised to induce Maillard browning for a different flavor and color profile. The malted grain is now stable until the destined end use (Delcour and Hoseney 2010d).

Traditionally barley, *Hordeum vulgare* L., has been the most commonly malted grain for use in the brewing or distilling industry. Malted barley is also a minor added ingredient to bread flour to ensure optimum enzyme activity, used in ready-to-eat cereals as a flavoring agent, and in baby food or malted candy (Do et al. 2015). Wheat, other cereal grains, pseudo-cereals and legumes are experiencing interest as new ingredients in their malted form.

Safety Considerations of Controlled Germination

The conditions which are conducive to germination can also support microbial growth. In barley malting, aerobic heterotrophic bacteria, mycelial fungi and yeast start to proliferate

during steeping and reach maximum levels after germination. Damaged kernels are especially susceptible targets for growth. Total microbial counts are greatly reduced during the kilning stage, but contamination is not entirely removed (Noots et al. 1999). Piernas and Guiraud (1997) investigated the microbial hazards during rice sprouting and found that no pathogenic microorganisms were detected. However, bacteria, yeast and mold all increased during sprouting, and coliforms were also present. When rice seeds were artificially contaminated with pathogenic *Bacillus cereus* and *Listeria innocua*, both rapidly grew on the germinating rice cereal grain (Piernas and Guiraud 1997). From 2010-2014 there were ten outbreaks, involving *Salmonella*, Shiga-toxin producing *E. coli* and *Listeria*, in the United States related to sprouts, accounting for 8% of total outbreaks (Crowe et al. 2015).

Disinfection of seeds prior to germination is proposed to be the best way to control this safety hazard. It can be difficult to completely disinfect grain due to the crease in the caryopsis that liquid treatments may have trouble accessing. Grain storage temperature, moisture, and atmosphere can be altered to inhibit growth. The addition of sodium hypochlorite up to concentrations during steeping of 0.1% does not adversely affect barley germination and reduces contamination (Noots et al. 1999). Piernas and Guiraud (1997) compared various chemicals at different concentrations and temperatures as well as contact times used to disinfect rice seeds, and they found that the method with the greatest reduction in microbial activity was 100 ppm sodium hypochlorite at 60 °C for five minutes. Another method of disinfection was the use of γ-irradiation that resulted in complete sterilization of the seed (Noots et al. 1999). Limiting potential hazards associated with grain germination is an important consideration in the process.

Nutrition of Germinated Grains

It is difficult to generalize how the nutrition profile of wheat is altered by controlled germination because these values are dependent on the exact germination conditions of the study and at what point the sprouted wheat is analyzed. Antioxidants, vitamins, and fiber are the main categories that have been investigated in terms of nutritional modification during germination. Overall, the changes in wheat during germination are viewed as beneficial in terms of nutrition.

Oxidative stress in the body from free radicals is related to numerous chronic diseases (obesity, diabetes, cancer, etc.) (Fardet 2010). Antioxidants provide protection to cells from oxidative stress and are present in cereal grains as phenolic compounds (Hubner and Arendt 2013). Yang et al. (2001) found that ferulic and vanillic acids decreased slightly when germination started and then significantly increased with maximum levels on day eight of germination after 24 or 48 hours of steeping. Another study found ferulic, isoferulic, p-coumaric, caffeic phenolic acid, total phenolics and total radical-scavenging capacity in wheat increased after 24 hours of steeping and five days of sprouting. Total radical-scavenging capacity also increased in this study for wheat sprouted for five days (Zilic et al 2014). Hung et al. (2011) detected increases in 4-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, and sinapic phenolic acids after 24 hours of steeping and two days of germination. Phytic acid is considered an antioxidant that is also anti-nutritional since it chelates with minerals so that they are not available for absorption. However, during germination, phytates are degraded, increasing mineral bioavailability (Nelson et al. 2013).

Vitamins are an essential part of the diet to maintain a properly functioning human body (Hubner and Arendt 2013). Yang et al. (2001) found no vitamin C content in dry wheat but a near linear increase in level during germination to a peak of 550 μg/g. Important in synthesis of

vitamin A in the body, β-carotene was also increased during wheat germination in the same study. Danisova et al. (1994) also found ascorbic acid (vitamin C) to increase to levels of 394 and 399 mg/kg of dry matter for germinated wheat compared to 0 in the un-germinated wheat. Yang et al. (2001) detected increases in vitamin E content with α-tocopherol and γ-tocopherol. Zilic et al. (2014) also observed an increase in vitamin E content after sprouting wheat and found α, β+γ, and δ tocopherols present. B vitamins were analyzed by Zilic et al. (2014) and found that niacin (B_3) represented the largest portion of B vitamins in wheat and was increased by 19% after sprouting. Riboflavin (B_2) also increased when wheat was sprouted, however thiamine (B_1) and pyridoxine (B_6) both decreased and the reduction was attributed to leaching out during steeping. Danisova et al. (1994) also found thiamine in germinated wheat to decrease 10-46% and riboflavin to increase 49%. In a different study, another B vitamin, folate, was reported by Koehler et al. (2007) to increase during germination of wheat with the highest content of 200 μg/100 g at 102 hours of germination at 20 °C compared to 58 μg/100 g in un-germinated wheat.

Fiber, in general, comprises the parts of the cell which are resistant to digestion and are associated with chronic disease reduction when consumed (Liu 2007). Koehler et al. (2007) found that total dietary fiber initially decreased during wheat germination for the first 48 hours and then increased. At 168 hours of germination, soluble dietary fiber had increased and insoluble dietary fiber had decreased (Koehler et al. 2007). Danisova et al. (1994) found a 37% increase in dietary fiber during wheat germination. Arabinoxylans are non-starch polysaccharides that contain a xylose backbone linked with single arabinose units and are found in cell walls (Sluimer 2005b; Nelson et al. 2013). Donkor et al. (2012) found that arabinoxylan content increased during germination of wheat after five days.

Studies of controlled wheat germination demonstrate that each constituent viewed as nutritionally beneficial for humans do not increase at the same rate. There are not optimum germination conditions or a length of germination time that will optimize all the desired quantities of antioxidants, vitamins, minerals, fiber, protein or starch alteration and enzyme activity. Furthermore, the best germinated wheat in terms of nutrition may not translate to a superior end-product in a baked good.

Yeast Leavened Bread

The essential ingredients in a yeast leavened bread are flour, yeast, water, and salt. Other ingredients such as sugar, amylase (germinated grain), fat, emulsifiers or oxidants may be included in a bread formula to enhance or alter dough handling or an attribute of the final product. The breadmaking process involves mixing, fermentation, and baking. Storage conditions and shelf-life of bread are also important considerations.

Essential Ingredients

Flour is the main ingredient in yeast leavened bread and is notated as 100% in baker's percentage while other ingredient amounts are listed as a percent of the flour weight (Sluimer 2005a). The percentages of other ingredients are listed on a flour weight basis (fwb). Hard wheat with a high protein content is generally used for bread making. Water is added to the formula in the optimized amount to hydrate gluten in the flour and create the viscoelastic dough. Optimized water absorption for most breads can vary from 50% up to 80% (fwb) (Sluimer 2005a). Yeast, *Saccharomyces cerevisiae,* is added at 1-6% (fwb) and consumes fermentable carbohydrates, with a preference for glucose, to produce carbon dioxide gas and ethanol; the carbon dioxide gas provides the volume increase and lightness to a loaf of bread (McWillaims 1989). Yeast also has an oxidizing effect and increases the elasticity of the dough. Salt is added

up to 2% (fwb) in the formula. Salt is a flavor enhancer and dough strengthener in addition to counteracting yeast activity and protease activity so the fermentation rate is not too fast, resulting in a weak dough (McWilliams 1989).

Nonessential Ingredients

Nonessential ingredients are added to the formula to generate beneficial characteristics in the breadmaking process or final bread. Sugar is added to impart a sweet flavor in the bread, provide additional substrates for fermentation, aid in Maillard browning reactions and improve crumb texture. White bread will generally have about 2% (fwb) sugar added in the formula and levels over 4% (fwb) of added sugar will begin to slow fermentation (Sluimer 2005c). Malted grains, most commonly barley, are often added at the mill to sound hard wheat flour as a minor ingredient to increase α-amylase enzyme activity in the dough. This increases the amount of fermentable sugars available for yeast fermentation, increases bread loaf volume and extends shelf life (Ral et al. 2016). A falling number around 250 seconds is often the specification for enzyme activity in bread flour.

Fat is added as a dough plasticizer to increase bread volume, increase shelf life and tenderness and increase palatability of baked products. Fat is often added around 1% (fwb) to common bread formulas and in much higher amounts in specialty breads, such as 5-10% (fwb) in hamburger buns (Sluimer 2005c). Emulsifiers such as diacetyl tartrate ester of monoglycerides (DATEM) or sodium stearoyl lactylate (SSL) are used up to 0.5% (fwb) to increase bread crumb softness, increase shelf life and strengthen the dough (Sluimer 2005c; Delcour and Hoseney 2010e). Oxidants produce stronger dough that is drier and more resilient and a final product with a finer crumb and larger volume while reducing agents act in the opposite way to weaken the dough structure when a shorter mixing time and less resilient dough are desired (Slumier 2005c).

Mixing

Mixing is the process of combining the bread formula ingredients and incorporating air. Water completely hydrates the protein and starch in the flour. The physical effect of mixing is to allow for interaction between hydrated gliadin and glutenin fractions in the protein matrix. The gliadin and glutenin interactions form the new disulfide bonds of the gluten network that is responsible for the viscoelastic nature of dough; gliadin contributes to viscosity without resistance to extension while glutenin adds elasticity and resistance to extension attributes to the dough (Sluimer 2005d; Delcour and Hoseney 2010e). Dough is optimally mixed when the flour particles are completely hydrated and exhibit strength and elasticity. If dough is mixed past optimum, the disulfide bonds in the gluten are broken down and result in a weak, wet and sticky dough (Sluimer 2005d). Mixing also incorporates air into the dough system. The gasses produced from yeast activity must enter existing gas cells for expansion. An increase in available air bubbles will result in a bread with a desirable fine grain (Sluimer 2005d).

Fermentation and Proofing

During fermentation yeast consumes glucose to produce ethanol, carbon dioxide and heat. Sucrose can be utilized by yeast because it contains invertase that can hydrolyze sucrose into consumable fructose or glucose, with yeast preferring glucose (Delcour and Hoseney 2010e). Yeast can be active in an aerobic or anaerobic environment. Initially, the air incorporated by mixing generates an aerobic environment that is quickly changed to an anaerobic environment when the oxygen is consumed. Fermentation is considered an anaerobic process. Carbon dioxide gas generated during fermentation enters the air cells incorporated during mixing and expands those cells. Punching during fermentation allows for gas cells to be divided into smaller cells and bring yeast in contact with fermentable sugars it did not have access to because

yeast is stationary in the dough system (Slumier 2005e). Low temperatures result in fermentation occurring slowly so an ideal temperature range for fermentation is 25-28°C (McWilliams 1989). During fermentation, yeast has an oxidizing effect on the dough so that it becomes more elastic, drier and easier to handle; the pH is also lowered from 6.0 to around 5.0 (Delcour and Hoseney 2010e). Before baking, dough is allowed to rest during a final proofing stage in a fermentation cabinet after the dough has been moulded and placed in the baking pan. Under proofed dough may produce capped bread, with a thick top that is almost separated from the loaf, while an over proofed bread will have large round cells with thick cell walls as the air cells merge together.

Baking

When placed in the oven, the dough experiences oven-spring, which is a rapid expansion in volume that is finished within eight minutes. During oven-spring, yeast is very active until the temperature is reached where it is inactivated, the carbon dioxide is less soluble and moves into air cells as well as expands as the temperature raises and ethanol generated during fermentation evaporates (Delcour and Hoseney 2010e). A crust on the surface begins to form that is dry and cool from the vaporizing water. Later in baking, reducing sugars and protein take part in Maillard browning reactions. During baking, starch is gelatinized and protein experiences a reduction in water and exhibits strain hardening and the interior changes from a gas-discontinuous phase in dough to a gas-continuous system that air can penetrate easily (Delcour and Hoseney 2010e).

Staling

Baked bread begins the process of staling as soon as it is removed from the oven. As the baked bread is cooling, amylose crystallizes which contributes to establishing initial crumb

firmness in the cooled bread. During further crumb firming, amylopectin begins retrogradation as intermolecular and intramolecular crystals form. While crumb firming and amylopectin retrogradation occur over a similar time frame, there is no specific evidence linking the two (Delcour and Hoseney 2010e). Crumb firming is also accompanied by a migration of water from gluten to starch and from the interior crumb to the crust (Bosmans et al. 2013). Firming rate and extent is affected by water present in the bread system, storage temperature, storage time, and reheating (Zeleznak and Hoseney 1986). Bread that has firmed can be reheated to temporarily melt the amylopectin crystals, however, the refreshed softness is lost when the bread cools or is heated further. Refrigerator storage temperature increases the firming rate faster than ambient temperature, while freezing can inhibit retrogradation (McWilliams 1989). Many ingredients have been investigated to slow bread firming and increase shelf life. One area of interest is the role of amylases in counteracting firming (Fadda et al. 2014).

Whole Wheat Bread

Whole wheat bread is made from whole wheat flour that contains all kernel components instead of refined white flour which mostly contains the starchy endosperm. The AACCI (1999) definition of whole grains is "Whole grain shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact caryopsis." Whole wheat flour is composed of about 10-14% bran, 2.5-3.0% germ and 80-85% endosperm depending on variety characteristics and milling (Fardet 2010). The germ provides an increase in unsaturated fatty acid content that creates a lipid content of 1.3-2.0% which makes whole wheat flour more susceptible to rancidity from oxidation (Jensen et al. 2011). With the inclusion of the germ and bran, whole wheat flour has a high level of fiber and bioactive components. There is a vast array

of phytochemicals in wheat retained in whole wheat flour such as α-linolenic fatty acid, oligosaccharides, lignin, minerals, vitamins (B and E), carotenoids, polyphenols, phytic acid, and others (Fardet 2010). Depending on age and gender, the 2016 USDA "My Plate" guidelines recommends five to eight, one-ounce servings of grains daily for adults, with half of them being whole grains. Consumption of whole grains is linked to a reduction of developing chronic diseases. Obesity, metabolic syndrome, Type 2 Diabetes, cardiovascular disease, and some forms of cancer are reduced with whole grain intake due to improved digestive health, antioxidant and anti-inflammatory effects on the body. Although there is a large quantity of data to correlate increased health with intake of whole-grains, the exact mechanisms are not entirely clear since so many phytochemicals are involved in tandem (Fardet 2010).

Whole wheat bread has some altered characteristics when compared to bread made from refined white flour. In general, whole wheat bread has a reduced volume, requires less mixing time and a shorter time for fermentation. The bran particles interfere with the gluten network and the dough cannot retain gas as well as the refined white flour counterpart (Boita et al. 2016). Phytic acid, mainly located in the aleurone and present in whole wheat at about 1-2%, acts as an antioxidant and is also viewed as an anti-nutrient because it complexes with minerals so that they are not bioavailable during consumption. Park et al. (2016) demonstrated that added phytate reduced bread volume by interfering with glutenin polymerization though phytate bonds with protein and iron-chelating antioxidant activity reducing glutenin cross-linking. Particle size of bran in whole wheat flour plays an important role in water absorption as a decrease in particle size requires an increase in added water (Cai et al. 2014). Consumers often view whole wheat products as less palatable than refined flour products due to perceived bitter notes and the visual dark color. Red wheat exhibits these characteristics due to the presence of phenols and tannins

while white wheats produce a sweeter tasting bread that is also lighter in color than red wheats (Talbert et al. 2013). In a study with whole wheat flour tortillas, substitution of sprouted hard white spring whole wheat flour for regular hard white spring whole wheat flour increased the flavor score on a 5-point scale from 2.57 for the control (100% regular whole wheat flour) and 3.70 for 100% sprouted whole wheat flour. Panelists reported the products were less grainy and less bitter then the control flour tortillas (Liu et al. 2017).

Starch: Gelatinization, Viscosity, and Testing Methods

Starch granules are highly ordered and exhibit birefringence in polarized light that is viewed as a cross image as well as containing semicrystalline regions (Sluimer 2005b; Delcour and Hoseney 2010b). In excess water, starch swells as water enters the granule for an overall volume increase of around 5%. Water absorption in this manner is reversible as long as it is not accompanied by a temperature increase (Delcour and Hoseney 2010b).

Gelatinization occurs when starch is heated in excess water. During gelatinization, each individual starch granule absorbs water, swells, loses birefringence, crystallinity is disrupted, and amylose is leached out of what remains of the granule structure. For wheat, gelatinization occurs from 54 to 63°C (Sluimer 2005b). In baking a bread, gelatinization occurs before cereal α-amylase is denatured at about 75°C and is able to hydrolyze starch in the oven over this temperature range: this gelatinization is beneficial to the crumb softness (Delcour and Hoseney 2010e; Sluimer 2005b).

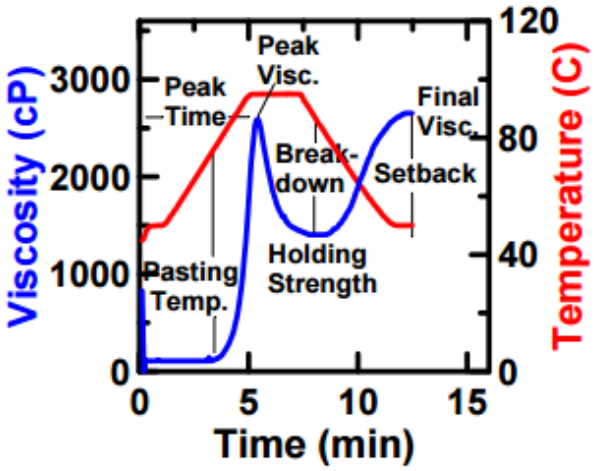


Figure 2-1 Rapid Visco Analyzer Temperature Profile (Perten 2017)

Rapid Viscoanalysis is a laboratory method used to investigate the gelatinization profile of starch. Figure 2-1 displays the temperature profile of the analysis and the parameters of the viscosity profile measured during the analysis. A Rapid Visco Analyser (RVA) measures viscosity while it gradually stirs and heats a sample in excess water to 95°C, holds this temperature while stirring, and then gradually cools the sample to 50°C (AACCI Method 76.21.01; Delcour and Hoseney 2010b). As temperature is increased, viscosity increases as starch is gelatinized. After gelatinization, pasting is the period of continued heating and further viscosity increase in the sample. Peak viscosity occurs as the internal temperature of the sample increases and the external RVA temperature reaches 95°C. As the external RVA profile temperature is held at 95°C and stirring continues, the starch molecules begin to move in the direction of stirring while other intact starch granules are broken apart by the shear action of stirring. This results in a decrease in viscosity that is referred to as shear-thinning until a minimum viscosity is reached during the breakdown portion of the rapid viscogram. As the temperature is cooled to 50°C, viscosity is increased during setback as hydrogen bonds are increased. At the end of the RVA test, the sample is a gel in which a large amount of water is

held by a much smaller amount of solid material and behaves as a solid (Delcour and Hoseney 2010b). Properties of the wheat flour, such as the presence of certain enzymes, will alter the pasting profile values. A study by Noda et al. (2003) demonstrated that α-amylase activity in wheat flour is an important factor in flour peak viscosity by lowering the peak viscosity as α-amylase activity is increased.

Falling Number (FN) is another analytical test involving viscosity that measures the cereal α-amylase present in wheat flour or ground wheat. The flour sample is mixed with water in a large glass test tube and placed in the hot water bath of the Falling Number apparatus. After a five-second delay, the sample is mixed with a stirring rod for 55 seconds. During the mixing time, the sample viscosity increases. At the end of the mixing, the stirring rod is raised to the top of the viscous sample and allowed to fall to the bottom of the test tube. The FN instrument reports the amount of time in seconds that it takes the stirring rod to travel through the heated sample and water. An increase in enzyme activity in the sample results in a lower falling number because starch is hydrolyzed by α-amylase to smaller molecules, and this reduces viscosity; 62 seconds is the lowest value attainable for falling number. A falling number of 400 seconds or above would indicate a sound wheat and a falling number less than 150 seconds demonstrates high amylase activity and sprout damage (Tilley et al. 2012). The ideal falling number for bread flour is generally regarded as between 225-275 seconds, and is made by adding malted barley to flour milled from sound grain. Fungal amylase may also be added to flour to obtain the same enzyme activity, but it is not detected by the FN test (Sluimer 2005b).

Effects of Growing Location

The location where wheat is grown has considerable effects on baking quality. Seed size, starch, and protein composition are altered due to environmental conditions after anthesis in

wheat. Temperature, water availability, and fertilizer application are examples of environmental factors which effect wheat quality and composition (Dupont and Altenbach 2003).

Temperatures over 30°C decrease the duration and rate of grain fill in wheat (Bewley and Black 1994b). The maximum duration of grain fill is associated with temperatures between 15 and 20°C. As duration of grain fill is increased, starch accumulation in the kernel is increased. At high temperatures, sucrose transforming to starch is reduced possibly from the heat denaturing starch synthase and the ratio of amylose to amylopectin is also altered (Dupont and Altenbach 2003). In terms of protein content, high temperature has demonstrated an increase in the ratio of gliadins to glutenins and causes a general increase in protein content (Dupont and Altenbach 2003).

Drought has been shown to result in a reduced grain size. When combined with high temperatures, the duration of grain fill was decreased more than either variable individually (Dupont and Altenbach 2003). Generalizations about the effects of fertilizer on grain development and quality can be difficult depending an amount applied, fertilizer composition, and timing in development. Protein content increase has been documented when application of a nitrogen fertilizer was increased. Fertilizer can also provide sulfur in addition to nitrogen to the plant. Nitrogen and sulfur additions affect the amount and composition of protein in the kernel. For example, insufficient sulfur may increase ω-glaidins and high molecular weight glutenin subunits as opposed to high sulfur α-gliadins, γ-gliadins and low molecular weight glutenin subunits (Dupont and Altenbach 2003).

Wheat genetics are an important factor during grain filling and end-use baking quality. Many studies have investigated the interaction of genetic variation of cultivars with the environment in wheat kernel development and subsequent quality. Peterson et al. (1998) found

that cultivar, environment, and their interaction were all statistically significant for the quality aspects tested. However, the effect of environment had a stronger influence than cultivar and the interaction of the two parameters on quality. Kaya and Akcura (2014) also demonstrated that yield and quality were affected more by environment then genetics. Growing environment exerts an important effect on wheat quality.

Chapter 3 - Materials and Methods

Small Scale Germination Series

Wheat Samples

Six hard red winter wheat varieties grown in three locations and harvested in 2016 were evaluated. The wheat varieties included WB 4458 (WestBred), WB Grainfield (WestBred), LCS Mint (Limagrain Cereal Seeds), LCS Wizard (Limagrain Cereal Seeds), SY Monument (Syngenta-Agripro Wheat), and T158 (Limagrain Cereal Seeds). The three Kansas growing locations were in Reno County, Republic County and Johnson County. All locations were grown without irrigation. Wheat variety T158 was not available from Johnson County.

Small-Scale Germination Method

Chaff, broken and damaged kernels were removed during a visual inspection. 25 grams of wheat was placed in a clear glass jar (diameter 5.0 cm, height 10.5 cm). The wheat was washed with deionized water by filling the jar with water, swirling it around by hand, and draining the water by using the lid to hold the wheat kernels back while tipping the jar into the sink. This was repeated so the wheat was washed two times. 50 mL of deionized water was added to the 25 grams of washed wheat in the glass jar. The lid was secured and the wheat was placed on the laboratory counter for 18 hours at 24°C for the steeping or soaking step (Figure 3.1).

Figure 3-1 Steeping

After 18 hours, the soaking water was drained from the wheat by using the lid to hold the imbibed wheat in the glass jar and pouring the water into the sink. Deionized water was then added to the imbibed wheat in the glass jar, swirled around, and drained off by using the lid to hold the imbibed wheat in the jar and pouring the water into the sink as a rinsing step. A 16.5 cm by 11.5 cm by 0.5 cm piece of Biostrate™ felt sprouting mat (Grow-Tech, LLC, Portland, ME) was wetted with deionized water. Excess water was squeezed from the felt mat before placing the mat in the bottom of a 17.5 cm by 11.5 cm by 5.7 cm aluminum tray. The imbibed wheat kernels were placed on top of the wetted felt mat in the tray and arranged in a single layer (Figure 3.2).

Figure 3-2 Imbibed Wheat Kernels on Felt Mat

The uncovered trays were placed into a fermentation cabinet (National Manufacturing Co., Lincoln NE) set at 30°C and 95% relative humidity for seven hours. After seven hours, the trays were removed from the fermentation cabinet, the germinated kernels were removed, and the wet felt mat was discarded. The germinated wheat kernels were placed back in a single layer in the tray and placed on the laboratory counter to dry for four days at room temperature (24°C) (Figure 3.3). During preliminary work, it was established that air drying was able to stop the germination process the same as drying the germinated kernels at low temperatures in an air

oven by checking that the falling number was constant 12 to 18 days after drying. Each variety/location sample was germinated with this method in triplicate.

Figure 3-3 Wheat Kernels Drying on Lab Counter

Falling Number Analysis

At the end of the four-day drying time, the germinated and dried wheat sample was ground in a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO). The entire ground sample was placed back into the tray and stirred with a spatula to create a uniform sample. 7.0 g of ground wheat was weighed and placed into the glass falling number test tube for the Falling Number 1700 (Perten Instruments, Huddinge, Sweeden). AACCI Method 56-81.03 was followed to determine the falling number. Two test tubes were filled with the same sample of wheat and 25 mL of distilled water was dispensed into each test tube. Tube were sealed with rubber stoppers and shaken vigorously, about ten times, or until the sample was a homogenous suspension without any dry particles remaining at the bottom of the test tube. The viscometer-stirrer was used to push down residue on the side of the test tube into the suspension and the test tubes were placed into the plastic test tube cassette. The test tubes were then placed in the falling number instrument water bath and the plastic guard was lowered to begin the test. The instrument analysis time in seconds for each sample was recorded. If the two analyses of the

same sample were more than 12 seconds apart, a third test was performed using the remaining ground wheat.

Adding Mold Control

The presence of mold was observed in some samples when removed from the fermentation cabinet after the germination step (Figure 3.4). The LCS Mint wheat sample from Republic County had the most instances of mold after the germination step. A disinfection step based on the methods of Yang et al. (2001) and Donkor et al. (2012) was added.

Figure 3-4 Mold Present After Germination

1.25% sodium hypochlorite (NaClO) was prepared using Clorox® Bleach (6% NaClO) (Appendix A). 25 g wheat was submerged in 50 mL 1.25% NaClO for 30 minutes in a glass jar (diameter 5.0 cm, height 10.5 cm) at lab ambient temperature (24°C). The wheat was then placed in a plastic half gallon bottle with a tubing outlet at the bottom so that water could exit the bottom of the bottle while keeping the wheat inside the container. The top of the bottle was cut off at a height of 19.5 cm (bottom diameter 10.6 cm). Cool tap water (about 10°C) was continually run over the wheat and allowed to exit the bottom of the container for 30 minutes. At the end of the tap water rinse, the wheat was placed into the soaking jar and rinsed once with distilled water, allowing the excess water to drain into the sink by holding the wheat back with the lid. After this disinfection step, the small-scale germination method was followed by adding 50 mL distilled water and steeping the wheat for 18 hours, germinating the wheat for 7 hours and

drying on the laboratory counter for four days before grinding the wheat and measuring the falling number. This analysis was performed with LCS Mint wheat from Republic County in triplicate.

Large-Scale Germination Series

Wheat Samples

Hard Red Winter Wheat samples were obtained from Ehmke Seed grown in Lane County Kansas from the 2017 crop year. Wheat varieties were Byrd, T158 and Tam 204. All were grown without irrigation and were treated with Storcide, for insect control, which does not affect germination and is safe for human consumption the same day as application. Byrd is noted for good baking quality for bread made from refined white flour, T158 has acceptable baking characteristics and Tam 204 has poor baking qualities since it was developed primarily for grazing (Haley et al. 2012; Ledbetter 2014; Ehmke 2016).

Large-Scale Germination Method

Wheat samples were cleaned with a Carter Dockage tester (Carter-Day Company, Minneapolis, MN). 700 g of wheat was weighed and placed into a plastic container (soaking jar) with a tight-fitting lid (bottom diameter 12 cm, height 51 cm). 1400 mL of 1.25% NaClO was prepared from Clorox Bleach (6% NaClO) and was added to the wheat sample (Appendix A). The lid was secured and the sample was allowed to rest for 30 minutes at room temperature (24°C). At the end of this time, the liquid was drained off by holding the wheat back with the lid. The wheat was then placed in a plastic half gallon bottle with a tubing outlet at the bottom so that water could exit the bottom of the bottle while keeping the wheat inside the container. The top of the bottle was cut off at a height of 19.5 cm (bottom diameter 10.6 cm). Cool tap water (about 10°C) was continually run over the wheat and allowed to exit the bottom of the

container for 30 minutes (Figure 3.5). The wheat was stirred every 10 minutes with a plastic stirring rod. After 30 minutes of rinsing, the tap water was turned off and allowed to drain from the rinsing container.

Figure 3-5 Rinsing Container and Process

The rinsed wheat was placed back into a cleaned soaking jar and rinsed twice with distilled water, holding the wheat back with the lid to drain the excess water from the sample. 1400 mL of distilled water was added to the disinfected wheat for the soaking phase (Figure 3.6). Soaking times were 18 or 24 hours.

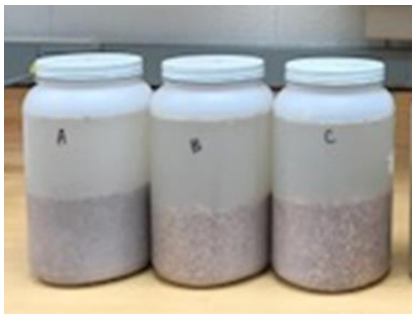


Figure 3-6 Soaking Jars

At the completion of the soaking phase, the water was drained from the wheat and the imbibed wheat was washed once with distilled water and the water drained away again. A 23 cm

by 51 cm by 0.5 cm piece of Biostrate™ felt sprouting mat was wetted with distilled water and excess water squeezed out. The felt mat was then placed in a black plastic growing tray without drain holes made by Living Whole Foods (Springville, UT) (bottom width 24 cm, bottom length 50.5 cm and height 6.5 cm). The imbibed wheat was spread evenly over the mat in the sprouting tray and placed uncovered into a fermentation cabinet (National Manufacturing Co., Lincoln NE) set at 30°C and 95% relative humidity (Figure 3.7). Germination times were 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 11 and 12 hours.

Figure 3-7 Imbibed Wheat in Germination Tray

At completion of the germination time in the fermentation cabinet (National Manufacturing Co., Lincoln NE), the germinated wheat was placed in a single layer on an aluminum bakery sheet pan (bottom width 42.5 cm, bottom length 63 cm, height 2.5 cm) in a bakery rack for two days (Figure 3.8). A small fan was used to circulate the air around the trays in the bakery rack. The air-dried and germinated wheat was spread on two Hefty™ E-Z Foil trays (bottom width 25.5 cm, bottom length 37.5 cm, height 2 cm) and placed into a Fisher Scientific Isotemp Oven Model 737F (Pittsburgh, PA) set at 55˚C. The wheat was oven-dried until it was about 12% moisture as measured by a DA 7200 NIR (Perten Instruments, Springfield, IL).

Figure 3-8 Air Drying Germinated Wheat

The oven-dried germinated wheat was then ground in a Laboratory Mill 3100 (Perten Instruments, Springfield IL). The ground wheat was mixed and placed in a cylindrical plastic container with a tight-fitting lid (bottom diameter 12.5 cm, height 19 cm). Two falling number results were obtained and then the sprouted flour was stored in the laboratory at ambient temperature (22-24˚C) until further testing was done. Two to six weeks after the initial falling numbers were measured (depending on the date that germination had been completed for each sample), a third falling number value was obtained to check if the sprouting reactions had been stopped and the samples were truly shelf stable.

The goal of the large-scale germination series was to obtain wheat samples with certain falling number ranges. The ranges were 350 ± 40, 250 ± 40, and less than 120 seconds (62 seconds is the minimum value attainable for falling number). Soaking and germination times were altered to obtain the specific falling number ranges for each variety. Three samples for each falling number range were made for each variety in addition to three control samples obtained from the ground sound wheat for each variety.

With the Byrd variety, additional samples were made from 700 g of sound wheat that was ground and then dry malted barley (King Arthur, Norwich, VT) was added to generate samples in the three falling number ranges. Malt was added in increments, mixed, and the falling number

was determined until the samples were in the proper range. For the 350±40 second range, 1.0 g of malt was added, for the 250±40 second range, 2.3 g of malt was added, and for the less than 120 second range, 12.0 g of malt was added.

Moisture Content

Moisture content of the germinated whole wheat flour was assessed by AACCI Method 44-15.02 (air oven method) and analyzed in duplicate. Approximately 2.0 g of flour was weighed into an aluminum moisture dish. The moisture dishes were place uncovered on an aluminum baking tray and placed in the Fisher Scientific Isotemp Oven Model 737F (Pittsburgh, PA) set at 130°C for 60 minutes after the oven returned to the set temperature. At the end of 60 minutes, the tight-fitting lids were secured on top of the moisture dishes and they were placed into a desiccator at ambient lab temperature (24°C). When the moisture dishes returned to room temperature, after 45-60 minutes, the weight of the flour was measured and the loss in weight was determined as the percent moisture by the equation:

$$\%\, moisture = \left(\frac{moisture\ loss\ in\ grams}{original\ weight\ of\ sample}\right) * 100$$

Ash Content

Ash content (inorganic residue) of the germinated whole wheat flour was quantified by AACCI Method 08-01.01 (muffle furnace). Approximately 4.0 g of whole wheat flour was weighed into a porcelain crucible and placed in a Fisher Scientific Isotemp Muffle Furnace Model 550-126 (Pittsburgh, PA) set at 575°C overnight (≥18 hours). The crucibles were then placed in a desiccator at ambient lab temperature (24°C) until the crucibles had attained room temperature after about 3 hours. The residue ash was weighed and the percent ash content was calculated by the equation:

$$\%\, ash\ (as-is) = \left(\frac{weight\ of\ residue}{sample\ weight}\right) * 100$$

Percent ash content adjusted to a 14% moisture basis was calculated by the equation:

$$\%\ ash\ (14\%\ mb) = \left(\frac{(100-14)}{100 - sample\ \%\ moisture\ content}\right) * \%\ ash\ (as-is)$$

Viscosity

A Rapid Visco Analyser (RVA) Model 4500 (Perten Instruments AB, Hägersten, Sweden) was used to determine the starch viscosity profile of the Byrd variety samples based on AACCI Method 76-21.01. 4.0 g samples were weighed (adjusted to a 14% moisture basis) and placed into an aluminum sample canister with 25 mL of distilled water. A polycarbonate stirring paddle was used to break up any sample clumps. The aluminum sample canister and stirring paddle were inserted into the RVA. Using Thermocline for Windows (TCW) software, the analysis was initiated for the Standard 1 profile (Table 3.1). Viscosity was measured in centipoise (cP) units in this analysis, where one cP equals one millipascal/second. Results generated included peak viscosity, holding strength, breakdown, setback, final viscosity, peak time, and pasting temperature. One sample for each Byrd germinated level (L, M, H), malted level (L, M, H) and one control (C) were analyzed.

Table 3-1 RVA Standard 1 Profile

Stage	Temperature / Speed	STD 1
1	50 ˚C	0 min, 0 sec
2	960 rpm	0 min, 0 sec
3	160 rpm	0 min, 10 sec
4	50 ˚C	1 min, 0 sec
5	95 ˚C	4 min, 42 sec
6	95 ˚C	7 min, 12 sec
7	50 ˚C	11 min, 0 sec
End of test		13 min, 0 sec
Time between readings		4 sec

Mixograph

Mixograph curves were obtained using AACCI Method 54-40.02 with a 10 g Mixograph (National Manufacturing Co., Lincoln NE) and MixSmart software. 10 g whole wheat flour (weighed on a 14% moisture basis) was added into the 10 g mixing bowl. Distilled water was added to obtain the optimum absorption. An optimum curve did not have wild pen strokes indicating the sample was too dry or a narrow, smeared look, suggesting that the sample was too wet. A wet dough also would fall away to the bottom or sides of the bowl during mixing. The software calculated the center line max peak time and the samples were allowed to run at least three minutes after the peak. Germinated whole wheat flour samples were analyzed to determine the optimum water absorption in addition to running the sample at the control absorption (un-germinated wheat of each variety).

Whole Wheat Bread Method

All samples were baked in duplicate based on the whole wheat bread method developed in the Wheat Quality Lab. The whole wheat bread method was based on the AACCI Optimized Straight-Dough Bread-Baking Method 10-10.03 with alterations for a whole wheat flour. Table 3-2 contains the whole wheat bread formula. The first alteration is that 120 g of whole wheat flour was used instead of 100 g of flour. The actual whole wheat flour weight was adjusted to a 14% moisture basis using the equation:

$$14\%\ mb\ Flour\ weight\ (g) = \left(\frac{100-14}{100-sample\ \%\ moisture\ content}\right) * 120\ g$$

Another alteration in the whole wheat bread formula is the addition of 1.0 g of DATEM to strengthen the dough. Shortening, active dry yeast, salt and sucrose weights remained the same in the whole wheat formula as for 100 g of refined flour.

Table 3-2 Whole Wheat Bread Formula

Ingredient	Formula (g)
Whole Wheat Flour	120[a]
Water	Optimum[b]
DATEM	1.0
Shortening	3.0
Salt[c]	1.5
Sucrose[c]	6.0
Active Dry Yeast	2.0

[a] Actual weight used based on 14% moisture basis adjustment
[b] Optimum absorption from mixograph
[c] Added in solution (Appendix A)

Whole wheat flour, DATEM, and shortening were weighed into small labeled metal cans with lids before baking. On baking day, active dry yeast stored in the refrigerator was brought to room temperature and placed in a dispenser that releases 2.0 g aliquots. Salt and sucrose solution and distilled water were at ambient lab temperatures (24°C) and dispensed with burettes.

Optimum water amounts and mixing times were based on mixograph data for each sample. All ingredients in the whole wheat bread formula were mixed in a 100 g pin mixer (National Manufacturing Co., Lincoln, NE) until the dough was developed. Mix time was optimized subjectively during baking. The developed dough was formed into a ball and placed into a greased bowl in the fermentation cabinet (National Manufacturing Co., Lincoln NE) set at 30°C and 95% relative humidity for a 90 minute fermentation. The "first punch" occurred after 52 minutes in the fermentation cabinet when the dough was placed in a sheeter (National Manufacturing Co., Lincoln, NE) with rolls set to a 3/16 inch (4.8mm) opening. White dusting flour was used to inhibit the dough sticking to the rolls. The "second punch" occurred 25 minutes later when the dough was cross sheeted to 3/16 inch (4.8mm). After an additional 13

minutes, the dough was sheeted to 5/16 inch (7.9 mm) and then 7/32 (5.6 mm) and placed on the moulder (National Manufacturing Co., Lincoln, NE) and rolled into a cylinder. The ends of the dough cylinder were pinched to seal and placed seam side down into metal pup loaf baking pans (14.3 x 7.9 cm top inside; 12.9 x 6.4 cm bottom outside; 5.7 cm inside depth) with the bottom and three sides greased (one long side left un-greased) and paper identification labels affixed to the top of the dough. The panned dough was returned to the fermentation cabinet and allowed to proof for 39 minutes.

The proofed dough was placed in a rotary baking oven (National Manufacturing Co., Lincoln, NE) set at 400°F (204°C) and baked for 30 minutes. Two beakers of water were placed into the oven before and during baking to provide steam. At the completion of baking, the bread was removed from the pans and the volume was immediately measured by rapeseed displacement following AACCI Method 10-05.01. Breads were allowed to cool on wire racks to ambient lab temperature (24°C) before being placed in plastic bags that were sealed with a plastic twist-tie and stored overnight at 24°C.

Evaluation of Whole Wheat Bread Crumb

The following day after baking, each loaf was sliced with the break and shred facing up so that the knife cut into the break and shred side. Four cuts with a bread knife were made using a bread slicing guide (National Presto Industries, Eau Claire, WI) at 2.5 cm increments from the end of the bread so that five slices were generated. The two heel ends were discarded and the three interior slices were evaluated. One slice was imaged using the C-Cell Imaging System (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). A high-resolution image was captured for the slice in addition to the quantification of crumb characteristics including number of cells, cell wall thickness, and cell diameter.

Each of the three interior slices (2.5 cm thick) were analyzed for crumb firmness and elasticity using the TA.XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA/Stable Micro Systems, Godalming, Surrey, UK) with a modified version of AACCI Method 74-10.02. A TA-3 cylindrical acrylic probe (diameter 2.5 cm and height 3.5 cm) was used. Each slice was positioned so that the center of the probe interacted with the center of the bread slice. The probe compressed the slice to 40% of the initial thickness at a rate of 1 mm/sec and held the compression for 30 seconds. Crumb firmness was measured as the force required to complete the 40% compression of the slice. Elasticity of the crumb was determined by dividing the force after 30 seconds of compression by the peak force and multiplied by 100 to provide a percent value. Higher values for elasticity suggest the crumb was able to spring back after compression while lower values indicate the crumb was gummy and did not spring back after compression.

Data Analysis

This study was designed as a two-way factorial experiment. Analysis of variance (ANOVA) was performed on samples using Proc Glimmix (SAS Institute Inc, version 9.4, Cary, NC) and least squares means were compared with Tukey-Kramer HSD at a $p<0.05$ level of significance. Dough, baking and crumb grain results were correlated with falling number in the large-scale germination series using Pearson Correlation Coefficients with Proc CORR (SAS Institute Inc, version 9.4, Cary, NC).

Chapter 4 - Results and Discussion

Small-Scale Germination Series

All wheat samples were sound before the small-scale germination series was conducted (Table 4.1). According to Tilley et al. (2012), wheat above a 400 sec falling number is considered sound. The lowest average falling number result (from two analyses) was 437 sec for SY Monument from Reno County, however, this is still above the minimum value for sound wheat.

Table 4-1 2016 Crop Wheat Falling Number Results Before Sprouting

Variety	Location (KS County)	Average Falling Number (sec)
T158	Reno	527
T158	Republic	507
LCS Mint	Reno	496
LCS Mint	Johnson	614
LCS Mint	Republic	500
LCS Wizard	Reno	537
LCS Wizard	Johnson	573
LCS Wizard	Republic	438
WB Grainfield	Reno	448
WB Grainfield	Johnson	636
WB Grainfield	Republic	506
WB 4458	Reno	530
WB 4458	Johnson	568
WB 4458	Republic	483
SY Monument	Reno	437
SY Monument	Johnson	661
SY Monument	Republic	505

Results Following the Germination Method

During October and November of 2016, the laboratory temperature dropped significantly below the 24°C standard setting due to repairs in the building. The room temperature was in the range of 16-18°C during this time and the falling number results obtained after germination were

higher, indicating less sprouting occurred at the lower temperatures. Thus, results obtained at the lower lab temperatures were removed from the study. Seeds germinated above or below the optimal temperature will delay germination without totally inhibiting it (Mayer and Poljakoff-Mayber 1989b). Once the laboratory temperature was restored to a constant 24°C, germination trials continued.

The two falling number values measured after the small-scale germination method and used for analysis for each sample can be found in Appendix B. When the two falling number results differed by more than 12 seconds, a third rep was analyzed and the two values closest to each other were used in the study.

Table 4-2 Average Falling Number (Sec)[a] after Germination for Six Wheat Varieties Grown in Three Kansas Locations

Location (KS County)	WB4458	LCS Mint	LCS Wizard	WB Grainfield	SY Monument	T158
Reno	317 a	308 ab	308 ab	276 abcd	166 de	151 e
Johnson	322 a	291 abc	226 abcde	225 abcde	188 cde	n/a
Republic	271 abcd	238 abcde	238 abcde	203 bcde	166 de	175 de

[a] Means in the same column or row with different letters are significantly different at $p<0.05$
n/a T158 from Johnson County was not available

Table 4-2 displays the average results of the small-scale germination method. The Tukey-Kramer method was used to compare the means of each treatment to every other treatment. When viewing these results, there are no significant differences for each wheat variety grown at different locations. This demonstrates that the growing location did not significantly influence the falling number value obtained after germination. However, looking at different varieties grown within a location showed significant differences in falling number. Thus, variety did affect the falling number after germination. Table 4-3 displays the results in terms of average for each wheat variety. The variety averages fell within three categories of

sprouting level based on falling number. The highest falling number results ranged from 257-303 seconds, the middle values ranged from 235-279 seconds, and the lowest falling number values ranged from 163-173 seconds.

Table 4-3 Average Falling Number[a] Results after Germination for Six Wheat Varieties Grown in Three Locations

Variety	Falling Number (Seconds)
WB 4458	303 a
LCS Mint	279 ab
LCS Wizard	257 ab
WB Grainfield	235 b
SY Monument	173 c
T158	163 c

[a] Means in the same column with different letters are significantly different at $p<0.05$

Mold Control

LCS Mint from Republic County had visible mold during each germination trial. When disinfection (1.25% NaClO) was added, mold was not visible after the germination step (when the wheat was removed from the fermentation cabinet). The addition of the disinfection step did significantly alter the average falling number (Table 4-4). This may have occurred because the wheat kernels were in contact with water one hour longer than the original method due to the additional half hour disinfectant soaking and half hour rinsing steps. Water availability affects the level of water uptake in a seed (Mayer and Poljakoff-Mayber 1989b). In the large-scale germination method, the disinfectant step was applied to all samples so there would be no difference in the subsequent germinations trials.

Table 4-4 Effect of Disinfectant Step on Falling Number[a] of LCS Mint Wheat grown in Republic County

Treatment	Average Falling Number (Seconds)
Regular Small-Scale Germination	238 a
Disinfectant Step	193 b

[a] Means in the same column with different letters are significantly different at $p<0.05$

Large-Scale Germination Series

Byrd, Tam 204 and T158 wheat samples were all sound before the large-scale germination series. The ground un-germinated wheats were considered to be the controls. Table 4-5 shows the average falling number results, analyzed in duplicate, for each wheat variety batch. The lowest falling number for the un-germinated wheat was batch 3 T158 with 532 sec falling number value, demonstrating that all wheat was sound prior to germination.

Table 4-5 Sound Wheat (Controls) Falling Number Results

Variety	Batch	Falling Number (sec)[a]
Byrd	1	590
Byrd	2	577
Byrd	3	594
Tam 204	1	588
Tam 204	2	569
Tam 204	3	589
T158	1	572
T158	2	570
T158	3	532

[a] Average of two falling number analyses

The Byrd, Tam 204 and T158 germinated samples with falling numbers of less than 120 seconds were low falling number samples (L), 250±40 seconds were medium falling number samples (M), and 350±40 seconds were high falling number samples (H). A lower falling

number corresponds to a higher degree of germination. Appendix C shows all batch soaking and germination times, including samples which did not fit into the ranges for this study. Table 4-6 displays the falling number results of germinated samples obtained after the drying step. An additional falling number was obtained after two to six weeks of storage at room temperature (22-24°C). The largest change in falling number after storage was 24 sec batch M3 for Tam 204. The falling number values after storage did not alter the targeted ranges for each sample and also demonstrated that the germination reaction was stopped during drying.

Table 4-6 Falling Number Results for Three Wheat Varieties after Germination to Low, Medium and High Falling Number Ranges

Variety	Falling Number Group[a]	Batch[b]	Falling Number after Germination[c] (sec)	Falling Number after Storage Time[d] (sec)	Difference in Falling Number (sec)
Byrd	L	1	100	108	8
Byrd	L	2	92	92	0
Byrd	L	3	110	109	1
Byrd	M	1	262	275	13
Byrd	M	2	256	253	3
Byrd	M	3	244	253	9
Byrd	H	1	381	360	21
Byrd	H	2	332	337	5
Byrd	H	3	331	324	7
Tam 204	L	1	101	96	5
Tam 204	L	2	89	95	6
Tam 204	L	3	108	113	5
Tam 204	M	1	254	230	24
Tam 204	M	2	287	276	11
Tam 204	M	3	264	253	11
Tam 204	H	1	376	362	14
Tam 204	H	2	340	318	22
Tam 204	H	3	343	338	5
T158	L	1	117	120	3
T158	L	2	78	76	2
T158	L	3	103	106	3
T158	M	1	237	229	8
T158	M	2	237	234	3
T158	M	3	266	269	3
T158	H	1	333	329	4
T158	H	2	358	359	1
T158	H	3	341	338	3

[a] "L" for low falling number value <120 sec, "M" for medium falling number value 250±40 sec, "H" for high falling number value 350±40 sec

[b] Independently sprouted batches (replicates)

[c] Average of two falling number analyses

[d] Falling number after 2-6 weeks of storage (22-24°C)

Whole Wheat Flour Properties

Table 4-7 displays moisture and ash contents of the germinated and control ground whole wheat flour. Overall, the moisture contents of all whole wheat flour samples were similar; the highest moisture contents were in Byrd H and T158 H samples with 11.74% and the lowest moisture content was the Byrd control sample with 10.60%. The ash content gives an overall value for the mineral residue in the sample. Minerals can be leached out during steeping into the soak water and decrease ash content (Danisova et al. 1994). However, there was no clear trend in ash content raising or lowering with controlled germination when compared to the un-germinated control samples. The only significant difference in ash within a variety was T158 between the control (1.273%) and the high falling number level (1.383%).

Table 4-7 Percent Moisture and Ash Content[a]

Variety	Level[b]	Moisture (%)[c]	Ash (%, 14% mb)[c]
Byrd	L	11.49 ab	1.278 abcd
Byrd	M	11.26 ab	1.262 cd
Byrd	H	11.74 a	1.216 d
Byrd	C	10.60 b	1.265 cd
Tam 204	L	11.13 ab	1.306 abcd
Tam 204	M	11.30 ab	1.269 bcd
Tam 204	H	11.59 ab	1.306 abcd
Tam 204	C	10.78 ab	1.375 ab
T158	L	11.04 ab	1.327 abc
T158	M	11.33 ab	1.334 abc
T158	H	11.74 a	1.383 a
T158	C	10.74 ab	1.273 bcd

[a] Means in the same column with different letters are significantly different at $p<0.05$
[b] "L" low fn <120 sec, "M" medium fn 250±40 sec, "H" high fn 350±40 sec, "C" control/un-germinated
[c] Average of two analyses

Whole Wheat Dough Properties

Water absorption was determined by mixograph analysis (Table 4-8). Absorption was weakly positively correlated with falling number at $r=+0.37$ ($p=0.03$). The only significant

difference within a variety was in Byrd where the control had significantly higher absorption than all germinated samples. However, this trend was not observed in the Tam 204 or T158 varieties.

For the Byrd variety, the dough mix time for the control was 6.51 minutes which was significantly longer than the L level of germination with 4.89 minutes. The other germination levels (M and H) were not significantly different from the control or the L level in Byrd. Tam 204 did not display any significant differences in dough mixing time between the control and any germination level. T158 showed significant differences between the control (4.35 min) and H levels of germination (2.85 min). However, there was no difference between the control and the L or M levels of germination. This does not follow the trend seen in the Byrd variety. The raw data for Table 4-8 can be found in Appendix C.

Table 4-8 Whole Wheat Mixing Properties[a]

Variety	Level[b]	Absorption (%)	Mixograph Peak Time (min)
Byrd	L	66.7 d	4.89 b
Byrd	M	67.0 cd	5.42 ab
Byrd	H	67.3 cbd	5.62 ab
Byrd	C	70.0 a	6.51 a
Tam 204	L	70.0 a	2.08 d
Tam 204	M	69.3 ab	2.36 d
Tam 204	H	68.0 abcd	2.22 d
Tam 204	C	70.0 a	3.07 cd
T158	L	68.3 abcd	2.92 cd
T158	M	68.3 abcd	2.93 cd
T158	H	69.0 abc	2.85 d
T158	C	68.7 abcd	4.35 bc

[a] Means in the same column with different letters are significantly different at $p<0.05$
[b] "L" low fn <120 sec, "M" medium fn 250±40 sec, "H" high fn 350±40 sec, "C" control/un-germinated

Whole Wheat Baking Properties

Optimized dough mix times, as determined subjectively as the point when the dough was developed, demonstrated a significant difference for the Byrd variety between the control (6.42 min) and the L level of germination (4.83 min) as shown in Table 4-9. T158 showed significant differences in mix time between the control (4.00 min) with the M and H levels of germination (2.58 and 2.50 min, respectively). Tam 204 did not exhibit any significant differences in dough mix time. There was no trend relating dough mix time and level of germination among all three wheat varieties.

Larger loaf volume values are associated with superior baking quality. Within each variety there were no significant difference in loaf volume between the control and germinated samples. Loaf volumes of white pan bread tend to increase with addition of malt flour (Sluimer 2004c) or lab sprouted white flour (Ariyama and Khan 1990). This was not observed in this study using the whole grain instead of a refined white flour.

Table 4-9 Whole Wheat Bread Mixing Time and Bake Volume[a]

Variety	Level[b]	Dough Mix Time (min)	Bake Volume (cc)
Byrd	L	4.83 bc	764.3 a
Byrd	M	5.33 ab	767.7 a
Byrd	H	5.58 ab	777.0 a
Byrd	C	6.42 a	756.0 a
Tam 204	L	1.83 e	610.3 c
Tam 204	M	2.08 e	658.0 bc
Tam 204	H	1.92 e	657.7 bc
Tam 204	C	2.67 e	627.7 bc
T158	L	2.83 de	660.3 bc
T158	M	2.58 e	696.7 abc
T158	H	2.50 e	695.3 abc
T158	C	4.00 cd	702.7 ab

[a] Means in the same column with different letters are significantly different at $p<0.05$
[b] "L" low fn <120 sec, "M" medium fn 250±40 sec, "H" high fn 350±40 sec, "C" control/un-germinated

For the crumb, an increase in cell number, a decrease in cell wall thickness, and a decrease in cell diameter are all indicators of a desirable fine crumb grain structure (Sluimer 2005a). A higher quality bread will have many small cells with thin cell walls resulting in a fine and tender crumb. Figure 4-10 shows that T158 had significantly fewer cells for the L level of germination with 2673 cells compared to the control, M, and H levels of germination (3071, 3108, and 3169 cells, respectively). Tam 204 level H had significantly higher number of cells (2937) than Tam 204 L level (2548). Within each variety there was no significant difference in cell wall thickness or cell diameter. Ariyama and Khan (1990) reported inferior crumb grain for heavily sprouted white bread including a decrease in grain uniformity and an increase in large cells. However, in this study, no trend for difference in cell number was evident across the three wheat varieties and no difference in cell wall thickness or cell diameter was shown.

An inferior bread will exhibit a higher value for firmness and a lower value for elasticity when it is not able to recover after compression (Slumier 2005a). Within each variety, there were no significant differences in bread slice firmness (Figure 4-10). For elasticity, Byrd showed a significant difference between the control (61.6%) and the L level of germination (56.8%). This trend continued with Tam 204, with the L level of germination exhibiting a significantly lower elasticity value (52.6%) compared to the control, M, and H levels (57.0, 55.6, 57.9%, respectively). T158 also showed a significant difference in elasticity between the control (60.1%) and the L level of germination (52.6%). In all varieties, the L level (falling number less than 120 sec) was significantly less elastic than the control samples. Elasticity was positively and significantly correlated with falling number with $r=+0.71$ ($p<0.0001$). For Byrd and Tam 204, the M and H levels of germination were not significantly different from the control showing that the crumb was able to recover from compression while the highly sprouted levels (low

falling number) were less able to do so with a lower elasticity of the bread crumb. High levels of sprouting can cause sticky and gummy bread crumb (McCleary and Sturgeon 2002).

Table 4-10 Whole Wheat Bread Crumb Properties[a]

Variety	Level[b]	Number of Cells	Cell Wall Thickness (mm)	Cell Diameter (mm)	Firmness (g)	Elasticity (%)
Byrd	L	3351 abc	0.443 abcd	2.79 ab	294 cde	56.8 bcd
Byrd	M	3567 a	0.431 cd	2.58 ab	266 e	59.1 abc
Byrd	H	3588 a	0.425 d	2.50 b	282 de	58.9 abcd
Byrd	C	3492 ab	0.433 cd	2.59 ab	268 e	61.6 a
Tam 204	L	2548 g	0.455 ab	3.14 a	387 ab	52.1 f
Tam 204	M	2796 defg	0.447 abc	2.93 ab	371 abc	55.6 de
Tam 204	H	2937 def	0.437 bcd	2.67 ab	355 abcd	57.9 bcd
Tam 204	C	2752 efg	0.446 abc	2.96 ab	416 a	57.0 bcd
T158	L	2673 fg	0.459 a	3.04 ab	313 bcde	52.6 ef
T158	M	3108 cde	0.441 abcd	2.75 ab	318 bcde	55.8 cde
T158	H	3169 bcd	0.439 abcd	2.69 ab	311 bcde	58.6 abcd
T158	C	3071 cde	0.447 abc	2.87 ab	311 bcde	60.1 ab

[a] Means in the same column with different letters are significantly different at $p<0.05$
[b] "L" low fn <120 sec, "M" medium fn 250±40 sec, "H" high fn 350±40 sec, "C" control/un-germinated

Byrd: Addition of Malted Barley Comparison

This section investigates any differences between the samples which were germinated to the three falling number ranges compared to adding commercial malted barley to obtain falling number values in the same ranges. Byrd samples with malt added were labeled "Malt" and the average of two falling number analyses can be found in Table 4-11.

Table 4-11 Byrd Samples with Added Malted Barley

Byrd	Falling Number (sec)
Malt H	350
Malt M	275
Malt L	115

Byrd Malted and Germinated Dough and Bread Characteristics

Table 4-12 displays the dough properties of Byrd germinated samples compared to Byrd with added malted barley. The control sample and all three malted samples had higher absorptions than the germinated samples. For mixograph peak times, all germinated samples were lower than the control and all malted samples were longer than the control. The highest peak time was the sample malted to the 250±40 sec range. For each falling number level, the malted mixograph peak time was longer then the corresponding germinated sample.

Table 4-12 Germinated and Malted Dough Characteristics

Byrd	Level[a]	Mixograph Absorption (%)	Mixograph Peak Time (min)
Malted	High	70.0	7.06
Germinated		67.3	5.62
Malted	Medium	72.0	9.79
Germinated		67.0	5.42
Malted	Low	70.0	7.08
Germinated		66.7	4.89
Control	Sound	70.0	6.51

[a] High=350±40 sec; Medium=250±40 sec; Low=<120 sec, Sound=>400 sec falling number ranges

After the samples were baked there were no significant differences among any of the Byrd samples in terms of loaf volume, number of cells, cell wall thickness, cell diameter, and firmness (Table 4-13). This indicates that malting compared to germinating in the Byrd variety did not affect these bread characteristics. In addition, malt level or the germination level also did not account for any significant differences in these bread quality parameters. Elasticity did display a significant difference between the control and the L level germinated samples. These samples germinated to less than 120 seconds had the lowest elasticity with 56.8%, suggesting this sample had a gummier crumb than the control which had elasticity of 61.6%. However, the malted sample in the same range was not significantly different than the control or the L sample.

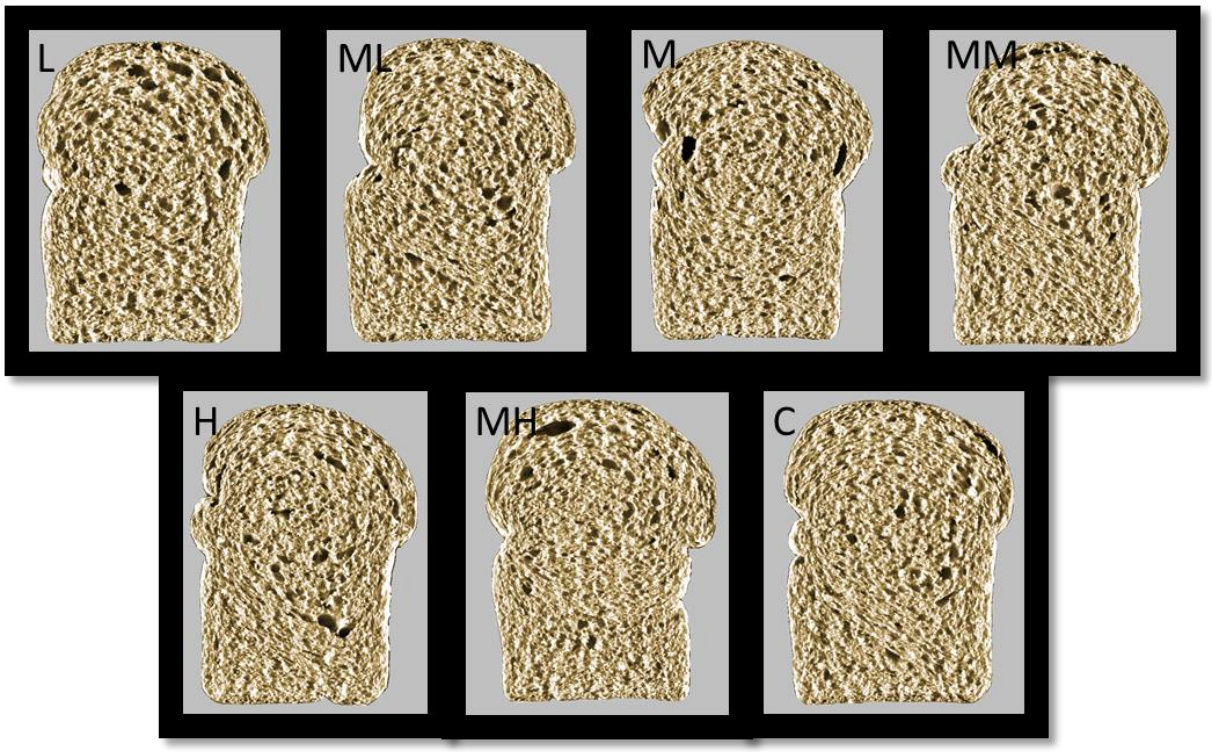
There were no other significant differences for elasticity in this comparison. Sluimer (2004c) states that low falling numbers obtained from adding malt to sound wheat behaves differently than sprouted wheat flour to a low falling number. White bread made with sprouted wheat flour had thick cell walls and a coarse crumb. This was not observed with the germinated whole wheat flour compared to the control or malted samples, except the L germinated sample had a significantly lower elasticity than the corresponding malted sample.

Table 4-13 Germinated and Malted Byrd Bread Characteristics[a]

Sample	Level [b]	Loaf Volume (cc)	Number of Cells	Cell Wall Thickness (mm)	Cell Diameter (mm)	Firmness (g)	Elasticity (%)
Malted	High	828 a	3859 a	0.430 a	2.51 a	250 a	60.3 ab
Germinated		777 a	3588 a	0.425 a	2.50 a	282 a	58.9 ab
Malted	Medium	788 a	3448 a	0.442 a	2.75 a	235 a	60.8 ab
Germinated		768 a	3567 a	0.431 a	2.58 a	266 a	59.1 ab
Malted	Low	765 a	3376 a	0.439 a	2.58 a	297 a	57.5 ab
Germinated		764 a	3351 a	0.443 a	2.79 a	294 a	56.8 b
Control	Sound	756 a	3492 a	0.433 a	2.59 a	268 a	61.6 a

[a] Means in the same column with different letters are significantly different at $p<0.05$

[b] High=350±40 sec; Medium=250±40 sec; Low=<120 sec, Sound=>400 sec falling number ranges



L=Germinated <120 sec, ML=Malted <120 sec, M=Germinated 250±40 sec, MM= Malted 250±40 sec, H=Germinated 350±40 sec, MH=Malted 350±40, C=Control

Figure 4-1 Representative C-Cell Brightness Images of Byrd Bread Slices

Figure 4-1 shows a representative bread slice image for each Byrd treatment. The L sample displays a crumb that does not have much organization or line of strength. Sample L stands out as a slightly lower quality bread in these images. This supports the instances of data (elasticity and mixing time) that indicate it is inferior to the control, the other levels of germination, and all levels of malt addition. Extreme starch breakdown from the germinated level L could cause a dense bread which is not able to support crumb strength. The Byrd sample with malt added to the same level (ML) appears to have more organized cells and some strength (from the bottom right of the photo to the mid left break and shred side of the bread) when compared to than the L sample. The control appears to show good strength with uniform and

organized cells. All other samples, except sample L, seem to follow the general appearance of the control.

Byrd Malted and Germinated RVA

Figure 4-2 displays a visual of the parameters measured during RVA. Byrd viscosity profiles obtained by RVA are displayed in Table 4-14. The pasting temperature is where the sample viscosity begins to increase. There was no significant difference among all Byrd samples for pasting temperature.

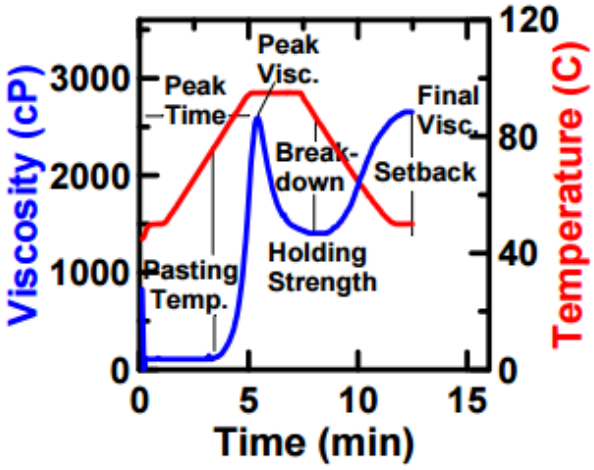


Figure 4-2 RVA Parameters (Perten 2017)

The peak viscosity and peak time are measurements at the point where viscosity increases due to starch granule swelling and viscosity decreases due to starch alignment with stirring and granule rupture are in equilibrium (Delcour and Hoseney 2010b). Higher values indicate greater water-binding capacity and potentially better end-product quality. The control had significantly higher values in peak time (7.00 min) and peak viscosity (2914 cP) than all other samples. For each malt and germination level, the corresponding samples had results that were not significantly different. For peak time, each corresponding malt and germination level was

significantly different than each other pair except the malted M sample which was also similar to the H level samples. There was more cross-over between levels with peak viscosity; H level samples were similar to the malted M sample and the germinated M sample was similar to the malted L sample. Noda et al. (2003) correlated a reduction in peak viscosity with an increase in α-amylase activity, which is evident with this study.

Holding strength or trough determines the samples ability to withstand mixing and high temperature (95°C) as viscosity reaches a minimum value. Breakdown is the difference between peak viscosity and holding strength (Perten 2013). The control had the highest holding strength (2389 cP) and the lowest values were the germinated and malted L samples (65.3 and 117.0 cP, respectively). The corresponding germinated and malted samples had similar holding strength and breakdown. Similar to peak viscosity, there were similarities in holding strength between the levels. Germinated and malted H level samples were similar to the malted M level and the malted L level was also similar to the germinated M level. Overall, increasing germination and malt addition decreased holding strength. The control had the highest breakdown (525 cP) and the lowest breakdown were found in the germinated and malted H levels. The malted H level and all other germinated and malted samples were also similar.

As the temperature cooled to 50°C, the sample experienced an increase in viscosity as it transformed into a paste or gel with the maximum stabilized value as final viscosity. Setback is the difference between holding strength and final viscosity (Perten 2013). The highest final viscosity was the control with a value of 3442 cP. Again, the corresponding germinated and malted levels were not significantly different from each other for final viscosity. Additionally, the malted M level was also similar to the germinated and malted H levels. For setback, there were significant differences between corresponding malted and germinated samples. The control

had the highest value for setback (1053 cP) followed by malted H and M samples (531 and 515 cP, respectively). Germinated H and M were lower and significantly different to each other (433 and 377 cP, respectively). Malted L and germinated L were also significantly different to each other (145 and 46 cP, respectively). Setback is the one parameter of the RVA gelatinization profile where corresponding malted and germinated samples were significantly different. However, in general there appears to be little difference in the gelatinization profile for samples that are malted or germinated to the same falling number range.

For this section, the RVA standard 1 profile was used, and one test lasts 13 minutes. The RVA standard 2 profile, follows the same profile but one test lasts 25 minutes. The RVA standard 1 profile may not have accurately elucidated differences in the samples because the internal sample temperature was not increased at the rate at which the test proceeded. The longer test time for RVA standard profile 2 would produce more accurate results.

Table 4-14 Byrd RVA Results[a]

Sample	Level [b]	Pasting Temp (°C)	Peak Time (min)	Peak Viscosity (cP)	Holding Strength (cP)	Break-down (cP)	Final Viscosity (cP)	Setback (cP)
Malted	High	69.9 a	6.33 b	1804 b	1519 b	285 bc	2050 b	531 b
Germinated		69.9 a	6.31 b	1674 b	1444 b	230 c	1877 b	433 c
Malted	Medium	69.9 a	5.60 bc	1236 bc	875 bc	361 b	1390 cb	515 b
Germinated		70.2 a	5.40 c	987 cd	671 cd	316 b	1048 c	377 d
Malted	Low	70.0 a	3.93 d	492 de	117 de	375 b	262 d	145 e
Germinated		70.1 a	3.64 d	396 e	65 e	330 b	111 d	46 f
Control	Sound	69.6 a	7.00 a	2914 a	2389 a	525 a	3442 a	1053 a

[a] Means in the same column with different letters are significantly different at $p<0.05$

[b] High=350±40 sec; Medium=250±40 sec; Low=<120 sec, Sound=>400 sec falling number ranges

Chapter 5 - Conclusion

The first objective of this study was to develop a small-scale laboratory method for wheat germination. There is no standard method employed across scientific studies for laboratory germination so it is difficult to expand laboratory generated data into useful generalized results. The small-scale germination method in this study presents a repeatable method with equipment generally found in a baking laboratory. The developed small-scale germination method was used to compare hard red winter wheats grown in three locations. The effect of growing environment did not have a significant effect on germination as determined by falling number analysis while the effect of variety was significant in terms of falling number post germination.

The second objective of this study was to analyze different varieties of hard red winter wheat for sprout related attributes, activity and baking characteristics. To perform this, a large-scale germination method was employed to sprout Byrd, Tam 204, and T158 varieties to distinct levels of germination based on falling number values. The levels were 350±40 seconds (H), 250±40 seconds (M) and less than 120 seconds (L). There were not many significant differences within each variety in terms of dough characteristics and no consistent trends between varieties. Byrd displayed a decrease in water absorption between the control and all sprouted levels in addition to a decrease in mix time between the control and highest level of sprouting (L). The other two varieties did not exhibit significant differences in absorption while T158 had significantly lower mix times for M and H (but not the L level) when compared to the control.

There were no significant differences in bread volumes within each variety at all levels of sprouting. One clear significant difference shared within all varieties was that bread baked with the highest level of sprouted wheat flour exhibited lower elasticity than the control, indicating a gummier crumb.

Further, focused analysis of Byrd indicated that there were few differences between flours altered by germinating or by adding malted barley. The lower elasticity for bread made with the highly germinated L level was not observed in the sample malted to the same falling number range. The RVA profile of the Byrd samples demonstrated that germinating and adding malted barley resulted in similar gelatinization profiles within the same falling number range.

Overall, this study indicates that wheat germination and the subsequent whole wheat flour does not alter dough or bread properties when compared to the same un-germinated wheat bread. Highly sprouting wheat to a falling number value below 120 seconds will result in some undesirable baking characteristics, most notably in an undesirable reduction in bread elasticity.

Chapter 6 - Future Work

- Determining how germination effects shelf-life would provide interesting additional information and possible insight about the role of amylases in staling.
- Nutritional analysis is important to continue to clarify the potential health benefits of germination.
- Sensory analysis (descriptive analysis) would elucidate any differences detected in bread due to germination. Potential differences in level of sweetness and crumb texture could be notable.
- Digestibility analysis would determine if germination altered this aspect in a final product.

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Appendix A - Solution Preparation

Sugar-Salt Solution

The sugar-salt solution was modified based on the AACC Method 10-10.03. The solution contains 6 g of sucrose and 1.5 g of sodium chloride (NaCl) in a 10 mL solution. To make the stock solution, 1200 g of sucrose and 300 g of NaCl were weighed and mixed with distilled water and continuously stirred until dissolved to obtain a 2 L stock solution in a volumetric flask. The sugar-salt solution is stable at room temperature for several weeks. If the solution becomes cloudy, it should be discarded.

1.25% Sodium Hypochlorite Solution

Clorox® Bleach contains 6% sodium hypochlorite (NaClO). For the small-scale germination method mold control trial, a 200 mL solution was made with 42 mL of Clorox Bleach and 158 mL of distilled water to generate a 1.25% NaClO solution. Each of the three samples were soaked in 50 mL of the solution. For the large-scale germination method, each sample was soaked in 1400 mL of 1.25% NaClO. The solution was made for each sample by combining 292 mL of Clorox Bleach with 1108 mL of distilled water.

Appendix B - Small-Scale Germination

Falling Number Data

The following table shows the two falling number results used for each variety and location in the triplicate germination trials. Each trial was a separately germinated batch following the general method of an 18-hour soak time, 7-hour germination time in a fermentation cabinet, and 4 days drying at room temperature (24°C). Samples were then ground two falling number results obtained.

Table B-1 Falling Number (seconds) for Each Location/Variety Trial

Variety	WB4458			LCS Mint			T158		LCS Wizard			WB Grainfield			SY Monument		
County	Reno	R[a]	J[b]	Reno	R[a]	J[b]	Reno	R[a]	Reno	R[a]	J[b]	Reno	R[a]	J[b]	Reno	R[a]	J[b]
Trial	296	288	319	319	255	285	146	188	361	250	286	302	170	208	180	187	135
1	303	282	317	318	262	295	147	194	352	248	295	312	181	199	184	185	137
Trial	336	270	357	304	260	326	151	211	284	285	192	265	245	266	147	180	213
2	343	276	358	301	257	334	146	210	283	300	204	261	254	265	149	183	217
Trial	307	252	287	298	192	246	154	122	355	175	186	255	180	206	166	125	213
3	315	257	293	303	201	258	157	126	364	170	189	261	188	206	169	135	214

[a] Republic
[b] Johnson

Appendix C - Large-Scale Germination

All Soaking and Sprouting Times Falling Number Results

The following tables display all of the falling number results for the soaking and sprouting times used when attempting to produce triplicates for each falling number range. If the samples were used in the study, "L" samples were in the less than 120 seconds range, "M" samples were in the 250±40 seconds range, and "H" samples were in the 350±40 seconds range. If there is no sample label, it was not used in this study.

Table C-1 Byrd Variety Soaking and Germination Times with Resulting Falling Number

Sample Label if Used in Study	Soaking Time (hr)	Germination Time (hr)	Falling Number (sec)	Falling Number (sec)
	18	7	466	468
	24	9	270	273
M1	24	9	262	261
H1	24	9	377	384
	24	11	244	239
L1	24	12	107	93
L2	24	9	93	91
L3	24	12	109	110
M2	24	6	253	259
	24	7	137	140
	18	9	285	282
	18	8	304	309
M3	18	6	241	247
H2	18	7	329	335
	18	5	421	431
	18	4	200	185
H3	18	8	330	331

Table C-2 Tam 204 Variety Soaking and Germination Times with Resulting Falling Number

Sample Label if Used in Study	Soaking Time (hr)	Germination Time (hr)	Falling Number (sec)	Falling Number (sec)
H1	18	9	371	381
M1	24	9	252	256
	24	9	192	195
M2	24	9	292	282
L1	24	11	103	99
	24	12	79	83
L2	24	9	87	90
	24	12	84	85
	24	6	143	142
L3	24	7	108	108
M3	18	9	260	267
H2	18	8	338	341
	18	4.5	315	312
H3	18	7	336	350

Table C-3 T158 Variety Soaking and Germination Times with Resulting Falling Number

Sample Label if Used in Study	Soaking Time (hr)	Germination Time (hr)	Falling Number (sec)	Falling Number (sec)
M1	24	9	236	237
M2	24	9	235	238
	24	9	198	204
	24	11	215	216
L1	24	12	115	119
L2	24	9	76	79
	24	12	76	76
	24	6	276	280
L3	24	7	102	103
H1	18	9	330	335
	18	8	194	198
M3	18	8	264	267
	18	7	201	209
	18	5	278	284
	18	4	249	249
	18	2	447	454
H2	18	2.5	342	373
	18	3	370	387
H3	18	3.5	340	342

Table C-4 Raw Data for Dough Characteristics

Sample	Water Absorption (%)	Peak Time (min)	Mix Time (min)
Byrd L1	68	5.83	5.50
Byrd L2	66	4.59	5.00
Byrd L3	66	4.24	4.00
Byrd M1	67	5.46	6.00
Byrd M2	67	5.04	4.75
Byrd M3	67	5.76	6.00
Byrd H1	67	5.00	4.75
Byrd H2	68	6.21	6.00
Byrd H3	67	5.66	5.25
Byrd C1	70	6.04	5.75
Byrd C2	70	7.61	7.00
Byrd C3	70	5.89	6.50
Byrd Malt H	70	7.06	7.00
Byrd Malt M	72	9.79	9.00
Byrd Malt L	70	7.08	7.00
Tam 204 L1	70	2.03	1.75
Tam 204 L2	70	2.06	1.75
Tam 204 L3	70	2.14	2.00
Tam 204 M1	68	2.21	2.00
Tam 204 M2	70	2.62	2.25
Tam 204 M3	70	2.26	2.00
Tam 204 H1	68	2.38	2.00
Tam 204 H2	68	2.21	2.00
Tam 204 H3	68	2.07	1.75
Tam 204 C1	70	2.82	2.50
Tam 204 C2	70	2.72	2.50
Tam 204 C3	70	3.66	3.00
T158 L1	67	2.58	3.00
T158 L2	69	2.78	2.50
T158 L3	69	3.41	3.00
T158 M1	67	2.76	2.50
T158 M2	69	3.10	2.75
T158 M3	69	2.94	2.50
T158 H1	69	2.93	2.50
T158 H2	69	2.76	2.50
T158 H3	69	2.87	2.50
T158 DC1	68	3.83	3.50
T158 C2	69	4.24	4.00
T158 C3	69	4.98	4.50

Appendix D - Commercially Available Sprouted Whole Wheat Flour

For preliminary work, five commercially available sprouted wheat flours were purchased or provided by the suppliers. The flours were analyzed to investigate some basic baking parameters of products on the market. There is no clear definition pertaining to products sold as "sprouted wheat" and there are obvious differences among available products. It would be difficult for a consumer to ensure they are purchasing a quality product.

The whole wheat bread method used to bake the large-scale germination samples was followed to make two loaves of each sample. Sample B had a falling number value of a sound wheat which would not indicate that this product was germinated. Sample A had a very coarse particle size and it was difficult to generate a bread at all. The variation among available sprouted wheat flour products was notable.

Table D-1 Commercially Available Sprouted Wheat Flour

Sample	Falling Number (sec)	Moisture (%)	Protein (%, 14% mb[a])	Absorption (%)	Mix Time (min)	Bread Volume (cc)
A	247	7.55	14.58	64	5.75	< 400
B	439	11.10	11.41	68	3.75	500
C	150	9.07	12.51	66	4.00	543
D	245	9.31	13.24	75	2.25	673
E	205	8.83	12.90	66	3.00	815

[a] Moisture Basis