CONTROLLED SPROUTING IN WHEAT INCREASES QUALITY AND CONSUMER ACCEPTIBILITY OF WHOLE WHEAT BREAD

by

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of

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DEDICATION

For my family and friends who put up with and encouraged me through this adventure.
I thank you all for your unconditional love and support.

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I would like to start by expressing my thanks to, Dr. Michael Giroux, Department Head and Professor of Plant Science and Plant Pathology, Montana State University. His encouragement to pursue a graduate degree and his support and guidance as my PI and advisor throughout this endeavor has been welcome and appreciated. I have the utmost respect for his willingness to let me fail until I succeeded. I would also like to express my gratitude to the rest of my committee, comprising of Dr. Jack Martin, Professor Emeritus, Department of Plant Science and Plant Pathology, Montana State University, and Dr. Carmen Byker Shanks, Associate Professor, Department of Health and Human Development, Montana State University, who were always willing to guide, advise and make their expertise available.

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Finally, I want to thank my family: My wonderful husband Jeff, who has been Super-Dad to Xander and Lyrin while I spent long nights studying, and who reminded me to schedule in family fun, my parents and in-laws who unconditionally supported this endeavor, and my sisters, who kept me sane while pursuing their own adventures. Thank you all for providing sustenance when I failed, and believing I would succeed.

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GLOSSARY or NOMENCLATURE

AACC – American Association of Cereal Chemists
FN(V) – Falling Number (Value)
LECO – Laboratory Equipment Corporation
NIR – Near-Infrared Reflectance
RT – Room Temperature
SKCS - Single Kernel Characterization System
SDS – Sodium Dodecyl Sulfate sedimentation
L* - Color Brightness (white)
*a** - Color Redness
*b** - Color Yellowness

ABSTRACT

Intentional sprouting of grain to modify grain products nutritional composition and flavor has been in practice for thousands of years. However, few studies have tested the impact of controlled sprouting on wheat flour functionality and flavor. In this study, grain of nine hard red spring (HRS) wheat (*Triticum aestivum* L) cultivars was sprouted with the goal of attaining a falling number (FN) value of 200 from a starting FN of 350 seconds. Paired samples of sprouted and sound HRS grain were then assayed for nutritional composition, functionality in bread baking, and in bread taste tests. Sprouting reduced grain hardness and test weight while increasing grain color brightness, yellowness, and redness. Whole sprouted grain flour had twice the alpha amylase activity and a large decrease in flour swelling power relative to sound grain flour. Sprouted flour also contained increased free amino acids and monosaccharides while having decreased sugar alcohol content. Total dietary fiber trended down in the sprouted grain flour while starch content remained unchanged. Whole grain flour color parameters were relatively unaltered by sprouting. Sprouting reduced dough mix times while increasing loaf volume. Sensory panel evaluations demonstrated that testers preferred bread prepared from sprouted grain flour to bread prepared from sound grain while also finding that sprouted grain bread tasted less bitter and grainy while also being perceived as sweeter and moister. The results demonstrate that controlled sprouting of wheat grain could be used to increase whole grain flour functionality in bread baking and consumer acceptability of whole grain foods.

CHAPTER ONE

INTRODUCTION

Wheat is a staple food around the world and is the 2nd most cultivated crop worldwide (USDA, June 2018). Wheat is the preferred food source for many baked products as wheat gluten has a unique viscoelasticity, allowing the entrapment of fermentation bubbles. This fermentation process is partially responsible for the light airy texture of baked products such as yeast breads, and the chewy texture of flat breads such as chapati or naan (reviewed in Morris and Rose, 1996). Whole grain wheat (*Triticum aestivum* L) products have known human health benefits including mitigating some disease risk (Van Hung et al., 2015). Whole grain wheat products are also low in fat and high in dietary fiber, and many micronutrients (Slavin et al., 2001; Donkor et al., 2012). However, consumption of whole grain products in the United States remains abysmally low, on average less than one serving per day (Albertson et al., 1995; Cleveland et al., 2000; Lang and Jebb, 2003; Slavin, 2004; Fardet, 2010).

Intentionally sprouting grain to increase nutrient availability has been in practice for thousands of years (Alexander, 1983). The re-discovery and increasing popularity of sprouted grain products in the Western hemisphere is a recent development. This is partly due to the perception of sprouted grains being more natural and nutritious, what many consumers are looking for (Pagand et al., 2017). This raises the question, can sprouted grains be considered whole grains? In 2008 the American Association of Cereal

Chemists (AACC) released a statement defining what could be marketed as sprouted whole grains:

> "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."

In essence, nutritive value of products made from sprouted grain must be comparable to a whole grain standard, the statement supports the sprouted grain industry, while ensuring/encouraging responsible production and marketing practices (AACC, 2008).

Describing how sprouting impacts grain nutritional composition is difficult because changes are dependent on germination conditions, level of germination, and drying methods (Hübner and Arendt, 2013; Nelson et al., 2013). Germination triggers enzymatic activity in sprouting seeds, leading to the breakdown of proteins, carbohydrates, and lipids (Chavan et al., 1989; Nout and Ngoddy, 1997). The enzymes mobilized during the sprouting process impact grain functionality and nutritional content. Several studies (Price, 1988; Slavin et al., 2001; Dziki, 2010) have described the biochemical changes that occur in the seed during germination, which in turn impact grain properties such as structure, nutrient bioactivity, flavor, and stability.

Wheat seeds are made up of three main components, including: bran, which make up 10-14% of the seed by weight; the germ, or the embryo which make up 2.5-3%; and the endosperm which is the remaining 80-85% (Fardet, 2010). Each of these components contains nutrients with bioactive potential ready to be mobilized in support of seedling growth. Germination alters the fundamental composition of the wheat kernel which in turn affects functional and nutritional components. In mature unsprouted (sound) wheat

seeds, grain protein ranges between 8-18% (Shewry et al., 2009), and is a vital structural component in wheat flour-based foods.

Protein content and quality in wheat is essential to producing acceptable wheat flour-based products such as bread, noodles, and cookies. Schofield (1994) noted the positive linear relationship between flour protein content and bread loaf volume. Studies in protein alterations show conflicting results, with some reprting increases in protein content (Steve, 2012; Pirvulescu et al., 2014; Van Hung et al., 2015), and others reporting no change(Žilić, et al. 2016). Despite the differences in consensus, the proportions of individual storage protein subunits changed. The changes in storage protein composition could readily modify wheat baking quality (Ibrahim and D`Appolonia, 1979). In terms of nutritional benefits, Hartmann et al. (2006) indicates that protein hydrolysis likely aids in digestibility as well as possibly being beneficial to gluten sensitive individuals. The majority of wheat seed storage protein resides in the endosperm distributed among the starch granules in protein bodies.

Starch is the primary reserve of nutrients in seeds and serve as a major source of calories in human diets. Approximately 80% of the wheat endosperm is composed of starch (Stone et al., 2009). The germination process activates α-amylase, which hydrolyzes the polysaccharide starch components amylose and amylopectin into energy available sugars (Hung et al., 2011; Nelson et al, 2013). Abbas (2014) found that carbohydrate content declined significantly over a six-day germination period. These findings are supported by the findings of several other studies who also report a decrease in carbohydrates with germination. Hydrolyzed starch is one form of damaged starch, the

other simply being starch granules that are damaged during the flour milling process. Whether the starch is damaged through germination or flour milling, the resultant flour is often limited in its uses (Schofield, 1994).

Compared to carbohydrates and proteins, lipids are present in relatively small amounts in wheat seeds, only 1.5-7% of seed weight (Lorenz and D`Appolonia, 1980; Chavan, 1989). Lorenz and D`Appolonia (1980) reviewed several studies and found that sprouted grain final lipid contents exceeded those of the sound grain. Abbas et al. (2014) and Van Hung et al. (2015) also found increases in total lipid content in sprouted grain support this. Although this component is only a small proportion of wheat seeds, lipids are important in the support and transport of some micronutrients, such as Vitamin E (Andersson et al., 2014).

Wheat is known to contain many of the B vitamins as well as Vitamin E. Availability of several vitamins including Vitamins C, E, and many of the B vitamins are significantly increased during germination (Pomeranz and Robbins, 1971; Finney, 1978; Finney 1983; Yang et al., 2001; Koehler at al 2007; Zilic et al., 2014; Laxmi et al., 2015). Vitamin C is not found in unsprouted wheat, although Yang et al. (2001) reported ascorbic acid synthesis in germinating wheat. Mineral bioavailability is limited in whole grains due to the chelating properties of some anti-nutritional compounds (Hübner, 2013). Phytic acid is known to interfere with the metabolism and absorption of Ca, Fe, and Zn (Finney, 1983; Singh et al., 2015). Anjum et al. (2012) performed a study that examined how germination affected mineral extractability using 0.03 N HCl to mimic conditions in the human stomach. Germination tended to increase extractability of Ca,

Cu, Fe, K, Mn, Na and Zn with significant increases in Ca and Fe. While micronutrients are important nutritionally, the macronutrients (protein, carbohydrates, and lipids) have significant impacts on baking quality.

Baking quality must be assessed when considering the possible uses of sprouted wheat. Falling number (FN) analysis (AACC 58-81.03) developed by Hagberg (1960, 1961) and Perten (1964), is a standard test in determining degree of sprout and grain functionality/quality. Evidence from numerous studies indicate that the germination process negatively impacts flour quality, resulting in poor mixing properties with decreased dough development and reduced water absorption (Hwang and Bushuk, 1973; Lorenz and D`Appolonia, 1980; Singh et al., 1987). Germination also results in doughs that are sticky and hard to handle (Ibrahim and D`Appolonia, 1979) and that have poor loaf volume (Lemar and Swanson, 1976; Abbas et al., 2014), with dark and gummy crumb (Kozmin 1933; Ranhorata et al., 1977; Lemar and Swanson, 1976; Ibrahim and D`Appolonia, 1979; Lorenz and D`Appolonia, 1980). These undesirable baking qualities affect sensory parameters such as flavor and texture.

Affective sensory testing measures a consumer's degree of liking or disliking of a choice of alternative products. To be successful, a representative sample of untrained participants must be offered distinct samples under conditions that can be generalized to consumers in the real world (Lawless and Heymann, 1999).

Heinio et al. (2001) explored the sensory alterations that occur in germinated oat seeds (*Avena sativa*). The results indicate that sensory profiles are dependent on drying speed, temperature, and method. Bellaio et al. (2013) noted that sprouting results in

increased sugar content which is likely responsible for the widely held belief that malted cereals have a sweeter flavor. Anjum et al. (2012), and Liu et al. (2017) both found that consumers responded favorably to the taste of tortilla and chapatti flatbread made from germinated wheat flour. It should be noted that additional sensory qualities of the tortilla and chapatti were judged to be poorer than the control loaves, resulting in an overall lower rating (Anjum et al., 2012). The sensory qualities were attained through affective testing with consumers responding to a hedonic scale.

REFERENCES

Abbas, T.A. 2014. Effect of germination on chemical composition of two wheat cultivars. J. of Genetic and Environmental Resources Conservation. **2**(2): 231-235.

Albertson, A.M. & Tobelmann, R.C. 1995. Consumption of grain and whole-grain foods by an American population during the years 1990 to 1992. J. Am Diet Assoc **95**: 703–704.

Alexander, J.C. 1983. Sprouts in our muffins? Nutrient content and quality of germinated cereals. Highlights of Agricultural Research in Ontario. **6**(3): 1-3.

American Association of Cereal Chemists. 2000. Approved Methods of the American Association of Cereal Chemists. (11th Ed) St. Paul, MN. AACC.

American Association of Cereal Chemists. 2008. https://www.aaccnet.org/initiatives/definitions/Pages/WholeGrain.aspx

Andersson, A.A., Dimberg, L., Åman, P., Landberg, R. 2014. Recent findings on certain bioactive components in whole grain wheat and rye. J. Cereal Sci. **59**(3): 294-311.

Anjum, F.M., Samrah, T., Shahzad, H., Khan, M.I., Arshad, M.S., Muhammad, N. 2012. Effect of Bioprocesses on Phenolic Compounds, Phytic Acid and HCl Extractability of Minerals in Wheat Cultivars. Food Sci and Technol Res. **18**(4): 555-562.

Bellaio, S., Kappeler, S., Rosenfeld, E.Z., Jacob, M. 2013. Partially germinated ingredients for naturally healthy and tasty products. Cereal Food World. **58**(2): 55-59.

Chavan, J.K., Kadam, S.S., Beuchat, L.R. 1989. Nutritional improvement of cereals by sprouting. Crit Rev Food Sci. **28**(5): 401-437.

Cleveland, L.E., Moshfegh, A.J., Albertson, A.M., Goldman, J.D. 2000. Dietary intake of whole grains. J Am Coll Nutr. **19:** 331S–338S.

Donkor, O.N., Stojanovska, L., Ginn, P., Ashton, J., Vasiljecic, T. 2012. Germinated grains--sources of bioactive compounds. Food Chem. **135**(3): 950-959.

Dziki, D. and Laskowski J. 2010. Study to analyze the influence of sprouting of the wheat grain on the grinding process. J. Food Eng. **96**(4): 562-567.

Fardet, A. 2010. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev. **23**(1): 65-134.

Finney, P. 1978. Potential for the use of germinated wheat and soybeans to enhance human nutrition. Pages 681-701 in: Nutritional Improvement of Food and Feed Proteins. Springer: New York.

Finney, P. 1983. Effect of germination on cereal and legume nutrient changes and food or feed value: A comprehensive review. Pages 229-305 in: Mobilization of Reserves in Germination. Springer: New York

Hagberg, S. 1960. A rapid method for determining alpha-amylase activity. Cereal Chem. **37**(2): 218-222.

Hagberg, S. 1961. Note on a simplified rapid method for determining alpha-amylase activity. Cereal Chem. **38**(2): 202-203.

Hartmann, G., Koehler, P., Wieser, H. 2006. Rapid degradation of gliadin peptides toxic for coeliac disease patients by proteases from germinating cereals. J. Cereal Sci. **44**(3): 368-371.

Heiniö, R.-L., Oksman-Caldentey, K-M., Latva-Kala, K., Lehtinen, P., Poutanen, K. 2001. Effect of drying treatment conditions on sensory profile of germinated oat. Cereal Chem. **78**(6): 707-714.

Hübner, F. and Arendt, E.K. 2013. Germination of cereal grains as a way to improve the nutritional value: a review. Crit Rev Food Sci **53**(8): 853-861.

Hung, P.V., Hatcher, D.W., Barker, W. 2011. Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. Food Chem. **126**(4): 1896-1901.

Hwang, P. and Bushuk, W. 1973. Some changes in the endosperm proteins during sprouting of wheat. Cereal Chem. 50: 147-160.

Ibrahim, Y. and D'Appolonia, B. 1979. Sprouting in hard red spring wheat. Bakers Dig. **53**(5): 17-19.

Koehler, P., Hartmann, G., Weiser, H., Rychlik, M. 2007. Changes of folates, dietary fiber, and proteins in wheat as affected by germination. J. Agr Food Chem. **55**(12): 4678-4683.

Kozmin, N. 1933. Biochemical characteristics of dough and bread from sprouted grain. Cereal Chem. **10**: 420-436.

Lang, R. and Jebb, S.A. 2003. Who consumes whole grains, and how much? Proc Nutr Soc. **62**: 123–127.

Laxmi, G., Chaturvedi, N., Richa, S. 2015. The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. J. Nutr Food Sci. **5**(5): 407.

Lawless, H.T. and Heymann, H. 1999. Introduction and Overview. Pages 7-26 in: Sensory Evaluation of Food: Principles and Practices. New York: Chapman & Hall.

Lemar, L.E. and Swanson, B.G. 1976. Nutritive value of sprouted wheat flour. J. Food Sci. **41**(3): 719-720.

Liu, T., Hou, G.G., Cardin, M., Marquart, L., Dubat, A. 2017. Quality attributes of whole-wheat flour tortillas with sprouted whole-wheat flour substitution. LWT-Food Sci Technol **77**: 1-7.

Lorenz, K. and D'Appolonia, B. 1980. Cereal sprouts: Composition, nutritive value, food applications. CRC Cr Rev Food Sci. **13**(4): 353-385.

Morris, C.F. and Rose, S.P. 1996. Wheat. Pages 3-54 in: Cereal Grain Quality. R. J. Henry and P. S. Kettlewell. Dordrecht, Springer Netherlands: Dordrecht

Nelson, K., Stojanovska, L., Vasiljevic, T., Mathai, M. 2013. Germinated grains: a superior whole grain functional food? Can J. Physiol Pharm. **91**(6): 429-441.

Nout, M.J.R. and Ngoddy, P.O. 1997. Technological aspects of preparing affordable fermented complementary foods. Food Control. **8**:279–287.

Pagand, J., Heirbaut, P., Pierre, A., Pareyt, B. 2017. The Magic and Challenges of Sprouted Grains. Cereal Foods World. **62**(5): 221-226.

Perten, H. 1964. Application of the falling number method for evaluating alpha-amylase activity. Cereal Chem. **41**(3): 127-140.

Pîrvulescu, P., Botău, D., Ciulca, S., Madosa, e., Alexa, E. 2014. Researches regarding the quality of some sprouted grain flours. J. of Hort, For, Biotechnol. **18**(4): 83-88.

Pomeranz, Y. and Robbins, G. 1971. Malt sprouts: Their composition and use. Brewers Dig. **46**(5): 58-64

Price, T. 1988. Seed sprout production for human consumption—a review. Can Inst F Sci Tec J. **21**(1): 57-65.

Ranhotra, G.S., Loewe, R.J., Lehmann, T.A. 1977. Breadmaking quality and nutritive value of sprouted wheat. J. Food Sci. **42**(5): 1373-1375.

Schofield, J. 1994. Wheat proteins: structure and functionality in milling and breadmaking. Pages 73-106 in: Wheat. Springer: New York.

Shewry, P.R., D'Ovidio, R., Lafiandra, D., Jenkins, J.A., Mills, E.N., Békés, F. 2009. Wheat grain proteins. Pages 223-298 in: Wheat: Chemistry and Technology (Ed. 4). Springer Netherlands: Dordrecht.

Singh, A.K., Rehal, J., Kaur, A., Jyot, G. 2015. Enhancement of Attributes of Cereals by Germination and Fermentation: A Review. Crit Rev Food Sci. **55**(11): 1575-1589.

Singh, N., Sekhon, K.S., Nagi, H.P.S. 1987. Laboratory sprout damage and effect of heat treatment on milling and baking properties of Indian wheats. J. Food Sci. **52**(1): 176-179.

Slavin, J.L., Jacob, D., Marquart, L., Wiemer, K. 2001. The role of whole grains in disease prevention. J. Acad Nutr Diet. **101**(7): 780-785.

Slavin, J. 2004. Whole grains and human health. Nutr Res Rev. **17**(1): 99-110.

Steve, I.O. 2012. Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (Triticum aestivum). J. of Cereals and Oilseeds **3**(3): 35-47.

Stone, B., and Morell, M.K. 2009. Carbohydrates. Pages 299-362 in: Wheat: Chemistry and Technology (Ed. 4). Springer Netherlands: Dordrecht.

USDA- Foreign Agricultural Service, https://apps.fas.usda.gov/psdonline/circulars/grain.pdf p.14.

Van Hung, P., Maeda, T., Morita, N. 2015. Improvement of nutritional composition and antioxidant capacity of high-amylose wheat during germination. J. Food Sci Tech. **52**(10): 6756-6762.

Yang, F., Basu, T.K., Ooraikul, B. 2001. Studies on germination conditions and antioxidant contents of wheat grain. Int J Food Sci Nutr. **52**(4): 319-330.

Žilić, S., Basić, C., Hadži-Tašković, Š.V., Maksimović, V., Janković, M., Filipović, M. 2014. Can the sprouting process applied to wheat improve the contents of vitamins and phenolic compounds and antioxidant capacity of the flour?. Int J Food Sci Tech. **49**(4): 1040-1047.

Žilić, S., Janković, M., Barać, M., Pešić, M., Konić-Ristić, A., Hadži-Tašković, Š.V. 2016. Effects of enzyme activities during steeping and sprouting on the solubility and composition of proteins, their bioactivity and relationship with the bread making quality of wheat flour. Food & Funct. **7**(10): 4323-4331.

CHAPTER TWO

CONTROLLED SPROUTING IN WHEAT INCREASES QUALITY AND CONSUMER ACCEPTABILITY OF WHOLE WHEAT BREAD

Contribution of Authors and Co-Authors

Manuscript(s) in Chapter(s) 2

Author: Rachel Johnston

Contributions: Literature review, performed analytical tests and carried out sensory panel. Analyzed data and drafted manuscript.

Co-Author: Dr. John M. Martin

Contributions: Provided support with calculations and interpretation of results as well as assistance with the preparation of the manuscript and figures.

Co-Author: Dr. Carmen Byker-Shanks

Contributions: Provided insight and direction in preparation for the affective sensory panel. Assisted with the manuscript.

Co-Author: Sean Finney

Contributions: Assisted in project design along with sprouting the grain used in this study. Assisted with the manuscript.

Co-Author: Dr. Michael J. Giroux

Contributions: Project design and provided guidance and support throughout, aided in sensory panel bakes and provided assistance with the preparation of the manuscript and figures.

Manuscript Information

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CONTROLLED SPROUTING IN WHEAT INCREASES QUALITY AND CONSUMER ACCEPTABILITY OF WHOLE WHEAT BREAD

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ABSTRACT

Intentional sprouting of grain to nutritional composition and flavor has been in practice for thousands of years. However, few studies have tested the impact of controlled sprouting on wheat flour functionality and flavor. In this study, nine hard red spring (HRS) wheat (*Triticum aestivum* L) cultivars was sprouted with the goal of attaining a falling number (FN) value of 200 from a starting FN of 350 seconds. Paired samples of sprouted and sound HRS grain were then assayed for nutritional composition, functionality in bread baking, and in bread taste tests. Sprouting reduced grain hardness and test weight while increasing grain color brightness, yellowness, and redness. Whole sprouted grain flour had twice the alpha amylase activity relative to sound grain flour. Sprouted flour contained increased free amino acids and monosaccharides. Total dietary fiber trended down in the sprouted grain flour while starch content remained unchanged. Sprouting reduced dough mix times while increasing loaf volume. Sensory panel

evaluations demonstrated that testers preferred bread prepared from sprouted grain flour to bread prepared from sound grain. The results demonstrate that controlled sprouting of wheat grain could be used to increase whole grain flour functionality in bread baking and consumer acceptability of whole grain foods.

INTRODUCTION

Wheat is a staple food around the world and is the 2nd most cultivated crop worldwide (USDA, June 2018). Wheat is the preferred food source for many baked products as wheat gluten has a unique viscoelasticity, allowing the entrapment of fermentation bubbles. This fermentation process is partially responsible for the light airy texture of baked products such as yeast breads, and the chewy texture of flat breads such as chapati or naan (reviewed in Morris and Rose, 1996). Whole grain wheat (*Triticum aestivum* L) products have known human health benefits, including mitigating diseases such as diabetes, colon cancer, and heart disease (Van Hung et al., 2015). Whole grain wheat products are also low-fat and high in dietary fiber, vitamins, antioxidants, and minerals (Slavin et al., 2001; Donkor et al., 2012). However, consumption of whole grain products in the United States remains abysmally low, on average less than one serving per day (Albertson et al., 1995; Cleveland et al., 2000; Lang and Jebb, 2003; Slavin, 2004; Fardet, 2010).

Wheat seeds are made up of three main components, including: bran, which includes the pericarp, testa, and the aleurone layer which together make up 10-14% of the seed by

weight; the germ, or the embryo which make up 2.5-3%; and the endosperm which is the remaining 80-85% (Fardet, 2010). Each of these components contains nutrients with bioactive potential ready to be mobilized in support of seedling growth. Germination alters the fundamental composition of the wheat kernel which in turn affects functional and nutritional components.

Intentionally sprouting grain to increase nutrient availability has been in practice for thousands of years, most notably in the Orient and Middle East (Alexander, 1983). The re-discovery and increasing popularity of sprouted grain products in the Western hemisphere is a recent development. This is partly due to the perception of sprouted grains being more natural and nutritious, what many consumers are looking for. This has led to controlled germination being used to produce sprouted grain flours and flour blends resulting in increased numbers of new products containing sprouted grain flour, an average of increase of 14% per year between 2006 and 2011 (Pagand et al., 2017).

Defining how sprouting impacts grain nutritional composition is difficult because changes are dependent on germination conditions, level of germination, and drying methods (Hübner and Arendt, 2013; Nelson et al., 2013). Germination triggers enzymatic activity in sprouting seeds, leading to the breakdown of proteins, carbohydrates, and lipids into simpler forms (Chavan et al., 1989; Nout and Ngoddy, 1997). The enzymes mobilized during the sprouting process impact grain functionality and nutritional content. Several studies (Price, 1988; Slavin et al., 2001; Dziki, 2010)

have described the biochemical changes that occur in the seed during germination, which in turn impact grain properties such as structure, nutrient bioactivity, flavor, and stability. In mature unsprouted (sound) wheat seeds, grain protein ranges between 8-18% (Shewry et al., 2009), and is a vital structural component in wheat flour-based foods.

Protein content and quality in wheat is essential to producing acceptable wheat flour-based products such as bread, noodles, and cookies. Schofield (1994) noted the positive linear relationship between flour protein content and bread loaf volume. Several studies (Lemar and Swanson, 1976; Reema et al., 2004; Steve, 2012; Pirvulescu et al., 2014; Van Hung et al., 2015) have reported that sprouting wheat increases protein content. The apparent increase in protein content may result from an increase in free Nitrogen due to the enzymatic degradation of proteins during germination. Žilić, et al. (2016) found that five days of germination did not alter sprouted wheat protein content. However, the proportions of individual storage protein subunits changed, with a 11% decrease in gliadin subunits, a 45% decrease in albumins and globulins, a 17.9% decrease in soluble glutenins, and a 62% increase in non-protein nitrogen. Changes in storage protein composition could readily modify wheat baking quality (Ibrahim and D`Appolonia, 1979). In terms of nutritional benefits, Hartmann et al. (2006) indicates that protein hydrolysis likely aids in digestibility as well as possibly being beneficial to gluten sensitive individuals. The majority of wheat seed storage protein resides in the endosperm distributed among the starch granules in protein bodies.

Starch is the primary reserve of nutrients in seeds and serve as a major source of calories in human diets. Approximately 80% of the wheat endosperm is composed of starch (Stone et al., 2009). The germination process activates α-amylase, which hydrolyzes the polysaccharide starch components amylose and amylopectin into energy available sugars (Hung et al., 2011; Nelson et al, 2013). Abbas (2014) found that carbohydrate content declined significantly over a six-day germination period. These findings are supported by the findings of Lemar and Swanson (1976), Hung et al., (2011), and Steve (2012) who also report a decrease in carbohydrates with germination. Hydrolyzed starch is one form of damaged starch, the other simply being starch granules that are damaged during the flour milling process. Whether the starch is damaged through germination or flour milling, the resultant flour is often limited in its uses (Schofield, 1994).

Compared to carbohydrates and proteins, lipids are present in relatively small amounts in wheat seeds, only 1.5-7% of seed weight (Lorenz and D`Appolonia, 1980; Chavan, 1989). Lorenz and D`Appolonia (1980) reviewed several studies and found that sprouted grain final lipid contents exceeded those of the sound grain. This is supported by Abbas et al. (2014) and Van Hung et al. (2015) who both found increases in total lipid content in sprouted grain. Although a small component in terms of volume, lipids are important in the support and transport of some micronutrients, such as Vitamin E (Andersson et al., 2014).

Availability of several vitamins including Vitamins C, E, and many of the B vitamins are significantly increased during germination (Pomeranz and Robbins, 1971; Finney, 1978; Finney 1983; Yang et al., 2001; Koehler at al 2007; Zilic et al., 2014; Laxmi et al., 2015). Wheat is known to contain many of the B vitamins as well as Vitamin E. Vitamin C is not found in wheat, although Yang et al. (2001) reported ascorbic acid synthesis in germinating wheat. The effects of vitamin C synthesis have been seen in early wheat-wort beers that were used to treat and prevent scurvy (Finney, 1983). Mineral bioavailability is limited in whole grains due to the chelating properties of some anti-nutritional compounds such as phytic acid (Hübner, 2013). Phytic acid is known to interfere with the metabolism and absorption of Ca, Fe, and Zn (Finney, 1983; Singh et al., 2015). Anjum et al. (2012) performed a study that examined how germination affected mineral extractability using 0.03 N HCl to mimic conditions in the human stomach. Germination tended to increase extractability of Ca, Cu, Fe, K, Mn, Na and Zn with significant increases in Ca and Fe. While micronutrients are important nutritionally, the macronutrients (protein, carbohydrates, and lipids) have significant impacts on baking quality.

Baking quality must be assessed when considering the possible uses of sprouted wheat. Falling number (FN) analysis (AACC 58-81.03) developed by Hagberg (1960, 1961) and Perten (1964), is a standard test in determining degree of sprout and grain functionality/quality. Wheat flour with a FN of > 300 sec is considered good quality while FN of <160 is considered unusable in bread making (Richter et al., 2014).

Evidence from numerous studies indicate that the germination process negatively impacts flour quality, resulting in poor mixing properties with decreased dough development and reduced water absorption (Hwang and Bushuk, 1973; Lorenz and D`Appolonia, 1980; Singh et al., 1987). Germination also results in doughs that are sticky and hard to handle (Ibrahim and D`Appolonia, 1979) and that have poor loaf volume (Lemar and Swanson, 1976; Abbas et al., 2014), with dark and gummy crumb (Kozmin 1933; Ranhorata et al., 1977; Lemar and Swanson, 1976; Ibrahim and D`Appolonia, 1979; Lorenz and D`Appolonia, 1980). These undesirable baking qualities affect sensory parameters such as flavor, texture, and staling.

Heinio et al. (2001) explored the sensory alterations that occur in germinated oat seeds (*Avena sativa*). The results indicate that sensory profiles are dependent on drying speed, temperature, and method. Bellaio et al. (2013) noted that sprouting results in increased sugar content which is likely responsible for the widely held belief that malted cereals have a sweeter flavor. Anjum et al. (2012), and Liu et al. (2017) both found that consumers responded favorably to the taste of tortilla and chapatti flatbread made from germinated wheat flour. It should be noted that additional sensory qualities of the tortilla and chapatti were judged to be poorer than the control loaves, resulting in an overall lower rating (Anjum et al., 2012). The sensory qualities were attained through affective testing with consumers responding to a hedonic scale.

Affective sensory testing measures a consumer's degree of liking or disliking of a choice of alternative products. To be successful, a representative sample of untrained participants must be offered distinct samples under conditions that can be generalized to consumers in the real world (Lawless and Heymann, 1999).

The objective of this study was to compare sprouted and sound grain and whole wheat flour prepared from hard red spring (HRS) wheat cultivars. Specifically, the impact of controlled sprouting upon nutrient composition, product quality, and consumer preference was compared to sound grain. The results demonstrate that controlled sprouting of wheat grain could be used to increase consumer acceptability of whole grain wheat foods without negatively impacting bread quality.

MATERIAL AND METHODS

Nine hard red spring wheat cultivars were used in this study. Foundation seed grown in the northern great plains in 2017 was obtained and divided with one portion of the grain being sprouted and an equal portion remained sound. The cultivars included in the study were; Bay State Milling variety 3 (BSM3) , 'Choteau', 'Climax', 'Dapps', 'Glenn', 'Glenville', 'Reader',' Utmost', and 'Vida'.

The sprouting process consisted of soaking the wheat kernels under excess moisture at 21 °C for a period of 24 hours. The goal of the sprouting was to double α-amylase values and decrease FN values to around 200. Sprouted samples were washed in an excess of

water to mitigate any pathogen buildup. The grain was then dried at 40 °C in a forced air oven. Kilning can be challenging, temperatures are ideally kept under 60 °C to retain gluten functionality, however, α-amylase can remain active up to 70 °C (Richter et al., 2014).

Quality Testing

All evaluation and testing were performed using two independently milled grain samples from the sound and sprouted grain for each cultivar. The grain was milled using a Perten 3100 laboratory hammer mill fitted with a 500 µm screen (Perten Instruments, Springfield, IL, U.S.A). Grain tests included whole grain protein and grain moisture, which were obtained by near-infrared reflectance (NIR) using a Foss Infratec 1241 grain analyzer (Foss North America, Eden Prairie, MN, U.S.A.) (AACC approved method 39-10.01). Kernel hardness and seed weight were determined using the Single Kernel Characterization System (SKCS) (Perten Instruments, Springfield, IL, U.S.A.) (AACC approved method 55-31.01). Whole grain ash was measured using AACC approved method 08-01. Grain color was measured using a Minolta CR-310 Chroma Meter (Minolta, Ramsey, NJ). The Minolta Chroma Meter uses the International Commission on Illumination (CIE) color system and was used to measure L* (brightness), a* (red-green), and b* (yellow-blue). More positive values of L*, a*, and b* indicate increasing white, red, and yellow, respectively.

Hagberg Falling Number (FN) analysis was performed to determine level of sprout. Whole wheat flour was obtained by processing grain through a Perten Laboratory Mill 3100 (Perten Instruments, Sweden) fitted with a 500 µm screen (AACC approved method 26-10.02). Whole wheat flour protein and moisture were measured on a homogenized flour sample by NIR using a Foss Infratec 1241 grain analyzer (Foss North America, Eden Prairie, MN, U.S.A.) (AACC approved method 39-11.01). Once moisture was determined, FN was evaluated by adding 7.0g ± 0.05 g of whole wheat flour to 25 mL room temp purified water, the water and flour were homogenized using a Perten-Shakematic 1095 (Perten Instruments, Sweden). Samples were immediately placed into a Perten-FN1000 (AACC method 56-81.03). The FN-1000 was programmed to account for altitude. Reported FN values are corrected to reflect a 14 % grain moisture basis using the following equation:

$$\mathrm{FN}(14\ \%\ \mathrm{mb}) = \frac{\text{measured FN}(100 - 14)}{(100 - \text{measured flour moisture})}$$

All FN analysis was performed within 24-36 hours of grinding.

Crude whole wheat flour protein was determined by the LECO combustion method (AACC Method 46-30.01), where 0.188 g of sample was incinerated, and measured N content was multiplied by a protein conversion factor of 5.7.

Sodium dodecyl sulfate sedimentation (SDS) analysis (AACC Method 56-63.01) was performed by measuring 0.45 g flour corrected to 14 % moisture into a 10 mL stoppered

tube. Five mL methylene blue dye was added, samples were vortexed and placed in a water bath. After 2 min the samples were vortexed again and replaced in the water bath. After 4 minutes samples were vortexed and 5 mL of SDS lactic reagent was added to the samples. The samples were then placed on a rocker, inverted on the rocker for four full cycles, and allowed to sit undisturbed for 2 minutes. This process was repeated twice more with sitting periods of 4 and 6 minutes respectively. After the final inversion cycle, after the 6-minute incubation, the tubes were set upright on a shelf to rest. Sedimentation readings were taken at 20 minutes.

Starch content was assayed using a protocol adapted from Smith and Zeeman (2006), and Rösti et al. (2006). Ten mg of whole wheat flour was weighed into 2 mL micro centrifuge tubes, and 1 mL of 80% ethanol was added to the samples. The samples were vortexed and incubated at 80 °C for 3 min while shaking at 1400 rpm on a Thermomixer (Thermo Fisher Scientific, Waltham, MA). After allowing the sample to cool to RT the samples were centrifuged at 2000 rpm for 1 minute and the ethanol aspirated off. This process was repeated twice more. The samples were then placed in a vacuum desiccator overnight to dry. The samples were re-weighed, and final sample weight was recorded. A solution of 100 uM sodium acetate was added to the samples to normalize them to 10 mg/mL starch. The samples were then incubated at 98 °C for 20 min to completely gelatinize the starch. After cooling to room temp, 10 ul of an α-amylase / amyloglucosidase enzyme mix was added to the samples and samples were incubated at 37 °C for a minimum of 4 hours to overnight while rocking. Samples were spun at

13,000 rpm for 5 min, the supernatant was extracted and diluted (1:5) with purified water. Five µL of diluted sample was added to individual wells of a clear bottomed 96 well spectrophotometer plate, along with a serial dilution of a starch standard, all samples were assayed in triplicate. Two hundred µL of reaction mix #1: an aqueous solution of 100 mM HEPES-KOH (pH 7.5), 5 mM MgCl2, 0.8 mM ATP, and 1.6 mM NADP, was added to each well. The plate was mixed on a MixMate (Eppendorf, Hamburg, Germany) at 1,000 rpm for 30 seconds. Plates were pre-read at 340 nm on a SpectraMax plus 384 microplate reader (Molecular Devices, San Jose, CA). Five µL of reaction mix #2: an aqueous solution of 100 mM HEPES-KOH (pH 7.5), 0.5 U Hexokinase and 0.5 U glucose 6-phosphate dehydrogenase, was added to each well. The plate was mixed on a MixMate (Eppendorf Ag, Germany) at 1,000 rpm for 20 minutes. The plate was read again on the SpectraMax spectrophotometer at 340 nm. The initial A_{340} value was subtracted from the second A_{340} value to obtain the final A_{340} value. The standard curve was plotted and the slope (m) calculated. Percent starch calculated by the following equation:

% Starch = ((Initial A_{340} - Final A_{340} / m) *5)/10

Where m= the slope of the standard curve, 5 is the dilution factor, and 10 is the mg/mL concentration.

α-Amylase activity was determined with a micro assay for flour Alpha Amylase using a modified protocol to accommodate smaller sample sizes using the Megazyme Ceralpha kit (K-CERA: Megazyme, Ireland; AACC method 22-02.01), where 500 µL of extraction

buffer was added to 100 mg whole wheat flour and mixed at 500 rpm at 40 °C for 20 min. The samples were then centrifuged at 13,000 x g for 1 min. 10 µL of the extracted substrate was dispensed into a 96 well 0.2 mL clear bottomed plate, 7.5 µL wheat amylase was added to each well, and samples were incubated at 40 °C for 20 min and then 0.15 µL stopping reagent was added. Readings were done at 400 nm on a SpectraMax plus 384 microplate reader (Molecular Devices, USA). All samples were assayed in triplicate. Results are reported in Ceralpha units, where one unit of activity is defined as the amount of enzyme required to release one micromole of *p*-nitrophenol from BNPG7 in one minute under defined assay conditions in the presence of excess thermostable α-glucosidase (K-CERA booklet pg. 9). Ceralpha scores were calculated using the MegaCalc Ceralpha calculation sheet using the equation:

Units/g flour = (ΔE_{400} / Incubation Time) x (Total Volume Assayed/ AliquotAassayed) x (1/$^{\varepsilon}$mM *p*-nitrophenol) x (Extraction Volume/ Sample Weight) x Dilution.

Where ΔE_{400} is the reaction absorbance – the blank absorbance, incubation time = 20 min, total volume assayed is 17.65 µL, aliquot assayed = 10 µ, $^{\varepsilon}$mM of *p*-nitrophenol = 18.1, Extraction volume = 500 µL.

Total dietary fiber was measured on a subset of the samples. Four cultivars; BSM3, Choteau, Dapps, and Vida, were assayed using a protocol adapted from the Megazyme Available Carbohydrate/Dietary Fiber Kit (K-ACHDF, Megazyme, Ireland) to accommodate a smaller sample size. Two hundred fifty mg whole wheat flour were weighed out in duplicate into 125 mL Erlenmeyer flasks. Ten mL 0.5 M MES-Tris and

12.5 µL heat stable alpha amylase (3,000 U/mL) was added to each sample which were then incubated in a 80 °C shaking water bath (70 rpm) for 35 min. The flasks were then removed and 25 µL of protease (50 mg/mL:~350 tyrosine U/mL) was added. The samples were incubated in a 60 °C shaking water bath (70 rpm) for 30 min. Samples were removed and 5 mL 3M acetic acid and 50 µL of amyloglucosidase (3,300 U/mL) was added to the samples, and they were incubated at 60 °C in the shaking water bath. Samples cooled for 20 min at RT before 40 mL of pre-warmed (60 °C) 95 % ethanol was added. Samples incubated at RT for 60 min allowing a precipitate to form. The samples were then transferred to 60 mL pre-weighed, pre-ashed glass crucibles (Pyrex 36060-60C, Corning Incorporated, New York), washing any residue from the flasks with 15 mL 78 % ethanol and adding the wash solution to the crucibles. Crucibles were attached to a vacuum aspirator and samples were washed 3 times with 15 mL of 78 % ethanol, followed by 2 - 15 mL washes with 95% ethanol and finished with a 15 mL acetone wash. Sample residues were dried overnight at 70 °C. One of the duplicate samples was incinerated at 595 °C for 5 hours to determine ash content, the other sample was used to determine protein using the LECO combustion method (AACC Method 46-30.01), where 0.188 g of sample was incinerated, and measured N content was multiplied by a protein conversion factor of 5.7. Protein content from the LECO was reported in % and was converted to g (percent protein/100 x sample 2 residue) and the following equation was used to determine Total Dietary Fiber:

$$\mathrm{TDF} = (((\text{Ave Residue wt}) - \text{Protein(in g)} - \text{Ash})/(\text{ Ave Sample wt}))*100$$

Metabolite Analysis

Whole grain flour for metabolite analysis was immediately subsampled after the samples were milled and stored at -80 °C until extraction could take place. The extraction process was as described by Schmidt et al. (2011) and consisted of the following: Samples were removed from -80 °C freezer and allowed to equilibrate to RT. Approximately 30 mg of each sample was weighed, and exact sample weight was recorded. Three hundred and fifty µL of pre-warmed methanol (75 °C) was added, samples were vortexed and incubated at 60 °C for ten minutes. After the samples were vortexed, they were incubated in a sonicating water bath for ten minutes. Three hundred and fifty µL of chloroform was added to each sample and samples were vortexed. Finally, 300 µL of ddH_2O was added and samples were vortexed, then centrifuged at 13,300 g for five min. The polar fraction was transferred to a GC-MS glass vial in a volume dependent manner (150 µL per 30 mg FW) and dried in a speed-vac concentrator. Samples were sent to the West Coast Metabolomics Center (UC Davis, Davis, CA) for analysis, where an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) was utilized for metabolite quantification. Data acquisition, metabolite identification and normalization were performed as in Fiehn et al. (2008).

Dough and Baking Tests

Mixograph (AACC method 54-40.01) analysis was used to determine ideal mix time, water absorbance and dough tolerance. All loaf bakes were conducted using AACC approved methods (American Association of Cereal Chemists, 2000).

Test loaves were prepared using optimal water and mix times (AACC method 10-09.01), proofed, baked, and volume immediately measured using rapeseed displacement (AACC method 10-05.01). Crumb grain scores were evaluated using a 1-9 scale where 9 is best and 1 is poor crumb grain.

Crust and crumb color were evaluated with the same Minolta Chroma Meter used for flour and grain color.

Sensory Panel

Whole wheat flour loaves were prepared by the lead author from whole wheat flour of BSM3, Choteau, Dapps, and Vida. Loaves were made from 298.2 g whole wheat flour, 5.4 g instant dry yeast (Red Star, Milwaukee,WI), 177 mL water, 11 mL sugar/salt solution (6 g sugar and 1.5 g non-iodized salt dissolved in 11 mL water), 10.5 g canola oil, 15 mL ascorbic acid solution (0.0225 g ascorbic acid dissolved in 15 mL water) and 9 mL Doh-Tone (Corbion, Lenexa, KS) enzyme supplement (0.054 g Doh-Tone enzyme supplement dissolved in 9 mL water). Additional flour was added in small quantities as needed.

Dough was mixed for 5 min after ingredients were incorporated in a 6 qt KitchenAid mixer (KitchenAid, Benton Harbor, MI) on speed setting 2, and proofed for 55 min at 37 °C, Dough was punched down and proofed an additional 25 min. Dough was again punched down and sheeted with a rolling pin with 3/8-inch-thick spacers. The dough was rolled and placed in an oiled 21.6 x 11.4 x 7 cm loaf pan and proofed 45 min at 37

°C. Loaves were baked at 218 °C (to prevent crust caramelization) for 30 min and cooled for 2 hours at RT. Crust was removed from each loaf with a bread knife and loaves were cut into 1-inch cubes and sealed in plastic bags overnight. All bakes were completed in Montana State University's Herrick Hall Food Lab.

Sensory panels were performed the following morning. Participants for an untrained affective panel were recruited from Montana State University faculty, staff, and students. The 31 subject panel ranged in age from 16 to 52 with an average age of 27, with a gender split of 20 females and 11 males. Participants were included in the study if they consumed bread regularly and were not allergic to wheat flour or any typical bread ingredients. Sensory panels for each of the four cultivars, BSM3, Choteau, Dapps, and Vida were conducted on two replicate days. Each of the four cultivars were offered on a separate plate with two samples of sound bread and two samples of sprouted bread presented in a random order. Water for before and between samples was provided to all participants. Each cultivar was presented on an individual plate, with the four cultivar specific plates presented to the participants in a randomized order.

Participants were asked to rate each sample on flavor and texture criteria. Sample criteria were the same as in Talbert et al. (2013) where participants were asked to rate each sample on a 1(low) to 5 (high) scale for the flavor parameters of wheat-like flavor, sweetness, and bitterness, as well as the texture parameters graininess and moisture. The

participants were then asked to rank the samples from 1 (most) to 4 (least) favorite, this procedure was repeated for all 4 varietal plates.

The proposal for this sensory study was reviewed by the Montana State University Institutional Human Subject Review Board and ruled exempt from full review on Feb 16, 2018.

STATISTICAL ANALYSIS

Response variables were analyzed using analysis of variance where the model included status (sound or sprouted), cultivar and the status by cultivar interaction. Test of significance for these sources were made using the random error, except where the status x cultivar interaction was significant ($P<0.05$). In those instances, the status x cultivar mean square was used as the error in tests of significance. The data from the sensory panel were analyzed using a *t* statistic to compare the sprouted versus sound samples for each cultivar.

RESULTS

Quality Testing

The sprouted grain was softer with lower test weight and lower ash than the sound grain ($P<0.01$) (Table I). The sprouted grain was also brighter (>L*), more red (>a*) and more yellow (>b*) than sound grain ($P<0.01$). The change from sprouting were all in the same direction for each cultivar for these traits, but one or more cultivars changed

proportionally more than others, giving rise to the significant cultivar x status interaction for some traits.

As expected, falling number decreased while alpha amylase activity increased in sprouted grain compared to sound grain ($P<0.01$) (Table II). The increased alpha amylase activity is expected as germination uses amylases to degrade starches in order to mobilize nutrients in support of seedling growth. Even with the significant changes in falling number and alpha amylase, starch content was not different between sprouted and sound grain. Sprouted grain had lower dietary fiber than sound grain, but the difference did not reach statistical significance ($P=0.09$). Flour swelling power decreased but SDS sedimentation increased in sprouted grain compared to sound grain. Sprouted grain flour was darker (<L*) and less yellow (<b*) than that from sound grain. Sprouting reduced FN for all cultivars, but the reduction in FN varied from 25 to 275 seconds, which resulted in a significant status x cultivar interaction.

Table I. Characteristics of sound grain and sprouted grain from select HRS Wheat

Cultivar	NIR[a] Grain Protein (%)	SKCS[b] Grain Hardness	Individual Seed weight (mg)	Seed diam. (mm)	Test Weight Kg/hL^{-1}	Grain Ash (%)	Grain Color Parameters[c]		
							L*	*a**	*b**
Sound	14.2	73.7	29.2	2.7	794.4	1.52	61.5	10.1	23.9
Sprouted	14.3	56.9	28.7	2.7	743.3	1.48	67.7	11.5	28.1
P-value	***	***	-		***	***	***	***	***
Interaction p-value[d]		***	**	*	***				

[a] Near infrared reflectance
[b] Single Kernel Characterization System
[c] Where L* is brightness, *a** is redness and *b** is yellowness
[d] variety x Sprout/sound - Indicates that the Wheat Variety influences the parameter.
Ash and protein expressed at 12% moisture level

-, *, **, ***, denotes significance in tests of cultivar x sprout/sound interaction in ANOVA at the 0.1, 0.05, 0.01, and 0.001 level, respectively, in comparisons of sprouted vs whole wheat seed.
All values are an average of 2 replicates of each on 9 varieties

Table II. Characteristics of sound whole-wheat flour and sprouted whole-wheat flour from select HRS wheat cultivars

Cultivar	NIR Flour Protein (%)	FNV[a] (sec)	FSP[b] (g/g)	Total Dietary Fiber[c] (%)	SDS[d] (ml)	Starch (%)	Amylase Activity ceralpha	Flour Color Parameters		
								L*	*a**	*b**
Sound	16.3	352.1	10.4	14.82	4.1	67.7	0.040	81.2	1.6	9.0
Sprouted	16.4	211.3	8.3	13.11	4.7	68.2	0.080	80.7	1.7	8.8
P-value		***	**	-	***		***	**		***
Interaction p-value		**					**			

[a] Falling Number Value
[b] Flour Swelling Power
[c] Total dietary fiber (*n*=4)
[d] Sodium dodecyl sulfate sedimentation

-, *, **, ***, denotes significance in tests of cultivar x sprout/sound interaction in ANOVA at the 0.1, 0.05, 0.01, and 0.001 level, respectively, in comparisons of sprouted vs whole wheat seed.
All values are an average of 2 replicates of each on 9 varieties

Dough and Baking Tests

Sprouted grain did not significantly alter the amount of water required for optimized absorption in either the mixograph analysis nor the water required for mixing the dough for bread baking (Table III). Sprouting did not significantly alter optimal mix time with the sprouted flour to reach optimal dough strength. The cultivars reacted differently to sprouting for mix time. For example, the difference between sound and sprouted grain varied from -0.6 to 2.9 min with sprouted grain being lower in each instance (no, sprouted was not always lower) for mixograph mix time. Loaf volume was also significantly altered with sprouting increasing the average loaf volume by 45.6 cm^3 (Table III, Image 1). Color of the upper crust was altered across all three parameters (Table IV). Brightness (L*), redness (a*) and yellowness (b*) on average, were decreased in the sprouted samples. Crumb color also showed a significant decrease in redness (a*) (Table IV).

Table III. Bread quality characteristics of sprouted and sound hard red spring wheat cultivars

Cultivar	Mixo time (min)	Mixo Absorb (ml)	Mixo Tolerance[a]	Bake Mix time (min)	Bake absorb (ml)	Loaf volume(cc)	Crumb score[b]
Sound	3.0	65.3	3.8	5.3	69.99	811.1	5.7
Sprouted	2.8	65.2	3.6	4.7	69.97	856.7	5.7
P-value				-		*	
Interaction p-value	**	*		**	-		

[a] Tolerance is measured at 3-4 minutes past the peak of a mixograph. T0 = 6mm tail to T9 = 33 mm tail with each Tolerance score increasing by 3 mm.
[b] Crumb score is judge 1-9 with 1 being poor interior structure and 9 being perfect interior structure.

-, *, **, ***, denotes significance in tests of cultivar x sprout/sound interaction in ANOVA at the 0.1, 0.05, 0.01, and 0.001 level, respectively, in comparisons of sprouted vs whole wheat seed.

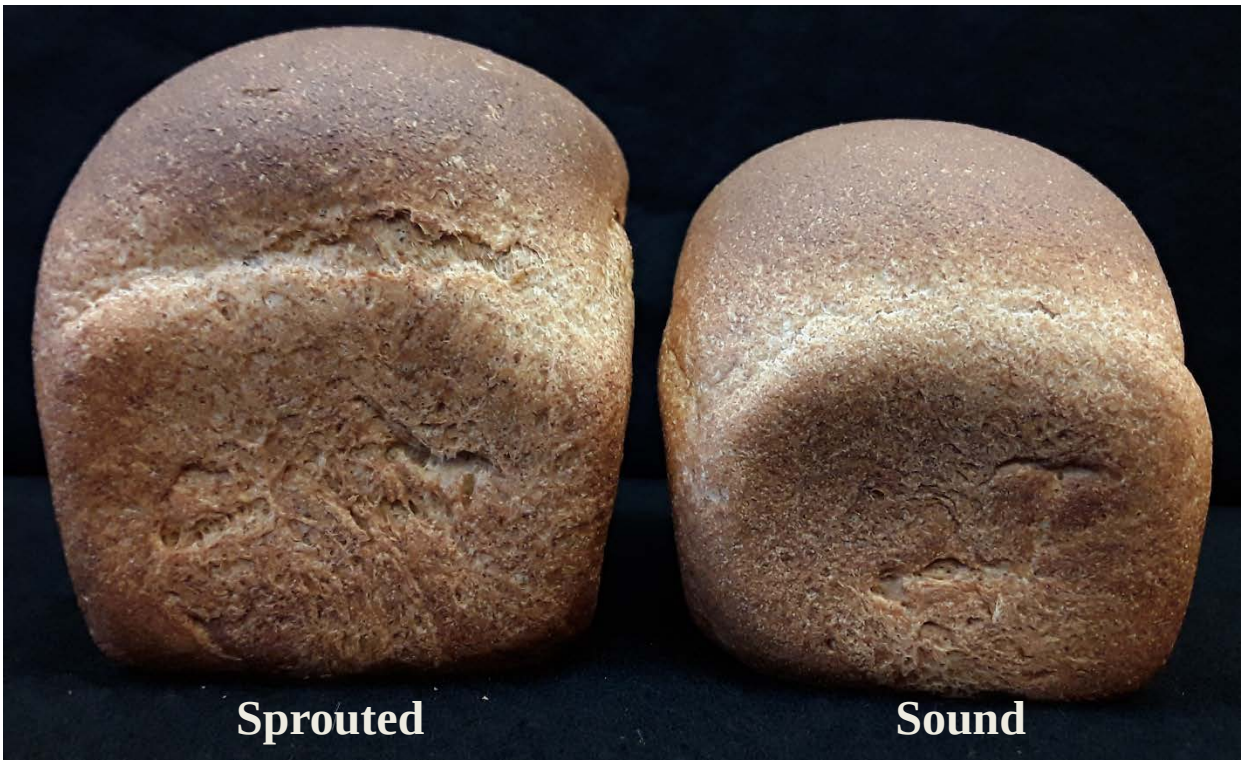


Image 1. Loaf volume difference between sprouted and sound test pup-loaves

Table IV. Color characteristics bread loaves of sprouted and sound hard red spring wheat cultivars

	Crumb Color Parameters[a]			Lower Crust Crust Color Parameters[a]			Upper Crust Crust Color Parameters[a]		
Cultivar	L*	*a**	*b**	L*	*a**	*b**	L*	*a**	*b**
Sound	59.7	6.1	20.3	38.0	11.3	16.5	36.7	11.1	14.8
Sprouted	59.4	6.0	19.8	38.3	11.2	16.6	36.0	10.5	13.6
P-value		***					*	**	**
Interaction p-value									

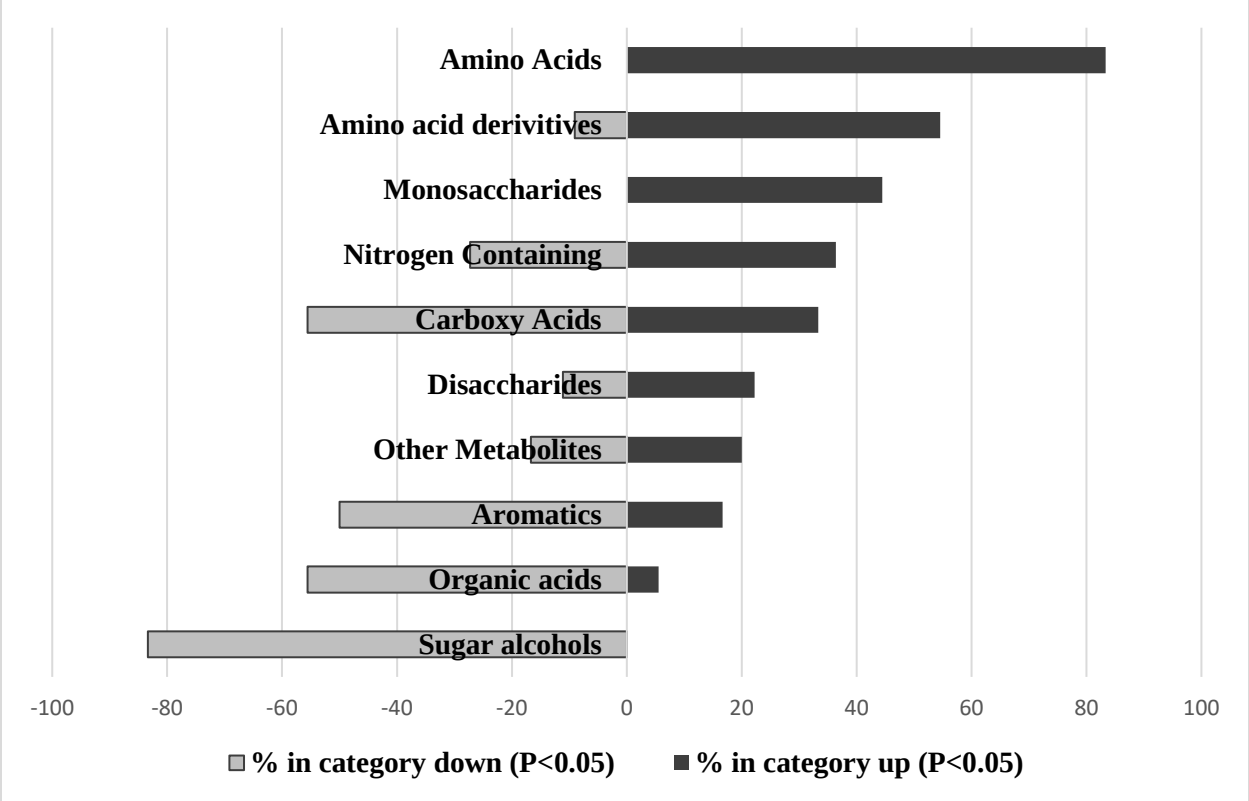
[a] Where L* is brightness, *a** is redness and *b** is yellowness

-, *, **, ***, denotes significance in tests of cultivar x sprout/sound interaction in ANOVA at the 0.1, 0.05, 0.01, and 0.001 level, respectively, in comparisons of sprouted vs whole wheat seed.

Metabolite Analysis

The 127 named metabolites that were reported were aggregated into 10 categories. The most striking alteration, as shown in Figure 1, is that sprouted flour had a marked increase in free amino acid content. Eighty-three percent of the primary amino acids (15 of 18) showed a significant increase ($P<0.05$), while 55% of the secondary amino acids and amino acid derivatives (6 of 11) were also increased ($P<0.05$). Saccharides fluctuated, with 45% of monosaccharides (4 of 9) being significantly increased in sprouted flour, while the disaccharides, and sugar alcohols saw significant decreases or no change. Many of the metabolite categories showed no net change, although sprouted flour organic acids and aromatic compounds had significantly more decreased compounds. Table V showed the breakdown of all each category into the number of metabolites significantly increased ($P<0.05$), significantly decreased ($P<0.05$), and those where sprouting did not impact their level.

Figure 1: Percent change in metabolite classes in sprouted wheat



Percent change is evaluated as percent of metabolite class up or down at an $\alpha = 0.05$

Table V. Breakdown of each of the metabolite classes in sprouted wheat

Metabolite Category	# in category up (P<0.05)	# in category down (P<0.05)	No significant change	Total Metabolites
Amino Acids	15	0	3	18
Amino acid derivatives	6	1	4	11
Monosaccharides	4	0	5	9
Nitrogen Containing	4	3	4	11
Carboxy Acids	3	5	1	9
Disaccharides	2	1	6	9
Other Metabolites	6	5	19	30
Aromatics	1	3	2	6
Organic acid	1	10	7	18
Sugar alcohols	0	5	1	6

Sensory Panel

The sprouted grain bread was preferred for each of the four cultivars included in the sensory panel with an average difference of 0.6 units in rank (Figure 2). Sweetness was perceived as significantly increased by sprouting across all the cultivars. None of the cultivars individually were judged as being significantly altered by sprouting for individual flavor or textural parameters, but collectively, sprouting impacted all measured parameters. Wheat-like flavor, bitterness, and graininess were decreased, while sweetness and moisture were increased. Figure 3 shows the relationship between the overall rank of the samples of each of the cultivars and FN. The correlation clearly shows a preference for the sprouted grain bread as the sprouted and sound samples are in distinct groups.

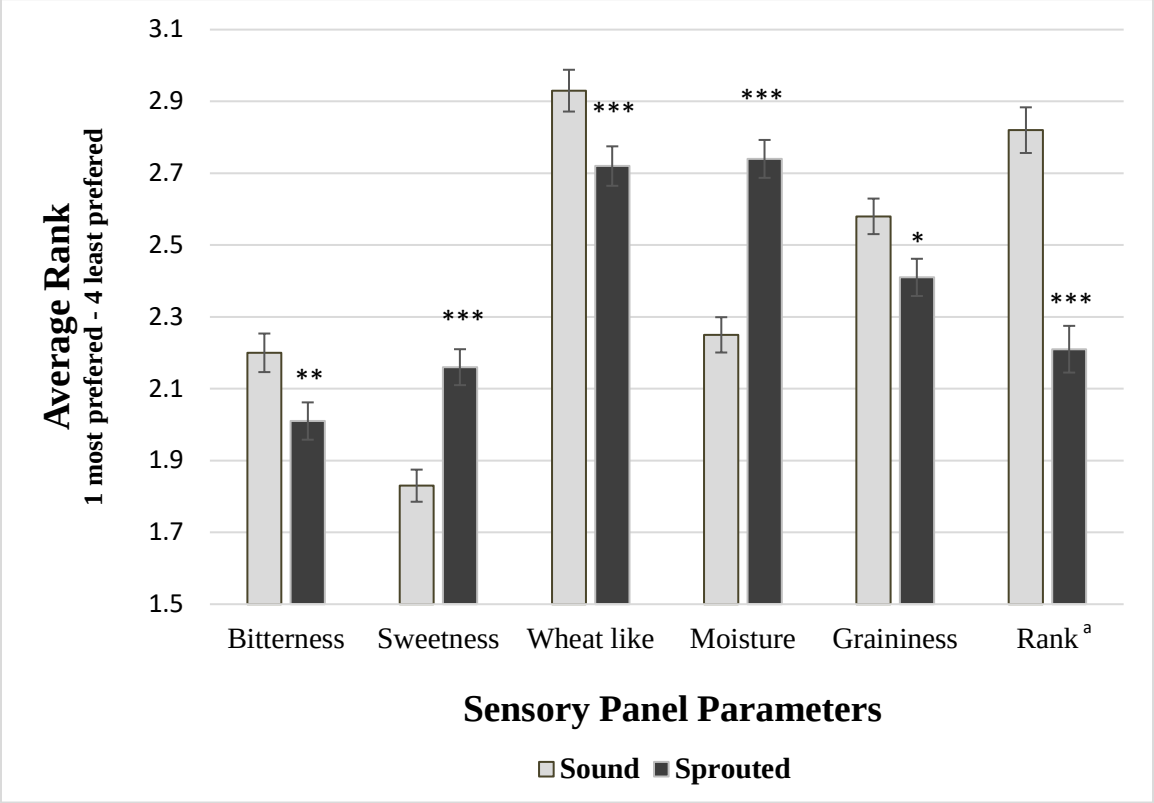


Figure 2. Comparison of sprouted and sound bread flavor and texture parameters as well as overall rank

All parameters, except rank, was scored 1(low) to 5(high).

[a] Rank was scored from 1(favorite) to 4(least favorite). The lower rank score indicates preference

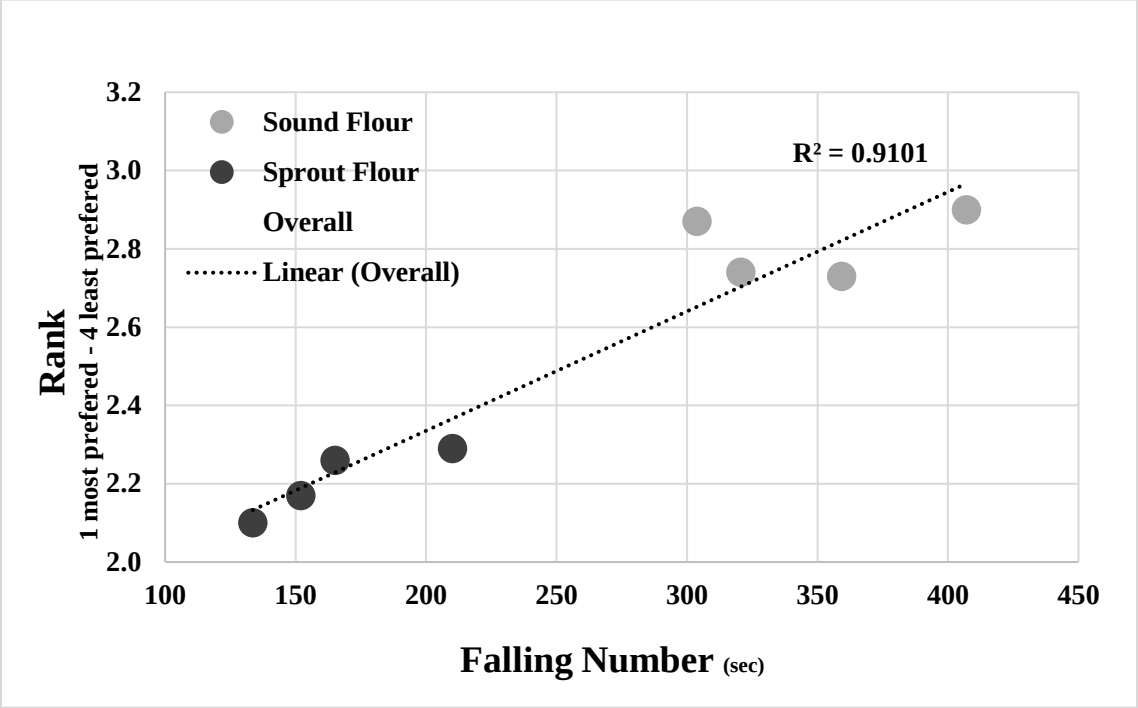


Figure 3. Correlation of sprouted and sound grain bread Rank in the sensory panel (1 = favorite 4 = least favorite) and Falling Number Value.

DISCUSSION

Finney (1978) indicated that food processing practices have trended toward efficiency, storability, and ease of use, at the detriment of nutritional value, whereas “time honored methods” produced more palatable, desirable, and nutritionally superior foods. Deora (2018) reiterates that many of the nutritional benefits in wheat seeds are found in its bran and aleurone layers, which are typically removed during the milling process. This study examined the potential of controlled sprouting to increase the desirability of whole grain wheat bread. Controlled sprouting resulted in significant alterations in several grain and many flour parameters. Overall protein content was not significantly altered. This is

consistent with the findings of Žilić et al. (2016) and Baranzelli et al. (2018), but not with most previous work. Falling Number values are consistent with Hagberg's (1961) descriptions of grain that has been sprouted and the relationship with alpha amylase concentrations. Starch content was not significantly altered which disagrees with the majority of the literature such as, Lemar and Swanson (1976), Abbas (2014), and Laxmi et al. (2015) however, the degree of sprouting used in our study was less than that of most or all previous studies.

Color in grain was altered in brightness (L*) as well as the red (*a**) and yellow (*b**) were significantly altered in sprouted grain, while flour brightness and yellowness were reduced. Bread crust color was also altered in with brightness redness and yellowness all being reduced. These are similar to results from Baranzelli et al (2018) where color alterations in bread after sprouting showed decreased brightness (L*), increased redness (*a**) though yellowness (*b**) was not altered. Ibraham and D'Appolonia (1979) and Lorenz and D'Appolonia (1980) also reported that sprouted grain bread crumb was less bright than that of non sprouted loaves.

The changes incurred during germination in turn altered the functional properties of the dough. Water absorption was unchanged which is in opposition to the previous literature. Hwang and Bushuk (1973) and Sighn et al. (1987) both found decreased absorption. Our findings of increased loaf volume are consistent with those of Richter et al. (2014) who found that sprouted wheat flour resulted in increased loaf volume. Controlled sprouting

of wheat seeds increase amylase and loaf volume could then potentially be used as a replacement for the barley (*Hordeum vulgare L*) malt often added to baked products as a source of amylase.

Amino Acid content increases support previous findings reviewed by Lorenz and D'Appolonia (1980), Donkor (2012) and Hung et al. (2012) who also report increases in amino acid content with germination.

The flavor profiles of whole grain sprouted wheat bread were significantly altered, with sweetness and moisture perceived as both different and preferred, while a decrease in bitterness and grainy texture was reported through an affective panel. Anjum et al (2012) and Liu et al (2017) had similar results with unleavened products. The sensory panels' perceived sweeter taste did not correlate highly with the measured sugar levels, as Bellaio et al (2013) reported. However, that sweeter taste correlates with the free amino acid content, indicating that amino acid content may influence flavor. The finding of increased free amino acid content is similar to the results reviewed by Lorenz and D'Appolonia (1980) where reported increases in some amino acids, namely lysine and tryptophan, though increases were found after a longer germination period and was somewhat dependent on germination temperature. More research is needed to determine if the perceived flavor alterations is in fact due to the total free amino acid content.

CONCLUSIONS

Controlled sprouting of wheat to a FN value of ~200 improves bread loaf volume while also creating whole wheat bread that is preferred by consumers and that is noticeably sweeter and less bitter than standard whole grain bread. This information could be beneficial to encourage increased whole grain consumption. More research is needed to determine if there is an ideal sprouted level for grain to optimize the beneficial alterations of sprouting while mitigating any negative effects such as reduced loaf volume if sprouting extended to the point that starch quality and quantity is reduced. Additional research into the changes that germination inflicts on the glutenin and gliadin proteins, as well as with both descriptive and affective panels added to varietal releases to improve flavor, negating the need for some of the baking additives.

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REFERENCES

Abbas, T.A. 2014. Effect of germination on chemical composition of two wheat cultivars. J. of Genetic and Environmental Resources Conservation. **2**(2): 231-235.

Albertson, A.M. & Tobelmann, R.C. 1995. Consumption of grain and whole-grain foods by an American population during the years 1990 to 1992. J. Am Diet Assoc **95**: 703–704.

Alexander, J.C. 1983. Sprouts in our muffins? Nutrient content and quality of germinated cereals. Highlights of Agricultural Research in Ontario. **6**(3): 1-3.

American Association of Cereal Chemists. 2000. Approved Methods of the American Association of Cereal Chemists. (11th Ed) St. Paul, MN. AACC.

Andersson, A.A., Dimberg, L., Åman, P., Landberg, R. 2014. Recent findings on certain bioactive components in whole grain wheat and rye. J. Cereal Sci. **59**(3): 294-311.

Anjum, F.M., Samrah, T., Shahzad, H., Khan, M.I., Arshad, M.S., Muhammad, N. 2012. Effect of Bioprocesses on Phenolic Compounds, Phytic Acid and HCl Extractability of Minerals in Wheat Cultivars. Food Sci and Technol Res. **18**(4): 555-562.

Baranzelli, J., Kringel, D.H., Colussi, R., Paiva, F.F., Aranha, B.C., Miranda, M.Z., Zavareze, E.de R., Dias, A.R.G. 2018. Changes in enzymatic activity, technological quality and gamma-aminobutyric acid (GABA) content of wheat flour as affected by germination. LWT- Food Sci Technol. **90**: 483-490.

Bellaio, S., Kappeler, S., Rosenfeld, E.Z., Jacob, M. 2013. Partially germinated ingredients for naturally healthy and tasty products. Cereal Food World. **58**(2): 55-59.

Chavan, J.K., Kadam, S.S., Beuchat, L.R. 1989. Nutritional improvement of cereals by sprouting. Crit Rev Food Sci. **28**(5): 401-437.

Cleveland, L.E., Moshfegh, A.J., Albertson, A.M., Goldman, J.D. 2000. Dietary intake of whole grains. J Am Coll Nutr. **19:** 331S–338S.

Deora, N.S. 2018. Whole Wheat Flour Stability: An Insight. Acta Sci Nutr Health. **2**: 08-18.

Donkor, O.N., Stojanovska, L., Ginn, P., Ashton, J., Vasiljecic, T. 2012. Germinated grains--sources of bioactive compounds. Food Chem. **135**(3): 950-959.

Dziki, D. and Laskowski J. 2010. Study to analyze the influence of sprouting of the wheat grain on the grinding process. J. Food Eng. **96**(4): 562-567.

Fardet, A. 2010. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev. **23**(1): 65-134.

Fiehn, O., Wohlgemuth, G., Scholz, M., Kind, T., Lee, D., Lu, Y., Moon, S., Nikolau, B. 2008. Quality control for plant metabolomics: reporting MSI-compliant studies. Plant J. **53**, 691–704.

Finney, P. 1978. Potential for the use of germinated wheat and soybeans to enhance human nutrition. Pages 681-701 in: Nutritional Improvement of Food and Feed Proteins. Springer: New York.

Finney, P. 1983. Effect of germination on cereal and legume nutrient changes and food or feed value: A comprehensive review. Pages 229-305 in: Mobilization of Reserves in Germination. Springer: New York

Hagberg, S. 1960. A rapid method for determining alpha-amylase activity. Cereal Chem. **37**(2): 218-222.

Hagberg, S. 1961. Note on a simplified rapid method for determining alpha-amylase activity. Cereal Chem. **38**(2): 202-203.

Hartmann, G., Koehler, P., Wieser, H. 2006. Rapid degradation of gliadin peptides toxic for coeliac disease patients by proteases from germinating cereals. J. Cereal Sci. **44**(3): 368-371.

Heiniö, R.-L., Oksman-Caldentey, K-M., Latva-Kala, K., Lehtinen, P., Poutanen, K. 2001. Effect of drying treatment conditions on sensory profile of germinated oat. Cereal Chem. **78**(6): 707-714.

Hübner, F. and Arendt, E.K. 2013. Germination of cereal grains as a way to improve the nutritional value: a review. Crit Rev Food Sci **53**(8): 853-861.

Hung, P.V., Hatcher, D.W., Barker, W. 2011. Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. Food Chem. **126**(4): 1896-1901.

Hung, P.V., Tomoko, M., Syota, Y., Naofumi, M. 2012. Effects of germination on nutritional composition of waxy wheat. J. Sci Food Agric. **92**(3): 667-672.

Hwang, P. and Bushuk, W. 1973. Some changes in the endosperm proteins during sprouting of wheat. Cereal Chem. 50: 147-160.

Ibrahim, Y. and D'Appolonia, B. 1979. Sprouting in hard red spring wheat. Bakers Dig. **53**(5): 17-19.

Koehler, P., Hartmann, G., Weiser, H., Rychlik, M. 2007. Changes of folates, dietary fiber, and proteins in wheat as affected by germination. J. Agr Food Chem. **55**(12): 4678-4683.

Kozmin, N. 1933. Biochemical characteristics of dough and bread from sprouted grain. Cereal Chem. **10**: 420-436.

Lang, R. and Jebb, S.A. 2003. Who consumes whole grains, and how much? Proc Nutr Soc. **62**: 123–127.

Laxmi, G., Chaturvedi, N., Richa, S. 2015. The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. J. Nutr Food Sci. **5**(5): 407.

Lawless, H.T. and Heymann, H. 1999. Introduction and Overview. Pages 7-26 in: Sensory Evaluation of Food: Principles and Practices. New York: Chapman & Hall.

Lemar, L.E. and Swanson, B.G. 1976. Nutritive value of sprouted wheat flour. J. Food Sci. **41**(3): 719-720.

Liu, T., Hou, G.G., Cardin, M., Marquart, L., Dubat, A. 2017. Quality attributes of whole-wheat flour tortillas with sprouted whole-wheat flour substitution. LWT-Food Sci Technol **77**: 1-7.

Lorenz, K. and D'Appolonia, B. 1980. Cereal sprouts: Composition, nutritive value, food applications. CRC Cr Rev Food Sci. **13**(4): 353-385.

Morris, C.F. and Rose, S.P. 1996. Wheat. Pages 3-54 in: Cereal Grain Quality. R. J. Henry and P. S. Kettlewell. Dordrecht, Springer Netherlands: Dordrecht

Nelson, K., Stojanovska, L., Vasiljevic, T., Mathai, M. 2013. Germinated grains: a superior whole grain functional food? Can J. Physiol Pharm. **91**(6): 429-441.

Nout, M.J.R. and Ngoddy, P.O. 1997. Technological aspects of preparing affordable fermented complementary foods. Food Control. **8**:279–287.

Pagand, J., Heirbaut, P., Pierre, A., Pareyt, B. 2017. The Magic and Challenges of Sprouted Grains. Cereal Foods World. **62**(5): 221-226.

Perten, H. 1964. Application of the falling number method for evaluating alpha-amylase activity. Cereal Chem. **41**(3): 127-140.

Pîrvulescu, P., Botău, D., Ciulca, S., Madosa, e., Alexa, E. 2014. Researches regarding the quality of some sprouted grain flours. J. of Hort, For, Biotechnol. **18**(4): 83-88.

Pomeranz, Y. and Robbins, G. 1971. Malt sprouts: Their composition and use. Brewers Dig. **46**(5): 58-64

Price, T. 1988. Seed sprout production for human consumption—a review. Can Inst F Sci Tec J. **21**(1): 57-65.

Ranhotra, G.S., Loewe, R.J., Lehmann, T.A. 1977. Breadmaking quality and nutritive value of sprouted wheat. J. Food Sci. **42**(5): 1373-1375.

Reema, Hera, C.K., Balwinder, S. 2004. Nutritional evaluation of supplementary foods prepared from germinated cereals and legumes. J. Food Sci Tech Mys. **41**(6): 627-629.

Richter, K., Chritiansen, K., Guo, G. 2014. Wheat sprouting enhances bread baking performance. Cereal Foods World **59**(5): 231-233.

Rösti, S., Rudi, H., Rudi, K., Opsahl-Sorteberg, H-G., Gahy, B., Denyer, K. 2006. The gene encoding the cytosolic small subunit of ADP-glucose pyrophosphorylase in barley endosperm also encodes the major plastidial small subunit in the leaves. J. Exp Bot. **57**(14): 3619-3626.

Schmidt M.A., Barbazuk W.B., Sandford M., May G., Song Z., Zhou W., Nikolau B.J., Herman E.M. 2011. Silencing of soybean seed storage proteins results in a rebalanced protein composition preserving seed protein content without major collateral changes in the metabolome and transcriptome. Plant Physiol. **156**, 330–345.

Schofield, J. 1994. Wheat proteins: structure and functionality in milling and breadmaking. Pages 73-106 in: Wheat. Springer: New York.

Shewry, P.R., D'Ovidio, R., Lafiandra, D., Jenkins, J.A., Mills, E.N., Békés, F. 2009. Wheat grain proteins. Pages 223-298 in: Wheat: Chemistry and Technology (Ed. 4). Springer Netherlands: Dordrecht.

Singh, A.K., Rehal, J., Kaur, A., Jyot, G. 2015. Enhancement of Attributes of Cereals by Germination and Fermentation: A Review. Crit Rev Food Sci. **55**(11): 1575-1589.

Singh, N., Sekhon, K.S., Nagi, H.P.S. 1987. Laboratory sprout damage and effect of heat treatment on milling and baking properties of Indian wheats. J. Food Sci. **52**(1): 176-179.

Slavin, J.L., Jacob, D., Marquart, L., Wiemer, K. 2001. The role of whole grains in disease prevention. J. Acad Nutr Diet. **101**(7): 780-785.

Slavin, J. 2004. Whole grains and human health. Nutr Res Rev. **17**(1): 99-110.

Smith, A.M. and Zeeman S.C. 2006. Quantification of starch in plant tissues. Nat Protoc. **1**: 1342-1345.

Steve, I.O. 2012. Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (Triticum aestivum). J. of Cereals and Oilseeds **3**(3): 35-47.

Stone, B., and Morell, M.K. 2009. Carbohydrates. Pages 299-362 in: Wheat: Chemistry and Technology (Ed. 4). Springer Netherlands: Dordrecht.

Talbert, L.E., Hofer, P., Nash, D., Martin, J.M., Lanning, S.P., Sherman, J.D., Giroux, M.J. 2013. Hard white versus hard red wheats: taste tests and milling and baking properties. Cereal Chem. **90**(3): 249-255.

USDA- Foreign Agricultural Service, https://apps.fas.usda.gov/psdonline/circulars/grain.pdf p.14.

Van Hung, P., Maeda, T., Morita, N. 2015. Improvement of nutritional composition and antioxidant capacity of high-amylose wheat during germination. J. Food Sci Tech. **52**(10): 6756-6762.

Yang, F., Basu, T.K., Ooraikul, B. 2001. Studies on germination conditions and antioxidant contents of wheat grain. Int J Food Sci Nutr. **52**(4): 319-330.

Žilić, S., Basić, C., Hadži-Tašković, Š.V., Maksimović, V., Janković, M., Filipović, M. 2014. Can the sprouting process applied to wheat improve the contents of vitamins and phenolic compounds and antioxidant capacity of the flour?. Int J Food Sci Tech. **49**(4): 1040-1047.

Žilić, S., Janković, M., Barać, M., Pešić, M., Konić-Ristić, A., Hadži-Tašković, Š.V. 2016. Effects of enzyme activities during steeping and sprouting on the solubility and composition of proteins, their bioactivity and relationship with the bread making quality of wheat flour. Food & Funct. **7**(10): 4323-4331.

CHAPTER THREE

CONCUDING REMARKS

Food processing practices have trended toward efficiency, storability, and ease of use, at the detriment of nutritional value, whereas “time honored methods” produced more palatable, desirable, and nutritionally superior foods (Finney, 1978). Deora (2018) reiterates that many of the nutritional benefits in wheat seeds are found in its bran and aleurone layers, which are typically removed during the milling process. The potential of controlled sprouting to increase the desirability of whole grain wheat products can be utilized to encourage more whole grain consumption, which continues to be extremely low, again, less than one serving per day in the United States (Albertson et al., 1995; Cleveland et al., 2000; Lang and Jebb, 2003; Slavin, 2004; Fardet, 2010).

Controlled sprouting of wheat to a FN value of ~200 improves bread loaf volume while also creating whole wheat bread that is preferred by consumers and that is noticeably sweeter and less bitter than standard whole grain bread. The flavor profiles of whole grain sprouted wheat bread were reported through an affective panel showing significant alterations, with sweetness and moisture perceived as both different and preferred, while also reporting a decrease in bitterness and grainy texture. Anjum et al (2012) and Liu et al (2017) had similar results with unleavened products. This information could be beneficial to encourage increased whole grain consumption. The functional differences that we found deviate from much of the previous work. This is

likely due to the chosen controlled sprouting process, which falls under the AACC definition of sprouted whole grain, resulting in minimal hydrolysis, nutrient breakdown and mobilization compared to the majority of the previous work.

More research is needed to determine if there is an ideal sprouted level for grain to optimize the beneficial alterations of sprouting while mitigating any negative effects such as reduced loaf volume is sprouting extended to the point that starch quality and quantity is reduced. Additional research into the changes that germination inflicts on the glutenin and gliadin proteins, as well as adding both descriptive and affective panels added to varietal releases to improve flavor, negating the need for some of the baking additives.

REFERENCES

Albertson, A.M. & Tobelmann, R.C. 1995. Consumption of grain and whole-grain foods by an American population during the years 1990 to 1992. J. Am Diet Assoc **95**: 703–704.

Anjum, F.M., Samrah, T., Shahzad, H., Khan, M.I., Arshad, M.S., Muhammad, N. 2012. Effect of Bioprocesses on Phenolic Compounds, Phytic Acid and HCl Extractability of Minerals in Wheat Cultivars. Food Sci and Technol Res. **18**(4): 555-562.

Cleveland, L.E., Moshfegh, A.J., Albertson, A.M., Goldman, J.D. 2000. Dietary intake of whole grains. J Am Coll Nutr. **19:** 331S–338S.

Deora, N.S. 2018. Whole Wheat Flour Stability: An Insight. Acta Sci Nutr Health. **2**: 08-18.

Fardet, A. 2010. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev. **23**(1): 65-134.

Finney, P. 1978. Potential for the use of germinated wheat and soybeans to enhance human nutrition. Pages 681-701 in: Nutritional Improvement of Food and Feed Proteins. Springer: New York.

Lang, R. and Jebb, S.A. 2003. Who consumes whole grains, and how much? Proc Nutr Soc. **62**: 123–127.

Liu, T., Hou, G.G., Cardin, M., Marquart, L., Dubat, A. 2017. Quality attributes of whole-wheat flour tortillas with sprouted whole-wheat flour substitution. LWT-Food Sci Technol **77**: 1-7.

Singh, N., Sekhon, K.S., Nagi, H.P.S. 1987. Laboratory sprout damage and effect of heat treatment on milling and baking properties of Indian wheats. J. Food Sci. **52**(1): 176-179.

Slavin, J. 2004. Whole grains and human health. Nutr Res Rev. **17**(1): 99-110.

REFERENCES CITED

Abbas, T.A. 2014. Effect of germination on chemical composition of two wheat cultivars. J. of Genetic and Environmental Resources Conservation. **2**(2): 231-235.

Albertson, A.M. & Tobelmann, R.C. 1995. Consumption of grain and whole-grain foods by an American population during the years 1990 to 1992. J. Am Diet Assoc **95**: 703–704.

Alexander, J.C. 1983. Sprouts in our muffins? Nutrient content and quality of germinated cereals. Highlights of Agricultural Research in Ontario. **6**(3): 1-3.

American Association of Cereal Chemists. 2000. Approved Methods of the American Association of Cereal Chemists. (11th Ed) St. Paul, MN. AACC.

Andersson, A.A., Dimberg, L., Åman, P., Landberg, R. 2014. Recent findings on certain bioactive components in whole grain wheat and rye. J. Cereal Sci. **59**(3): 294-311.

Anjum, F.M., Samrah, T., Shahzad, H., Khan, M.I., Arshad, M.S., Muhammad, N. 2012. Effect of Bioprocesses on Phenolic Compounds, Phytic Acid and HCl Extractability of Minerals in Wheat Cultivars. Food Sci and Technol Res. **18**(4): 555-562.

Baranzelli, J., Kringel, D.H., Colussi, R., Paiva, F.F., Aranha, B.C., Miranda, M.Z., Zavareze, E.de R., Dias, A.R.G. 2018. Changes in enzymatic activity, technological quality and gamma-aminobutyric acid (GABA) content of wheat flour as affected by germination. LWT- Food Sci Technol. **90**: 483-490.

Bellaio, S., Kappeler, S., Rosenfeld, E.Z., Jacob, M. 2013. Partially germinated ingredients for naturally healthy and tasty products. Cereal Food World. **58**(2): 55-59.

Chavan, J.K., Kadam, S.S., Beuchat, L.R. 1989. Nutritional improvement of cereals by sprouting. Crit Rev Food Sci. **28**(5): 401-437.

Cleveland, L.E., Moshfegh, A.J., Albertson, A.M., Goldman, J.D. 2000. Dietary intake of whole grains. J Am Coll Nutr. **19:** 331S–338S.

Deora, N.S. 2018. Whole Wheat Flour Stability: An Insight. Acta Sci Nutr Health. **2**: 08-18.

Donkor, O.N., Stojanovska, L., Ginn, P., Ashton, J., Vasiljecic, T. 2012. Germinated grains--sources of bioactive compounds. Food Chem. **135**(3): 950-959.

Dziki, D. and Laskowski J. 2010. Study to analyze the influence of sprouting of the wheat grain on the grinding process. J. Food Eng. **96**(4): 562-567.

Fardet, A. 2010. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev. **23**(1): 65-134.

Fiehn, O., Wohlgemuth, G., Scholz, M., Kind, T., Lee, D., Lu, Y., Moon, S., Nikolau, B. 2008. Quality control for plant metabolomics: reporting MSI-compliant studies. Plant J. **53**, 691–704.

Finney, P. 1978. Potential for the use of germinated wheat and soybeans to enhance human nutrition. Pages 681-701 in: Nutritional Improvement of Food and Feed Proteins. Springer: New York.

Finney, P. 1983. Effect of germination on cereal and legume nutrient changes and food or feed value: A comprehensive review. Pages 229-305 in: Mobilization of Reserves in Germination. Springer: New York

Hagberg, S. 1960. A rapid method for determining alpha-amylase activity. Cereal Chem. **37**(2): 218-222.

Hagberg, S. 1961. Note on a simplified rapid method for determining alpha-amylase activity. Cereal Chem. **38**(2): 202-203.

Hartmann, G., Koehler, P., Wieser, H. 2006. Rapid degradation of gliadin peptides toxic for coeliac disease patients by proteases from germinating cereals. J. Cereal Sci. **44**(3): 368-371.

Heiniö, R.-L., Oksman-Caldentey, K-M., Latva-Kala, K., Lehtinen, P., Poutanen, K. 2001. Effect of drying treatment conditions on sensory profile of germinated oat. Cereal Chem. **78**(6): 707-714.

Hübner, F. and Arendt, E.K. 2013. Germination of cereal grains as a way to improve the nutritional value: a review. Crit Rev Food Sci **53**(8): 853-861.

Hung, P.V., Hatcher, D.W., Barker, W. 2011. Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. Food Chem. **126**(4): 1896-1901.

Hung, P.V., Tomoko, M., Syota, Y., Naofumi, M. 2012. Effects of germination on nutritional composition of waxy wheat. J. Sci Food Agric. **92**(3): 667-672.

Hwang, P. and Bushuk, W. 1973. Some changes in the endosperm proteins during sprouting of wheat. Cereal Chem. 50: 147-160.

Ibrahim, Y. and D'Appolonia, B. 1979. Sprouting in hard red spring wheat. Bakers Dig. **53**(5): 17-19.

Koehler, P., Hartmann, G., Weiser, H., Rychlik, M. 2007. Changes of folates, dietary fiber, and proteins in wheat as affected by germination. J. Agr Food Chem. **55**(12): 4678-4683.

Kozmin, N. 1933. Biochemical characteristics of dough and bread from sprouted grain. Cereal Chem. **10**: 420-436.

Lang, R. and Jebb, S.A. 2003. Who consumes whole grains, and how much? Proc Nutr Soc. **62**: 123–127.

Laxmi, G., Chaturvedi, N., Richa, S. 2015. The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. J. Nutr Food Sci. **5**(5): 407.

Lawless, H.T. and Heymann, H. 1999. Introduction and Overview. Pages 7-26 in: Sensory Evaluation of Food: Principles and Practices. New York: Chapman & Hall.

Lemar, L.E. and Swanson, B.G. 1976. Nutritive value of sprouted wheat flour. J. Food Sci. **41**(3): 719-720.

Liu, T., Hou, G.G., Cardin, M., Marquart, L., Dubat, A. 2017. Quality attributes of whole-wheat flour tortillas with sprouted whole-wheat flour substitution. LWT-Food Sci Technol **77**: 1-7.

Lorenz, K. and D'Appolonia, B. 1980. Cereal sprouts: Composition, nutritive value, food applications. CRC Cr Rev Food Sci. **13**(4): 353-385.

Morris, C.F. and Rose, S.P. 1996. Wheat. Pages 3-54 in: Cereal Grain Quality. R. J. Henry and P. S. Kettlewell. Dordrecht, Springer Netherlands: Dordrecht

Nelson, K., Stojanovska, L., Vasiljevic, T., Mathai, M. 2013. Germinated grains: a superior whole grain functional food? Can J. Physiol Pharm. **91**(6): 429-441.

Nout, M.J.R. and Ngoddy, P.O. 1997. Technological aspects of preparing affordable fermented complementary foods. Food Control. **8**:279–287.

Pagand, J., Heirbaut, P., Pierre, A., Pareyt, B. 2017. The Magic and Challenges of Sprouted Grains. Cereal Foods World. **62**(5): 221-226.

Perten, H. 1964. Application of the falling number method for evaluating alpha-amylase activity. Cereal Chem. **41**(3): 127-140.

Pîrvulescu, P., Botău, D., Ciulca, S., Madosa, e., Alexa, E. 2014. Researches regarding the quality of some sprouted grain flours. J. of Hort, For, Biotechnol. **18**(4): 83-88.

Pomeranz, Y. and Robbins, G. 1971. Malt sprouts: Their composition and use. Brewers Dig. **46**(5): 58-64

Price, T. 1988. Seed sprout production for human consumption—a review. Can Inst F Sci Tec J. **21**(1): 57-65.

Ranhotra, G.S., Loewe, R.J., Lehmann, T.A. 1977. Breadmaking quality and nutritive value of sprouted wheat. J. Food Sci. **42**(5): 1373-1375.

Reema, Hera, C.K., Balwinder, S. 2004. Nutritional evaluation of supplementary foods prepared from germinated cereals and legumes. J. Food Sci Tech Mys. **41**(6): 627-629.

Richter, K., Chritiansen, K., Guo, G. 2014. Wheat sprouting enhances bread baking performance. Cereal Foods World **59**(5): 231-233.

Rösti, S., Rudi, H., Rudi, K., Opsahl-Sorteberg, H-G., Gahy, B., Denyer, K. 2006. The gene encoding the cytosolic small subunit of ADP-glucose pyrophosphorylase in barley endosperm also encodes the major plastidial small subunit in the leaves. J. Exp Bot. **57**(14): 3619-3626.

Schmidt M.A., Barbazuk W.B., Sandford M., May G., Song Z., Zhou W., Nikolau B.J., Herman E.M. 2011. Silencing of soybean seed storage proteins results in a rebalanced protein composition preserving seed protein content without major collateral changes in the metabolome and transcriptome. Plant Physiol. **156**, 330–345.

Schofield, J. 1994. Wheat proteins: structure and functionality in milling and breadmaking. Pages 73-106 in: Wheat. Springer: New York.

Shewry, P.R., D'Ovidio, R., Lafiandra, D., Jenkins, J.A., Mills, E.N., Békés, F. 2009. Wheat grain proteins. Pages 223-298 in: Wheat: Chemistry and Technology (Ed. 4). Springer Netherlands: Dordrecht.

Singh, A.K., Rehal, J., Kaur, A., Jyot, G. 2015. Enhancement of Attributes of Cereals by Germination and Fermentation: A Review. Crit Rev Food Sci. **55**(11): 1575-1589.

Singh, N., Sekhon, K.S., Nagi, H.P.S. 1987. Laboratory sprout damage and effect of heat treatment on milling and baking properties of Indian wheats. J. Food Sci. **52**(1): 176-179.

Slavin, J.L., Jacob, D., Marquart, L., Wiemer, K. 2001. The role of whole grains in disease prevention. J. Acad Nutr Diet. **101**(7): 780-785.

Slavin, J. 2004. Whole grains and human health. Nutr Res Rev. **17**(1): 99-110.

Smith, A.M. and Zeeman S.C. 2006. Quantification of starch in plant tissues. Nat Protoc. **1**: 1342-1345.

Steve, I.O. 2012. Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (Triticum aestivum). J. of Cereals and Oilseeds **3**(3): 35-47.

Stone, B., and Morell, M.K. 2009. Carbohydrates. Pages 299-362 in: Wheat: Chemistry and Technology (Ed. 4). Springer Netherlands: Dordrecht.

Talbert, L.E., Hofer, P., Nash, D., Martin, J.M., Lanning, S.P., Sherman, J.D., Giroux, M.J. 2013. Hard white versus hard red wheats: taste tests and milling and baking properties. Cereal Chem. **90**(3): 249-255.

USDA- Foreign Agricultural Service, https://apps.fas.usda.gov/psdonline/circulars/grain.pdf p.14.

Van Hung, P., Maeda, T., Morita, N. 2015. Improvement of nutritional composition and antioxidant capacity of high-amylose wheat during germination. J. Food Sci Tech. **52**(10): 6756-6762.

Yang, F., Basu, T.K., Ooraikul, B. 2001. Studies on germination conditions and antioxidant contents of wheat grain. Int J Food Sci Nutr. **52**(4): 319-330.

Žilić, S., Basić, C., Hadži-Tašković, Š.V., Maksimović, V., Janković, M., Filipović, M. 2014. Can the sprouting process applied to wheat improve the contents of vitamins and phenolic compounds and antioxidant capacity of the flour?. Int J Food Sci Tech. **49**(4): 1040-1047.

Žilić, S., Janković, M., Barać, M., Pešić, M., Konić-Ristić, A., Hadži-Tašković, Š.V. 2016. Effects of enzyme activities during steeping and sprouting on the solubility and composition of proteins, their bioactivity and relationship with the bread making quality of wheat flour. Food & Funct. **7**(10): 4323-4331.