Summer 8-2021

Studies on milling and baking quality and in-vitro protein digestibility of historical and modern wheats

Sujun Liu
University of Nebraska-Lincoln, willow@huskers.unl.edu

Studies on milling and baking quality and *in-vitro* protein digestibility of historical and modern wheats

by

Sujun Liu

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Food Science and Technology

Under the Supervision of Professor Devin Rose

Lincoln, Nebraska

August 2021

STUDIES ON MILLING AND BAKING QUALITY AND *IN-VITRO* PROTEIN DIGESTIBILITY OF HISTORICAL AND MODERN WHEATS

Sujun Liu, M.S.

University of Nebraska, 2021

Advisor: Devin Rose

There is considerable controversy among the public and the scientific community about whether modern wheats are harmful for human health and responsible for the increase of Celiac disease compared with historical varieties. Therefore, the milling and baking quality, protein digestibility, and protein composition of historical and modern wheats adapted to the Great Plains of the US were evaluated in this thesis.

One objective of this thesis was to determine how end-use quality of wheat changed with wheat cultivar release year. Kernel physical characteristics, milling yield, whole-wheat flour quality, flour protein content, mixing quality and baking quality of 23 hard winter wheat cultivars released in the Great Plains region of the US between 1870 and 2013 were evaluated. Several quality characteristics improved across release year, which is evidence of the impact of plant breeding efforts over the years. Specifically, wheat kernels have become harder, moister, more uniform in hardness but more variable in shape over a century of breeding. Bran quality decreased, which may have implications for whole grain quality and milling productivity. The baking quality remained constant despite a strong decrease in protein concentration.

Another objective was to determine the change in *in vitro* protein digestibility during breeding and its relationship between wheat end-use quality. Digestibility of bread increased with release year and was significantly positively correlated with kernel diameter standard deviation, milling yield, Mixograph mixing peak time, and loaf firmness while negatively

correlated with white flour protein content, Mixograph mixing peak value, and loaf volume. Flour protein digestibility had no relationship with release year and no correlation with end-use quality characteristics. High molecular weight protein increased while low molecular weight protein decreased as a function of release year. Several gluten proteins were associated with high digestibility which may need further study. In conclusion, the end-use quality improved during breeding somehow and some of them have positive correlation with protein digestibility which can be used for future wheat breeding selection.

ACKNOWLEDGEMENTS

Dr. Rose is the person I want to say thanks to firstly. He is such an excellent advisor. He helped me figure out many problems with my experiments, and I learned a lot from him. I hope I can be a person like him in the future. It was so lucky for me to have the opportunity to join his lab.

I also want to thank the members of my committee Dr. Danao, Dr. Downs, and Dr. Majumder, for their suggestions and help. I appreciate Dr. Majumder let me work in his lab, which allowed me to better understand the *in vitro* digestion process. Emerson and Snigdha provided helpful instructions during that time, and I feel grateful for their help.

I want to thank my previous and present lab mates: Erasme, Hollman, Jagrani, Kristina, Lei, Marissa, Mallory, Shuen. I would like to thank Hollman for his help. He is the person who introduced everything to me when I first came to the lab and helped me solved many problems with my research in these two years. I would also like to thank Shuen because although she only joined our lab half a year earlier than me, she is still a good model. She encouraged me many times when I was depressed and urged me to continue my study.

I would like to thank my boyfriend Yifan for his love. Thank you for taking care of my daily life, listening to all my words, supporting me, and making me happy every day. I would like to thank my friends Shengnan, Dongxu, Zijin, and Nannan. Their friendship helps me get through all the difficulties and keep optimism even though the situation is turning worse.

In the end, I would like to thank my family. Thank you for bringing me up and providing suggestions for each step in my life. Your love gives me the courage to face all the difficulties.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 MILLING, BAKING QUALITIES AND PROTEIN COMPOSITION OF HISTORICAL AND MODERN WHEAT

1.1. ABSTRACT

Wheat is a widely grown crop in temperate countries and is used for both human and livestock feed. Its popularity is based on its adaptability and high yield capacity, as well as the gluten protein fraction, which provides the viscoelastic properties that enable dough to be processed into bread, pasta, noodles, and other foods. Many controversies have arisen around whether modern wheat is harmful to humans compared with historical wheats. This review summarizes research on the differences between historical and modern wheat and discusses breeding efforts during the past century in an effort to better characterize the differential properties of modern versus historical wheats. In short, breeding efforts make historical and modern wheat very different, but no evidence shows that historical wheat is better than modern wheat for human health.

1.2. INTRODUCTION

Wheat is a staple food in many parts of the world. Over the past century, plant breeders have used traditional and modern breeding strategies to improve agronomic and end-use quality characteristics of wheat. For example, changes in kernel physical characteristics during breeding have been included in many different studies (Bordoni, Danesi, Nunzio, Taccari, & Valli, 2017; Dinu, Whittaker, Pagliai, Benedettelli, & Sofi, 2018; Kulathunga, Reuhs, Zwinger, & Simsek, 2021; Mefleh et al., 2019). Several findings suggest that the bread baking quality has improved successfully over the last century (Bassignana et al., 2015; Call et al., 2020; Guarda, Padovan, & Delogu, 2004; Konvalina, Bradova, Capouchova, Stehno, & Moudry, 2013).

Yet with the rise in prevalence of celiac disease, an allergic disease related to gluten protein (Shewry, Peter R., Pellny, & Lovegrove, 2016), as well as other diet-related public health concerns such as increases in obesity and type 2 diabetes, some consumers and researchers have speculated about whether some inadvertent changes have been made to modern wheats compared with historical wheats to cause these negative effects. Thus, many researchers are working on the changes in wheat during breeding in many ways. Many studies are working on how wheat proteins have changed and whether they should be blamed for negative health issues (Geisslitz, Longin, Scherf, & Koehler, 2019; Gulati, Brahma, Graybosch, Chen, & Rose, 2020; Lorgeril & Salen, 2014; Malalgoda, Ohm, Meinhardt, & Simsek, 2018; Prandi, Tedeschi, Folloni, Galaverna, & Sforza, 2017). Therefore, this review summarizes research on the differences between historical and modern wheat and discusses breeding efforts during the past century in an effort to better characterize the differential properties of modern versus historical wheats.

1.3. WHEAT

1.3.1. Historical and modern wheats

Wheat is one of the most significant cultivatable plants in terms of human nutrition today. The term "historical wheat" refers to kinds of wheat domesticated and grown by ancient civilizations generally. The earliest cultivated forms were diploid (genome AA) (einkorn) and tetraploid (genome AABB) (emmer) wheats (Shewry, P. R., 2009). *Triticum turgidum* L. spp. *dicoccum* Schrank ex Schubler (emmer wheat) is the ancient durum wheat that represented the transition from the wild tetraploid spp. *dicoccoides* (wild emmer wheat) to durum wheat (Mefleh et al., 2019). The expansion of emmer cultivation promoted hybridization with *Aegilops tauschii* (genome DD) and the emergence of the hexaploid bread wheat (*Triticum aestivum* L. subsp. *aestivum*, genome BBAADD) (Mastrangelo & Cattivelli, 2021). Unlike einkorn and emmer,

which originated from natural domestication, bread wheat has only existed in cultivation, having been created by hybridizing cultivated emmer with the unrelated wild grass *Triticum tauschii* (also called *Aegilops tauschii* and *Ae. squarosa*). This hybridization probably occurred several times independently with the novel hexaploid (genome AABBDD) being selected by farmers for its superior properties which caused the evolution of modern wheats (Shewry, 2009).

1.3.2. Wheat kernel

The wheat kernel is comprised of three essential constituents: bran, endosperm, and germ (Figure 1.1). The endosperm, which makes up 81-84% of the grain, is primarily made up of starch granules embedded in a protein matrix. The embryo and scutellum are contained in the germ, which makes up 2-3% of the grain. The aleurone layer is part of the endosperm but usually separated along with other bran layers which makes up the outer layers of grain during milling (Marchini et al., 2016).

1.3.3. Wheat protein

Generally, wheat protein can be divided into gluten and nongluten proteins. Gluten proteins in wheat are unique in that they can form a viscoelastic dough, which allows for the formation of a solid, cohesive dough that maintains gas and results in a light, aerated baked product. Gluten is made up of two major protein fractions, glutenin and gliadin, which work together to form gluten. Gliadin proteins are insoluble in water but can be extracted with aqueous alcohols (Delcour & Hoseney, 2010). Glutenin is insoluble in both water and aqueous alcohols but can be extracted into alcoholic solution by treating flour with a disulfide-reducing agent. High-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) are two types of glutenin subunits (Veraverbeke & Delcour, 2002). Both fractions contribute to the rheological properties of dough, although they serve different

purposes. Hydrated gliadins have less elasticity and are less cohesive than glutenins; they primarily contribute to the viscosity and extensibility of the dough system. Hydrated glutenins are both cohesive and elastic and are responsible for dough strength and elasticity (Wieser, 2007)

The amino acid composition of gluten is low in basic amino acids such as lysine but high in proline, accounting for around 14% of the protein. The amino acid composition of gluten proteins also reveals that hydrophobic side chains make up about 35% of the overall amino acids (Delcour & Hoseney, 2010). About 30% of gluten's amino acid residues are hydrophobic and these residues play an important role in the protein's capacity to form protein aggregates and bind lipids and other nonpolar substances by hydrophobic interactions. The water binding capabilities of gluten are due to its high glutamine and hydroxyl amino acid contents. Furthermore, the cohesion–adhesion properties of gluten polypeptides are enhanced by hydrogen bonding between glutamine and hydroxyl residues. Cysteine and cystine residues account for 2–3 mol% of the total amino acid residues in gluten. These residues conduct sulfhydryl–disulfide interchange reactions, resulting in extensive polymerization of gluten proteins (Damodaran, Parkin, & Fennema, 2007).

Researchers over the world focused on the functional properties of wheat protein. For example, they investigated solubility, foaming capability, water holding capacity, fat absorption capacity, and emulsifying capacity (Ahmedna, Prinyawiwatkul, & Rao, 1999; Krull & Wall, 1969; Veraverbeke & Delcour, 2002). Previous research also reported the role of wheat protein quality in baking (Macritchie, 1984; Tronsmo, Færgestad, Schofield, & Magnus, 2003; Veraverbeke & Delcour, 2002). Gluten gives dough its coherent and viscoelastic property, which is necessary to produce leavened products, especially bread. The molecular weight distribution of

the gluten protein controls the rheological properties of dough, as it does with polymer structures in general.

In those factors that may make historical and modern wheat different, protein is an exciting field to study because it not only can relate with milling quality and baking quality, but also can influence human health directly. When combined with water, modern wheat flour can produce a more viscoelastic dough because of its protein composition. On the other hand, the weak gluten quality in historical wheat flours results in softer dough with low elasticity and high extensibility (Geisslitz et al., 2019). Thus, modern wheat is better for bread making. Malalgoda et al. (2018) showed that breeding efforts improved dough properties, which could be linked to quantitative variation in glutenin polymeric proteins and certain subfractions of ω-gliadins. To determine whether wheat breeding led to the rise of celiac disease prevalence, Broeck et al. (2010) compared the genetic diversity of gluten proteins for the existence of two celiac disease epitopes. They did not find a clear difference between historical wheat and modern wheat protein because they discovered that one of the celiac disease epitopes is higher in modern wheat and the other one is lower, and that some modern cultivars have reduced levels of both epitopesIn addition, the impact of breeding on the protein composition was also investigated. Call et al. found that historical wheat did not contain less immunotoxic components than modern wheat (Call et al., 2020). Similar results can be found from many other studies (Malalgoda, Meinhardt, & Simsek, 2018; Prandi et al., 2017; Pronin, Börner, & Scherf, 2021). All of these reports indicate that historical wheat is not better for celiac disease sufferers. Breeding efforts have improved wheat gluten quality and increased high molecular weight protein content which could have an impact on digestibility (Santis et al., 2017). Protein digestibility has been shown to increase with release year. High molecular weight protein fractions increase, and low molecular

weight protein fractions decreased with release year (Gulati et al., 2020). A cluster analysis of historical and modern wheat based on parentage has shown that over the last 100 years of hard red spring wheat breeding, the dough mixing and bread making quality have been improved without changing the composition of gliadin protein (Malalgoda, Ohm, Meinhardt, Chao, & Simsek, 2017).

1.4. CONTROVERSY AROUND HISTORICAL AND MODERN WHEAT

There have been many controversies around historical and modern wheat in the past decade. Internet search records about the health effects of wheat have increased during the last two decades (Shewry et al., 2016). An apparent shopping trend is that gluten-free food products have become increasingly popular in recent years, even with customers who do not have a medical need opting for gluten-free diets (Pellegrini & Agostoni, 2015).

In the last 50 years, the prevalence of coeliac disease in the United States has increased by a factor of 4-5. Some specialists believe that is because of the use of modern wheat, but some researchers are trying to prove that celiac disease has not become more common due to modern wheat varieties (Ribeiro & Nunes, 2019). There is some hypothesis that breeding may have unwittingly modified the immunoreactive potential of wheat, which might explain the rising frequency of celiac disease in recent decades (Singh et al., 2018). Pronin et al. (2021) selected four celiac disease active peptides and quantitated their content in historical and modern wheat. They concluded that historical and modern wheat tend to have similar immunoreactive potential and that the harvest year (i.e., environmental conditions during growing) had a greater impact on the concentrations of celiac disease-active peptides than the cultivar

According to a number of studies, historical wheat may provide health benefits when compared to modern bread wheat (Lachman, Hejtmánková, & Kotíková, 2013; Ruibal-Mendieta

et al., 2005). In recent years, there has been increasing interest in historical wheats due to claims that they are rich sources of bioactive components and hence appropriate for making high-value food products with improved health benefits (Shewry, Peter, 2018). In a clinical experiment, the effects of adding historical wheat (Khorasan wheat) and modern wheat in human diet were compared. Results showed that adding historical wheat to the diet improved 24-hour systolic blood pressure, endothelium reactivity, fasting triglycerides, and glucose levels in healthy volunteers with inadequate blood pressure control (Cicero et al., 2018).

On the other hand, many researchers believe there is no advantage of historical wheat. They insist historical and modern wheat have similar health benefits, and modern wheat is even better. According to a Belgian research group, after being processed into bread, modern wheat was still equally nutritious as historical wheat. Nutritional variations can be found at the kernel level: modern wheat contains more total carbohydrates, protein, minerals, and fat. However, when nutrition characteristics for consumable bread are assessed, only slight variances can be found in terms of nutritional content. Historical wheat bread contains more protein, but no evidence showed that historical wheat was more healthy than modern wheat (Boxstael et al., 2020).

Based on all those controversies, many researchers are committed to determining how modern cultivars have changed during breeding and their relationship with human health. They have researched many different factors such as kernel physical quality, milling quality, baking quality, protein component, nutrients, and so on (Boxstael et al., 2020; Gulati et al., 2020; Kiszonas & Morris, 2018; Lovegrove et al., 2020; Murphy, Reeves, & Jones, 2008; Pronin et al., 2021; Simsek, Budak, Schwebach, & Ovando-Martínez, 2019; Singh et al., 2018). There were some trends with regards to historical versus modern wheats: there was a decreasing trend for

most amino acids, and a rising trend for dietary fiber (arabinoxylan), soluble sugars (particularly sucrose, maltose, and fructose), and betaine (Lovegrove et al., 2020). Simsek et al. (2019) found minerals, such as phosphorus, potassium, and zinc were significantly correlated with wheat cultivar release year among genotypes

1.5. WHEAT KERNEL PHYSICAL AND MILLING QUALITY

Wheat can be milled into refined flour to produce white bread with high volume (Posner & Hibbs, 2005). One of the primary goals of wheat flour milling is to remove the bran. Because it disrupts the gluten-starch network, it will negatively impact white bread quality, such as loaf volume, texture, color, and flavor (Gan, Galliard, Ellis, Angold, & Vaughan, 1992). To mill the kernel, it is first cleaned to eliminate any non-wheat components. Wheat is then tempered by adding water and letting it sit for a period of time. This can help toughen the bran while softening the endosperm, making grinding easier (Kweon, Martin, & Souza, 2009). The grinding may then occur in a roller mill, which uses pairs of rollers rotating in opposite directions to grind the material.

Many factors can be used to evaluate milling quality. The most common evaluation factor is milling yield which is the edible flour yield when milled by the usual roller process. Since flour is the most valuable milling product, wheat varieties that produce the highest proportions of flour have the highest economic value. Many factors can influence milling yield, such as kernel hardness, shape, size, and mill type (Baasandorj, Ohm, Manthey, & Simsek, 2015; Marshall, Mares, Moss, & Ellison, 1986). Large kernels can yield more flour than small kernels, but small kernels can help improve the quality of bread flour (Gaines, Finney, & Andrews, 1997).

Changes in kernel physical characteristics and milling quality during breeding have been studied (Bordoni et al., 2017; Dinu et al., 2018; Kulathunga et al., 2021; Mefleh et al., 2019).

According to the International Center for Maize and Wheat Improvement, wheat milling quality has been an important component in improving the end-use quality of all bread wheat products worldwide. A comparison study of the physical properties of historical and modern bread wheat investigated kernel weight, hardness, and diameter. According to the findings, old cultivars were usually softer than the modern cultivars (Cetiner, Tömösközi, Török, Salantur, & Koksel, 2020). A similar study looked at the kernel quality and chemical composition of historical and modern wheat which found that historical wheat had lower kernel weight, hardness, and protein content, and higher total dietary fiber content (Kulathunga et al., 2021). Rozo-Ortega et al. (2021) studied the changes in milling and bread making quality parameters in historical and modern wheat in the presence of foliar diseases. Foliar diseases had a stronger negative effect on modern cultivars than old cultivars. The negative effect is reduced the protein content in grain and probably associated with higher yield and lower source: sink ratio respect to the old cultivars.

1.6. BREAD BAKING QUALITY

Bread is the most popular wheat flour product in our daily life because wheat flour can make dough when combined with water. Furthermore, the gas produced during fermentation or chemical leavening can be retained in wheat dough resulting in a leavened product. The popularity of wheat products is due to these features of wheat flour dough (Delcour & Hoseney, 2010). There are a lot of studies that reported improving bread baking quality of wheat flour, which includes dough properties, loaf volume, loaf texture, surface color, crumb color, and uniformity of the crumb (Lacko-Bartošová & Korczyk-Szabó, 2011; Macritchie, 1978; Macritchie, 1984; Therdthai, Zhou, & Adamczak, 2002; Zhang & Datta, 2006).

Baking quality depends heavily on wheat protein quality and wheat protein content. Protein quality relates to the composition of glutenin and gliadins, the relative proportions of

different protein classes, and the molecular weight distribution of the glutenin polymers. It will affect the loaf volume, shape ratio, crumb and crust structure. Protein quality can be measured using dough property and protein size distribution measurements (Tronsmo et al., 2003). As mentioned before, changes in wheat proteins during breeding may influence bread baking quality. Several findings suggest that the bread baking quality has improved successfully over the last century (Bassignana et al., 2015; Call et al., 2020; Guarda et al., 2004; Konvalina et al., 2013). The cultivar release year has a significant impact on dough properties. Historical flour has higher amounts of protein but lower glutenin, which is the major gluten protein fraction that is a suitable predictor for baking volume. Historical wheat dough can reach the maximum viscosity quickly; thus, it is characterized by low consistency and poor resistance to over mixing (Geisslitz et al., 2019). In general, breeding efforts enhanced dough properties, which could be linked to quantitative variations in glutenin polymeric proteins and ω-gliadin subfractions (Malalgoda et al., 2018). Konvalina et al. (2013) performed an experiment on the baking quality of historical and modern wheat varieties. They found that the historical wheat cultivars are a valuable material with high protein content and may be more suitable for nonyeast-leavened products, such as pasta, biscuits, and so on, than modern wheat. Six historical wheats were chosen to compare with the modern commercial wheat for milling, rheological, and bread making performances in Italy. Compared with modern wheat, the bread made by historical wheat exhibited an overall lower specific volume (loaf weight divided by loaf volume) and lower firmness (Bassignana et al., 2015).

1.7. CONCLUSION

The difference between historical and modern wheat and which is better for human health have been studied extensively. In conclusion, modern wheat has changed from historical wheat

in terms of milling quality such as hardness, size, and milling yield. Modern wheat has better baking performance with enhanced dough properties and greater loaf volume compared with historical wheat. As an important factor that affects baking quality, protein content is higher in historical wheat but with lower glutenin content. Though celiac disease cases are increasing recently, many studies indicate that historical wheat is not better for celiac disease suffers.

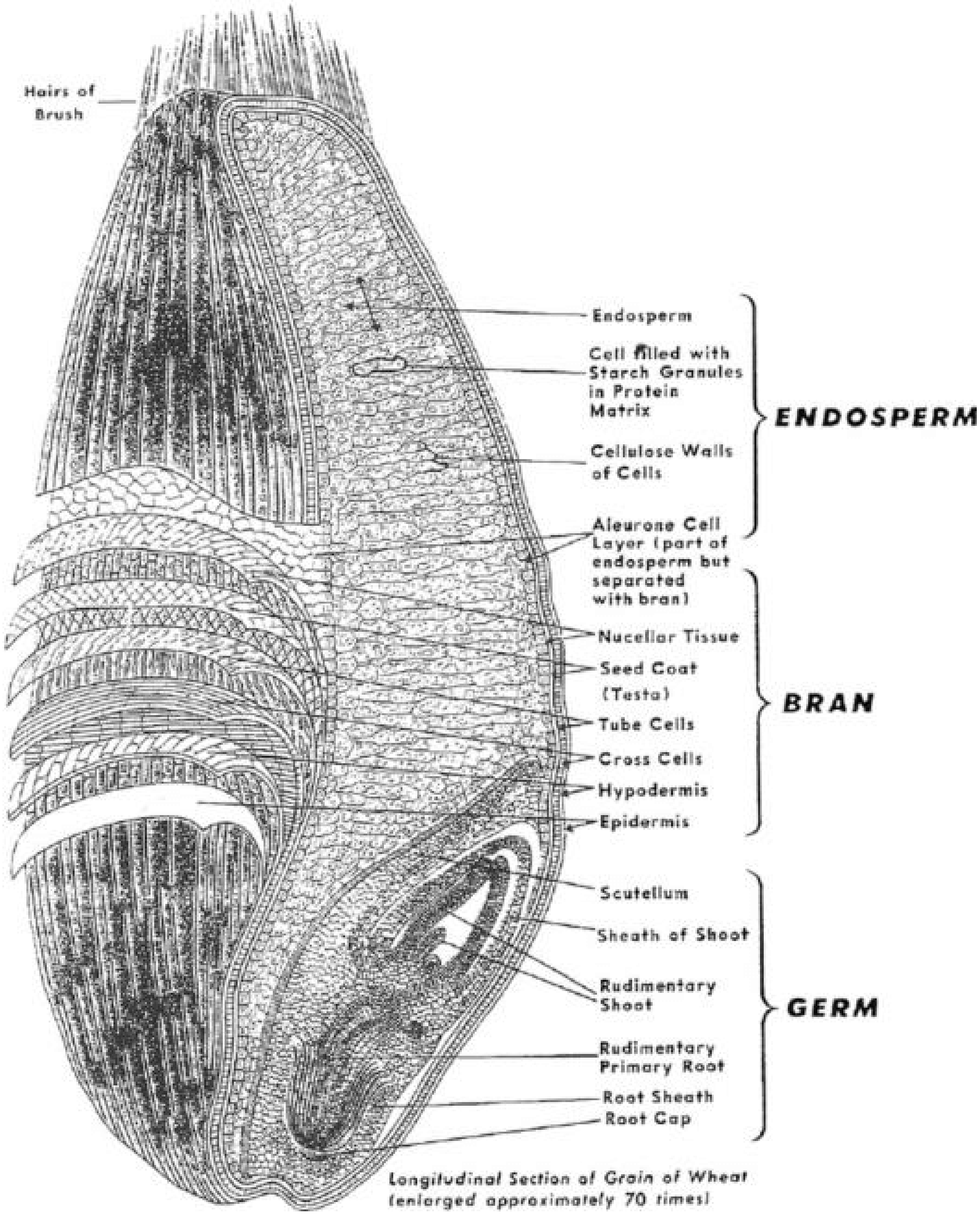

Figure 1.1. Typical wheat kernel structure and composition (Marchini et al., 2016).

1.8. REFERENCES

Ahmedna, M., Prinyawiwatkul, W., & Rao, R. M. (1999). Solubilized wheat protein isolate: Functional properties and potential food applications. Journal of Agricultural and Food Chemistry, 47(4), 1340-1345. doi:10.1021/jf981098s

Baasandorj, T., Ohm, J., Manthey, F., & Simsek, S. (2015). Effect of kernel size and mill type on protein, milling yield, and baking quality of hard red spring wheat. *Cereal Chemistry, 92*(1), 81-87. doi:https://doi.org/10.1094/CCHEM-12-13-0259-R

Bassignana, M., Arlian, D., Marti, A., Morandin, F., Zanoletti, M., & Pagani, M. (2015). Characterization of ancient wheat varieties and evaluation of their bread-making performances.

Bordoni, A., Danesi, F., Nunzio, M. D., Taccari, A., & Valli, V. (2017). Ancient wheat and health: A legend or the reality? A review on KAMUT khorasan wheat. *International Journal of Food Sciences and Nutrition, 68*(3), 278-286. doi:10.1080/09637486.2016.1247434

Boxstael, F. V., Aerts, H., Linssen, S., Latré, J., Christiaens, A., Haesaert, G., . . . Keyzer, W. D. (2020). A comparison of the nutritional value of einkorn, emmer, khorasan and modern wheat: Whole grains, processed in bread, and population-level intake implications. *Journal of the Science of Food and Agriculture, 100*(11), 4108-4118. doi:https://doi.org/10.1002/jsfa.10402

Broeck, H., Jong, H., Salentijn, E., Dekking, L., Bosch, D., Hamer, R., . . . Smulders, M. (2010). Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: Wheat breeding may have contributed to increased prevalence of celiac disease. *Theoretical and Applied Genetics, 121*(8), 1527-1539. doi:10.1007/s00122-010-1408-4

Call, L., Kapeller, M., Grausgruber, H., Reiter, E., Schoenlechner, R., & D'Amico, S. (2020). Effects of species and breeding on wheat protein composition. *Journal of Cereal Science, 93*, 102974. doi:10.1016/j.jcs.2020.102974

Cetiner, B., Tömösközi, S., Török, K., Salantur, A., & Koksel, H. (2020). Comparison of the arabinoxylan composition and physical properties of old and modern bread wheat (triticum aestivum L.) and landraces genotypes. *Cereal Chemistry, 97*(2), 505-514. doi:https://doi.org/10.1002/cche.10265

Cicero, A. F. G., Fogacci, F., Veronesi, M., Grandi, E., Dinelli, G., Hrelia, S., & Borghi, C. (2018). Short-term hemodynamic effects of modern wheat products substitution in diet with ancient wheat products: A cross-over, randomized clinical trial. *Nutrients, 10*(11), 1666. doi:10.3390/nu10111666

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2007). *Fennema's food chemistry* CRC Press.

Delcour, J. A., & Hoseney, R. C. (2010). *Principles of cereal science and technology, third edition* (3rd ed.) American Association of Cereal Chemists.

Dinu, M., Whittaker, A., Pagliai, G., Benedettelli, S., & Sofi, F. (2018). Ancient wheat species and human health: Biochemical and clinical implications. *The Journal of Nutritional Biochemistry, 52*, 1-9. doi:10.1016/j.jnutbio.2017.09.001

Gaines, C. S., Finney, P. L., & Andrews, L. C. (1997). Influence of kernel size and shriveling on soft wheat milling and baking quality. *Cereal Chemistry, 74*(6), 700-704. doi:https://doi.org/10.1094/CCHEM.1997.74.6.700

Gan, Z., Galliard, T., Ellis, P. R., Angold, R. E., & Vaughan, J. G. (1992). Effect of the outer bran layers on the loaf volume of wheat bread. *Journal of Cereal Science, 15*(2), 151-163. doi:10.1016/S0733-5210(09)80066-0

Geisslitz, S., Longin, C. F. H., Scherf, K. A., & Koehler, P. (2019). Comparative study on gluten protein composition of ancient (einkorn, emmer and spelt) and modern wheat species (durum and common wheat). *Foods, 8*(9), 409. doi:10.3390/foods8090409

Guarda, G., Padovan, S., & Delogu, G. (2004). Grain yield, nitrogen-use efficiency and baking quality of old and modern italian bread-wheat cultivars grown at different nitrogen levels. *European Journal of Agronomy, 21*(2), 181-192. doi:10.1016/j.eja.2003.08.001

Gulati, P., Brahma, S., Graybosch, R. A., Chen, Y., & Rose, D. J. (2020). In vitro digestibility of proteins from historical and modern wheat cultivars. *Journal of the Science of Food and Agriculture, 100*(6), 2579-2584. doi:https://doi.org/10.1002/jsfa.10283

Kiszonas, A. M., & Morris, C. F. (2018). Wheat breeding for quality: A historical review. *Cereal Chemistry, 95*(1), 17-34. doi:https://doi.org/10.1094/CCHEM-05-17-0103-FI

Konvalina, P., Bradova, J., Capouchova, I., Stehno, Z., & Moudry, J. s. (2013). Baking quality and high molecular weight glutenin subunit composition of emmer wheat, old and new varieties of bread wheat.(30)

Krull, L. H., & Wall, J. S. (1969). Relationship of amino acid composition and wheat protein properties. Retrieved from https://pubag.nal.usda.gov/catalog/32066

Kulathunga, Reuhs, Zwinger, & Simsek. (2021). *Comparative study on kernel quality and chemical composition of ancient and modern wheat species: Einkorn, emmer, spelt and hard red spring wheat* MDPI AG. doi:10.3390/foods10040761

Kweon, M., Martin, R., & Souza, E. (2009). Effect of tempering conditions on milling performance and flour functionality. *Cereal Chemistry, 86*(1), 12-17. doi:https://doi.org/10.1094/CCHEM-86-1-0012

Lachman, J., Hejtmánková, K., & Kotíková, Z. (2013). Tocols and carotenoids of einkorn, emmer and spring wheat varieties: Selection for breeding and production. *Journal of Cereal Science, 57*(2), 207-214. doi:10.1016/j.jcs.2012.05.011

Lacko-Bartošová, M., & Korczyk-Szabó, J. (2011). Indirect baking quality and rheological properties of spelt wheat (triticum spelta l.). *Research Journal of Agricultural Science, 43*(1)

Lorgeril, M. d., & Salen, P. (2014). Gluten and wheat intolerance today: Are modern wheat strains involved? *International Journal of Food Sciences and Nutrition, 65*(5), 577-581. doi:10.3109/09637486.2014.886185

Lovegrove, A., Pellny, T. K., Hassall, K. L., Plummer, A., Wood, A., Bellisai, A., . . . Shewry, P. R. (2020). Historical changes in the contents and compositions of fibre components and polar metabolites in white wheat flour. *Scientific Reports, 10*(1), 5920. doi:10.1038/s41598-020-62777-3

Macritchie, F. (1978). Differences in baking quality between wheat flours. *International Journal of Food Science & Technology, 13*(3), 187-194. doi:https://doi.org/10.1111/j.1365-2621.1978.tb00794.x

Macritchie, F. (1984). Baking quality of wheat flours. In C. O. Chichester, E. M. Mrak & B. S. Schweigert (Eds.), *Advances in food research* (pp. 201-277) Academic Press. Retrieved from https://www.sciencedirect.com/science/article/pii/S0065262808600580

Malalgoda, M., Meinhardt, S. W., & Simsek, S. (2018). Detection and quantitation of immunogenic epitopes related to celiac disease in historical and modern hard red spring wheat cultivars. *Food Chemistry, 264*, 101-107. Retrieved from https://www.sciencedirect.com/science/article/pii/S0308814618307799

Malalgoda, M., Ohm, J., Meinhardt, S., Chao, S., & Simsek, S. (2017). Cluster analysis of historical and modern hard red spring wheat cultivars based on parentage and HPLC analysis of gluten-forming proteins. *Cereal Chemistry, 94*(3), 560-567. doi:https://doi.org/10.1094/CCHEM-08-16-0223-R

Malalgoda, M., Ohm, J., Meinhardt, S., & Simsek, S. (2018). Association between gluten protein composition and breadmaking quality characteristics in historical and modern spring wheat. *Cereal Chemistry, 95*(2), 226-238. doi:https://doi.org/10.1002/cche.10014

Marchini, D., Bottani, E., Tagliavini, G., Zurich, E., Marchini, D., Montanari, R., . . . Digiuni, S. (2016). *Lifecycle modelling of an innovative durum wheat debranner lifecycle modelling of an innovative durum wheat debranner*

Marshall, D. R., Mares, D. J., Moss, H. J., & Ellison, F. W. (1986). Effects of grain shape and size on milling yields in wheat. II. experimental studies. *Australian Journal of Agricultural Research, 37*(4), 331-342. doi:10.1071/ar9860331

Mastrangelo, A. M., & Cattivelli, L. (2021). What makes bread and durum wheat different? *Trends in Plant Science, 26*(7), 677-684. doi:10.1016/j.tplants.2021.01.004

Mefleh, M., Conte, P., Fadda, C., Giunta, F., Piga, A., Hassoun, G., & Motzo, R. (2019). From ancient to old and modern durum wheat varieties: Interaction among cultivar traits, management, and technological quality. *Journal of the Science of Food and Agriculture, 99*(5), 2059-2067. doi:https://doi.org/10.1002/jsfa.9388

Murphy, K., Reeves, P., & Jones, S. (2008). Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. *Euphytica, 163*(3), 381-390. doi:10.1007/s10681-008-9681-x

Pellegrini, N., & Agostoni, C. (2015). Nutritional aspects of gluten-free products. *Journal of the Science of Food and Agriculture, 95*(12), 2380-2385. doi:10.1002/jsfa.7101

Posner, E. S., & Hibbs, A. N. (2005). Wheat flour milling. *Wheat Flour Milling.,* (Ed.2) Retrieved from https://www.cabdirect.org/cabdirect/abstract/20043213266

Prandi, B., Tedeschi, T., Folloni, S., Galaverna, G., & Sforza, S. (2017). Peptides from gluten digestion: A comparison between old and modern wheat varieties. *Food Research International, 91*, 92-102. doi:10.1016/j.foodres.2016.11.034

Pronin, D., Börner, A., & Scherf, K. A. (2021). Old and modern wheat (triticum aestivum L.) cultivars and their potential to elicit celiac disease. *Food Chemistry, 339*, 127952. doi:10.1016/j.foodchem.2020.127952

Ribeiro, M., & Nunes, F. M. (2019). We might have got it wrong: Modern wheat is not more toxic for celiac patients. *Food Chemistry, 278*, 820-822. doi:10.1016/j.foodchem.2018.12.003

Rozo-Ortega, G. P., Serrago, R. A., Lo Valvo, P. J., Fleitas, M. C., Simón, M. R., & Miralles, D. J. (2021). Grain yield, milling and breadmaking quality responses to foliar diseases in old and modern argentinean wheat cultivars. *Journal of Cereal Science, 99*, 103211. doi:10.1016/j.jcs.2021.103211

Ruibal-Mendieta, N. L., Delacroix, D. L., Mignolet, E., Pycke, J., Marques, C., Rozenberg, R., . . . Larondelle, Y. (2005). Spelt (triticum aestivum ssp. spelta) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of Agricultural and Food Chemistry, 53*(7), 2751-2759. doi:10.1021/jf048506e

Santis, M. A. D., Giuliani, M. M., Giuzio, L., Vita, P. D., Lovegrove, A., Shewry, P. R., & Flagella, Z. (2017). Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in italy. *European Journal of Agronomy, 87*, 19-29. doi:10.1016/j.eja.2017.04.003

Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany, 60*(6), 1537-1553. doi:10.1093/jxb/erp058

Shewry, P. R. (2018). Do ancient types of wheat have health benefits compared with modern bread wheat? *Journal of Cereal Science, 79*, 469-476. doi:10.1016/j.jcs.2017.11.010

Shewry, P. R., Pellny, T. K., & Lovegrove, A. (2016). Is modern wheat bad for health? *Nature Plants, 2*(7), 16097. doi:10.1038/nplants.2016.97

Simsek, S., Budak, B., Schwebach, C. S., & Ovando-Martínez, M. (2019). Historical vs. modern hard red spring wheat: Analysis of the chemical composition. *Cereal Chemistry, 96*(5), 937-949. doi:https://doi.org/10.1002/cche.10198

Singh, P., Arora, A., Strand, T. A., Leffler, D. A., Catassi, C., Green, P. H., . . . Makharia, G. K. (2018). Global prevalence of celiac disease: Systematic review and Meta-analysis. *Clinical Gastroenterology and Hepatology, 16*(6), 823-836.e2. doi:10.1016/j.cgh.2017.06.037

Therdthai, N., Zhou, W., & Adamczak, T. (2002). Optimisation of the temperature profile in bread baking. *Journal of Food Engineering, 55*(1), 41-48. doi:10.1016/S0260-8774(01)00240-0

Tronsmo, K. M., Færgestad, E. M., Schofield, J. D., & Magnus, E. M. (2003). Wheat protein quality in relation to baking performance evaluated by the chorleywood bread process and a hearth bread baking test. *Journal of Cereal Science, 38*(2), 205-215. doi:10.1016/S0733-5210(03)00027-4

Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition, 42*(3), 179-208. doi:10.1080/10408690290825510

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology, 24*, 115-119. Retrieved from https://agris.fao.org/agris-search/search.do?recordID=US201301105004

Zhang, J., & Datta, A. K. (2006). Mathematical modeling of bread baking process. *Journal of Food Engineering, 75*(1), 78-89. doi:10.1016/j.jfoodeng.2005.03.058

CHAPTER 2 END-USE QUALITY OF HISTORICAL AND MODERN WHEAT CULTIVARS

2.1. ABSTRACT

Improving milling and baking properties is important during wheat breeding. To determine changes in milling and baking quality of hard winter wheat, 23 adapted cultivars released in the Great Plains between 1870 and 2013 were grown in triplicate in a single location (Mead, NE USA) over two crop years (2018 and 2019). Kernel hardness index and moisture content increased by release year. The observed increase in hardness index was accompanied by a decrease in percent soft kernels and an increase in precent semi-hard kernels. A decreasing trend was observed for hardness index standard deviation in 2018. Although diameter and weight did not change with the release year, their standard deviation increased with the release year. Flour protein content decreased with release year and mixing quality increased. No significant relationship was found for baking property variables, but bran water retention capacity (BWRC), which is correlated with whole wheat bread quality, increased with release year. In conclusion, wheat kernels have become harder, moister, more uniform in hardness but more variable in shape over a century of breeding; mixing quality showed significant improvements and loaf volume and firmness remained constant, even in the presence of a strong decrease in protein concentration. Bran quality decreased across release year, which may have implications for whole grain baking quality and milling productivity.

2.2. INTRODUCTION

For nearly a century, modern breeding efforts have been applied to wheat to improve yield, disease resistance, end-use quality, and other factors (Bockus et al., 2011; Guarda et al., 2004; Kulathunga et al., 2021; Mefleh et al., 2019). Although buffered by the polyploid nature of

wheat, modern wheat has changed considerably from its ancestors. For example, modern wheat improved in spike shattering, wind-scattering, and harvesting compared to historical cultivars (Dubcovsky & Dvorak, 2007).

Many studies show there is a relationship between wheat kernel physical properties and milling quality. For instance, the most important milling property is milling yield. A recent study about the effect of kernel size and milling yield and baking quality reported that small kernels contribute to enhancing the quality of bread making but have a negative effect on milling yield (Baasandorj et al., 2015). Sutton et al. also showed that as wheat kernel size increased, flour yield increased. (Sutton et al., 1992). The effect of wheat kernel size on milling yield may be because the forces for large kernel during grinding are larger. Thus, small kernels are more difficult to grind than large kernels (Dziki & Laskowski, 2004). Kernel size uniformity is critical in wheat milling industry since it is difficult to identify the best machine operating parameters for varied kernel sizes. In the presence of large kernels, small kernels pass through the roller mills unground or merely partially broken (Dziki & Laskowski, 2005; Yoon et al., 2002). Kernel hardness can also influence milling process. For example, harder kernels require more energy to mill (Dziki & Laskowski, 2005). Many studies suggested that uniformity of kernel hardness is desirable for good milling performance (Campbell et al., 2007; Ohm et al., 1998).

Bran friability and bran water retention capacity (BWRC) are two new wheat quality measurements use to assess the potential quality of whole wheat flour. To assess bran friability, the proportion of bran retained on a no. 20 sieve relative to that retained on a no. 60 sieve was measured and termed “bran-friability”. In whole grain milling and baking application, bran friability and BWRC affect wheat bran functionality. Higher bran friability indicates lower whole wheat bread specific volume (Seyer & Gélinas, 2009). BWRC is the weight of water

retained by bran after centrifugation and is negatively correlated with whole grain baking quality (Navrotskyi et al., 2020).

Baking quality includes flour mixing properties and bread loaf quality. During the wheat breeding process, milling, and baking properties are analyzed and considered important for breeding decisions. Several studies have examined how breeding has affected these quality parameters over time (Bassignana et al., 2015; Call et al., 2020; Guzman et al., 2016; Hucl et al., 2015; Kulathunga et al., 2021; Malalgoda et al., 2018; Morgounov et al., 2013; Underdahl et al., 2008). Compared with historical wheats, modern wheats improved in grain yield and physical dough quality and stability (Morgounov et al., 2013; Underdahl et al., 2008). Kernel quality and chemical composition have been compared between ancient and modern wheat species. It was reported that historical cultivars are very different compared with modern hard red spring wheat, with modern wheat being harder and heavier in test weight (Kulathunga et al., 2021). Milling, rheological and bread-making performances of six historical wheat varieties have been investigated and compared with common Italian wheat. Compared with modern wheat, the bread made by historical wheat exhibited an overall lower specific volume (loaf weight divided by loaf volume) and lower firmness (Bassignana et al., 2015). Kernel weight, grain protein concentration, sodium dodecyl sulfate sedimentation volume, farinograph absorption, and dough development time all rose over time, indicating that Canada western red spring wheat has made progress in key agronomic and end-use traits (Hucl et al., 2015). Breeding efforts improved dough properties, such as farinograph peak time and stability, which could be associated with quantitative variations in glutenin polymeric proteins, and certain subfractions of ω-gliadins (Malalgoda et al., 2018).

While these studies report numerous significant improvements in functionality of modern wheat compared with historical cultivars, there is little information on the changes that have occurred in winter wheats adapted to the Great Plains of the US. Therefore, this study aimed to assess the quality of winter wheat cultivars adapted to this region. We analyzed the most important kernel physical characteristics, milling, mixing, and baking qualities to determine how wheat changed during breeding and how the different quality variables were associated.

2.3. MATERIALS AND METHODS

2.3.1. Materials

Twenty-three hard winter wheat cultivars released in the US between 1870 and 2013 were grown at the University of Nebraska Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE USA and harvested in 2018 and 2019 (Table 2.1.). Before sowing, 1 m^2 plots were prepared by applying a nitrogen fertilizer (90 kg/ha). Materials were planted in a randomized complete block design with three replications (field replicates) with the exceptions of ‘Anton’ with 4 replications and ‘Wesley’ with 2 replications in the 2019 harvest year (2018 planting). The weather data including precipitation and temperature was obtained from the *High Plains Regional Climate Center* (2020).

2.3.2. Kernel quality

2.3.2.1. Single kernel characterization system (SKCS)

Kernel physical characteristics were recorded using a single kernel characterization system (SKCS4100, Perten, Sweden). Kernel hardness index, hardness distribution, kernel moisture, kernel diameter, kernel weight, and their standard deviations were recorded following the manufacturer’s instructions.

2.3.2.2. Milling quality

Kernels were tempered and milled using a Quadrumat Jr laboratory mill (Cereals & Grains Association, Method 26-10.02). All samples were tempered at 15% moisture content overnight before milling. Flour and bran were separated using a no. 70 sieve with 212 µm openings. Milling yield was calculated as the weight of flour recovered divided by the weight of the starting wheat.

2.3.3. Flour quality

2.3.3.1. Flour properties

Moisture content of flour was determined following (Cereals & Grains Association, Method 44-15.02). Flour protein concentration was measured by a nitrogen analyzer (FP 528, Leco, St Joseph, MI, USA) with a nitrogen conversion factor of 5.7 (Cereals & Grains Association, Method 46-30.01).

2.3.3.2. Mixing properties

Flour mixing quality was assessed using a Mixograph (National, Lincoln, NE, USA) (Cereals & Grains Association, Method 54-40.02). Midline peak time (MPT), midline peak value (MPV), midline peak width (MPW), midline right slope (MRS) and midline time max area (MTA) were recorded from the Mixograph to evaluate the mixing quality of white flour.

2.3.4. Baking quality

2.3.4.1. Baking process

Bread was baked according to a standard straight-dough method using 30 g of flour and a fermentation time of 90 min (Cereals & Grains Association, Method 10-10.03). The mixing time and water absorption of flours were determined from the Mixograph results. Baked bread was cooled to room temperature for 1-4 h before further testing.

2.3.4.2. Loaf texture, volume, and specific volume

Loaf volume and specific volume (cm^3/g) was determined by the rapeseed displacement method (Cereals & Grains Association, Method 10-05.01). Firmness was obtained using a texture analyzer equipped with a 1 cm cylindrical probe and a 2 kg load cell (TA-XT2, New York, USA) (Cereals & Grains Association, Method 56-36.01).

2.3.5. Bran quality

2.3.5.1. Bran friability

Friability of bran was measured by sieving the bran obtained after milling through two testing sieves stacked on top of each other for 60 s (no. 20 and no. 60 containing 850 µm and 250 µm openings, respectively). Friability was calculated as the weight fraction of fine bran remaining on sieve No. 60 (fine bran particles) divided by the combined weights of bran on the No. 20 and No. 60 sieves (coarse and fine bran particles), expressed as a percentage (Navrotskyi, S. et al., 2019).

2.3.5.2. Bran water retention capacity (BWRC)

Water retention capacity of bran was obtained as described by Navrotskyi et al. (Navrotskyi et al., 2019). In short, 1 g of bran was mixed with 5 mL of water. After vortex mixing for 5 s, samples were shaken on a horizontal shaking platform at room temperature and 100 rpm for 20 min. Then, samples were centrifuged at 1000 x g for 15 min and the supernatant was discarded. After draining the pellet upside down over paper towels for 10min, the weight of the wet pellet was recorded. BWRC was calculated as the ratio of the weight of the wet pellet by the weight of dry bran, expressed as a percentage.

2.3.6. Statistical analysis

Data were analyzed using a mixed model two-way ANOVA with interaction. The main effects were the release year of cultivars and harvest year. Release year was modelled as a continuous variable and harvest year was a fixed variable. Replication nested within planting year was a random effect. Statistical significance was determined by $p<0.05$.

Partial correlation using harvest year as the partial variable was used to determine the relationships among variables. All statistics were performed using SAS software (version 9.4, Cary, NC USA). Data were plotted using the 'ggplot2', 'cowplot', and 'corrplot' packages in R (version 4.0.3) (Claus, 2019; R Core Team., 2020; Wei & Simko, 2017; Wickham, 2016).

2.4. RESULTS AND DISCUSSION

This study aimed to assess changes in wheat kernel physical characteristics, milling quality, mixing quality, and baking quality of hard winter wheat cultivars adapted to the Great Plains of the US during the past century of breeding. We compared our results with those obtained in previous studies using different market classes of wheat or adaptation environments.

The cultivars used in this study originated from the US states of Kansas, Nebraska, Texas, and Oklahoma and ranged in release years from 1870 to 2013 (Table 2.1). They were selected based on their relevance for grain production during their time, known adaptation to the climatic conditions of the study location, and their contribution to the pedigrees of modern genotypes widely grown in the Great Plains today.

The environmental conditions varied between harvest years (Figure 2.1). In 2019, conditions were not as favorable for wheat production due to the cold winter (with little snow

cover). Additionally, ample rain early in the spring gave way to excessive dryness during grain filling. This may explain some of the differing trends across release years between the two planting years as described hereafter (Table 2.2).

2.4.1. Kernel quality

Kernel physical characteristics included the mean and standard deviation of kernel hardness index, moisture content, diameter, weight, and hardness distribution. Kernel texture (hardness index, % soft kernels, and % semi-hard kernels) varied by release year with no interaction with harvest year (Table 2.2). Scatterplots of the least-squares means of these variables indicated that kernels from modern cultivars tended to be harder (Figure 2.2).

Similar results were reported in another study of spring wheat time (Malalgoda et al., 2018). Kernel hardness is an important milling parameter that should be measured before milling. Kernel hardness is significant to the milling process because kernel texture can impact the power consumption during milling, with harder kernels requiring more power (Dziki & Laskowski, 2005). Hard kernels also require a longer tempering time and need to reach a higher moisture content before milling than soft kernels. Harder wheat is more difficult to break down and may produce larger particles after milling (Muhamad & Campbell, 2004). The relationship between hardness index standard deviation and release year can show how the kernel hardness variation changed during breeding, which means the kernel hardness was more uniform. The kernel hardness uniformity is desirable in milling industry because it is positive correlated with milling scores, which is calculated based on kernel test weight, milling yield, ash content, and protein content (Campbell et al., 2007; Ohm et al., 1998).

Kernel moisture and dimensions (diameter and weight and standard deviation of diameter and weight) varied as a function of the interaction between release year and harvest year.

However, the interactions appeared to be due to differences in magnitude of the effect in the two harvest years, where the effect was stronger in one year than the other, but the trends were in the same direction (Figure 2.2). Overall, there was an increasing trend in kernel moisture content (Figure 2.2).

Because moisture content is a transient property of wheat kernels that changes depending on humidity, it is remarkable that a significant relationship existed between moisture content and release year, given that all the kernels were produced, harvested, and stored under the same conditions. This occurred despite samples being analyzed in random order. The apparent tendency of modern cultivars to equilibrate to elevated moisture contents could be a contributing factor to the increased number of microbial food safety issues caused by wheat flour in recent years (Myoda et al., 2019; Sabillón & Bianchini, 2016). The possible mechanisms behind this relationship merit further investigation.

The trends for kernel dimensions revealed that kernels have become smaller and less uniform in size across release years (Figure 2.2). This is in contrast to a previous study where kernel diameter was shown to increase with release year (Malalgoda et al., 2018). This could reflect differences in priorities in terms of quality. It has been reported that smaller kernels can have lower flour yield than large kernels because they have lower proportion of endosperm relative to bran (Baasandorj et al., 2015); however, smaller kernels can have better bread baking quality in terms of loaf volume and Mixograph peak time than larger kernels (Baasandorj et al., 2015). The decrease in kernel size uniformity across release year is not desirable. Kernel size uniformity has many effects on wheat milling. It can affect the flour yield, ash content, and the grinding process in the first bread. High kernel variability also causes higher attrition to the milling machine (Dziki & Laskowski, 2005).

No trend was found between milling yield with release year in this population (Table 2.2). Another study reported that milling yield has a positive correlation with release year due to the increase in the kernel size over time with spring wheat (Malalgoda et al., 2018).

2.4.2. Flour quality

The protein concentration varied as a function of the interaction between release year and harvest year (Table 2.2). Analysis by year indicated a strong decrease in protein concentration in the 2018 harvest year that was not evident in 2019 (Figure 2.3). The decreasing of protein content in modern wheat has been reported by many studies (Boxstael et al., 2020; Rozo-Ortega et al., 2021; Shewry et al., 2016).

Flour mixing quality variables also varied with release year interacting with harvest year. Midline peak time (MPT) and dough tolerance to overmixing (MRS) had a significant increasing relationship with release year in both harvest year. MTA had a crossover effect in two harvest years (Figure 2.3).

Overall, the trends associated with mixing time (MPT) and dough tolerance to overmixing (MRS) showed improvements with release year. Thus, protein mixing quality increased even while total protein decreased. The MPT is the time when the dough has optimum elasticity. Generally, a longer MPT is desirable to allow for adequate mixing of ingredients into the dough before it is developed. The longer the peak time (MPT) and lower midline right slope (MRS) means the dough has a higher tolerance to overmixing. In our study, MPT increased and MRS decreased with release year which means the dough elasticity and tolerance increased during breeding. Peak height (MPV) is indicative of dough consistency, peak widths (MPW) are indicative of mixing tolerance, and peak areas are indicative of dough strength. Small slope values indicate a flatter curve, which is preferable to large slope values, indicating poor tolerance

to mixing. Peak height is reached when optimum hydration has occurred; therefore, peak height is a function of protein content and water absorption (Miles, 2018). The improvement of mixing quality in terms of longer the development time and better the tolerance has been reported in many studies (Bassignana et al., 2015; Geisslitz et al., 2019; Malalgoda et al., 2018).

No significant relationship was found between bread volume, specific volume, and firmness with release year (Table2.2). This result is different from a previous study that reported a negative correlation between loaf firmness and release year but is in accordance with the same study that showed no correlation between loaf volume and release year (Malalgoda et al., 2018). Thus, although the physical characteristics, milling quality, mixing quality of wheat kernel results have shown changes over time, their influence was not enough to influence overall baking quality.

Notably, even though flour protein content decreased with release year, there was no decreasing trend was found between baking quality and release year (Table 2.2 & Figure 2.3). Thus, the protein in modern wheat must be more functional in terms of having better elasticity and pseudoplastic behavior. This is reflected in the improvements in some of the mixing quality parameters. The reason we did not observe improvements in baking quality may be because breeding programs typically evaluate kernel characteristics, milling, and mixing quality in early generation lines, but the baking quality is usually only evaluated in late generation lines because it uses so much more flour and is so much more time consuming (Pollak & Scott, 2005).

2.4.3. Bran quality

In this study, we evaluated two bran quality traits that are related to whole wheat bread baking quality: BWRC and bran friability (Seyer & Gélinas, 2009). No trend was found between bran friability and release year; however, BWRC had a significant increasing relationship with

release year (Table 2.2 & Figure 2.4). BWRC is inversely related to whole wheat bread quality (Navrotskyi et al., 2019). Therefore, the increasing BWRC in this study suggests a decrease in bran quality over time and thus a decrease in whole wheat bread quality. According to the increases of whole grain consumption, it would be important to include bran of whole grain quality parameters in breeding decisions in order to maintain or improve the quality of whole grain foods.

2.4.4. Correlation among quality parameters

Correlations among each variable are shown in Figure 2.5. Several expected correlations were observed between hard and soft kernels and kernel dimensions. Apart from these, moisture content was positively correlated with BWRC. Bran friability had a positive correlation with % hard kernel and a negative correlation with % soft kernel and a negative correlation with milling yield, BWRC, and MPT. Thus, bran friability can be used as an indicator to evaluate the end-use quality in the milling and baking industry because it is associated with many quality factors. Loaf specific volume had a strong positive correlation with load volume. Both loaf specific volume and volume has a significant negative relationship with loaf firmness, which means in the baking process, the greater the loaf volume, the greater loaf specific volume, lower the firmness.

It has been reported that larger kernels can have higher flour yield and friability, and lower endosperm separation index (Baasandorj et al., 2015; Breseghello & Sorrells, 2006; Li & Posner, 1987). In our study, the correlations between kernel size and milling quality are not very high when using harvest year as the partial variable. Because we did not observe a significant trend between size and release year, it is reasonable to not have a significant relationship between milling yield and release year. Brorsen et al. also found kernel diameter is significantly correlated with MPT (Brorsen et al., 2011). A previous study reported that grain hardness, flour

water absorption and whole wheat bread volume were strongly associated (Hrušková et al., 2006). Kernels with higher bran friability are more difficult to mill and can have lower milling yield (Gaines et al., 1997). High friability also means a lower specific volume which can have a negative effect on the whole wheat bread quality (Seyer & Gélinas, 2009).

2.5. CONCLUSION

In the present study on winter wheat adapted to the Great Plains of the US, wheat kernels are becoming harder, moister, and more variable in shape over nearly a century of wheat breeding. Mixing quality improved and baking quality was unchanged even with a decrease in flour protein concentration, indicating an improvement in protein functional quality. Future research should address the peculiar and potentially impactful finding that moisture content appears to be increasing over time. Additional research should consider the relationship between release year and nutritional quality parameters, including digestibility of macromolecules, to study further the relationships between historical and modern wheats.

Table 2.1. Release year, origin, and plant introduction (PI) or cereal introduction (CI) number for the wheat cultivars used in this study.[a]

Cultivar	Year of introduction, release, or PVP	Year of introduction category	Place of origin (US)	PI or CI number
Turkey	1870	Landrace	Landrace	CItr 5757
Kharkof	1900	Landrace	Landrace	PI 5641
Cheyenne	1933	Very old	UNL	CItr 8885
Red Chief	1940	Very old	Kansas	CItr 12109
Wichita	1944	Very old	KSU	CItr 11952
Warrior	1960	Old	UNL	CItr 13190
Lancer	1963	Old	USDA/UNL	CItr 13547
Triumph 64	1964	Old	OSU	CItr 12132
Sturdy	1966	Old	TAMU	CItr 13684
Scout 66	1967	Old	UNL	CItr 13996
Clark's Cream	1972	Old	Kansas	PI 476305
Centurk 78	1978	Old	UNL	CItr 17724
Centura	1983	Old	UNL	PI 476974
Siouxland	1984	Old	UNL	PI 483469
TAM 107	1984	Old	TAMU	PI 495594
Wesley	1998	Modern	USDA/UNL	PI 605742
Jagalene	2002	Modern	Monsanto	PI 631376
Anton	2007	Modern	USDA	PI 651044
Overland	2007	Modern	UNL	PI 647959
Camelot	2008	Modern	UNL	PI 653832
Settler CL	2008	Modern	UNL	PI 653833
Mattern	2012	Modern	USDA/UNL	PI 665947
Freeman	2013	Modern	UNL	PI 667038

[a]PVP, plant variety protection; USDA, US Department of Agriculture, UNL, University of Nebraska-Lincoln; TAMU, Texas A&M University; OSU, Oklahoma State University; KSU, Kansas State University; PI or CI obtained from the USDA-Agricultural Research Service National Plant Germplasm System Database: https://npgsweb.ars-grin.gov/gringlobal/search.aspx

Table 2.2. ANOVA (mean squares) among kernel physical characteristics and release year, harvest year and their interaction

Variable	HY			RY	RY*HY
	2018	2019	Both		
Hardness index	111.12	373.74*	426.8*	29.78	45.35
% soft kernel	1221.66**	626.05**	1800**	54.09	48.16
% semi-soft kernel	56.07	82.23	1.096	120.0	139.3
% semi-hard kernel	371.13**	1.36	208.0*	135.9	163.0
% hard kernel	65.08	1083.86**	842.0	232.9	310.9
Hardness std	58.17**	0.04	27.10**	25.98**	30.27**
Moisture (%)	4.89**	0.311**	3.650**	0.7735	1.253*
Moisture std (%)	0.01*	0.008**	0.0000	0.0106*	0.0174**
Diameter (mm)	0.0129	0.13**	0.0288	0.0992**	0.1121**
Diameter std (mm)	0.0429**	0.0039*	0.0370**	0.0120**	0.0109**
Weight (mg)	9.12	85.024**	18.58	68.07**	76.66**
Weight std (mg)	33.98**	5.96**	34.88**	6.468**	5.985**
Milling yield (%)	0.002	0.0004	0.0003	0.0021	0.0020
Bran friability (%)	0.0003	0.003	0.0007	0.0034	0.0028
BWRC (%)	3213.32*	1502.97	4549.25*	352.56	154.13
Protein content (%)	15.30**	1.66	13.50**	3.81**	3.41**
MPT (min)	32.94**	3.046**	27.95**	7.20**	7.92**
MPV (%)	149.76**	17.17	32.50	130.26**	133.92**
MPW (%)	2.18	293.92**	123.33*	163.36**	173.91**
MRS (%/min)	6.10**	3.25*	9.13**	0.16	0.22*
MTA (%TQ*min)	7738.10**	2776.10**	612.62	9651.88**	9882.23**
Loaf Volume (cm^3)	46.065	91.41	4.02	133.25	133.80
Loaf specific volume (cm^3/g)	0.00537	0.0533	0.046	0.012	0.013
Texture (F)	1893.06	870.287	94.29	2559.48	2661.33

ns, *, ** non-significant and significant at the 0.05 and 0.01 probability levels, respectively.

RY: release year; HY: harvest year; BWRC: bran water retention capacity; MPT: Midline peak time; MPV: Midline peak value; MPW: Midline peak width; MTA: Midline time max area; MRS: Midline right slope.

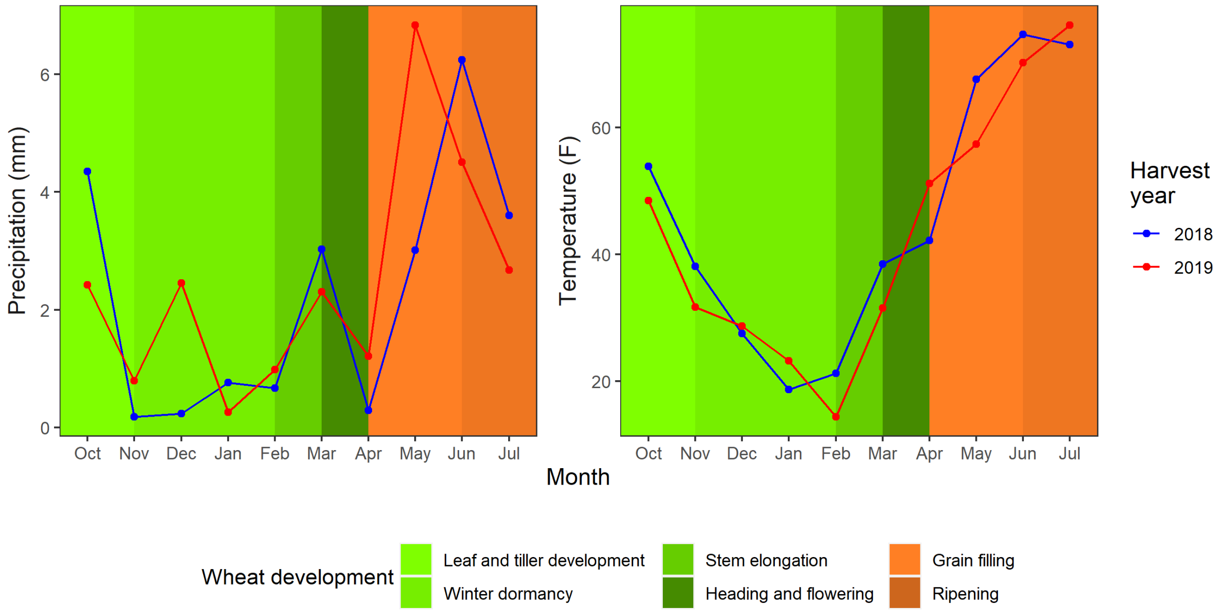

Figure 2.1. Precipitation (left) and temperature (right) condition and wheat development at Mead, NE, USA in 2018 and 2019

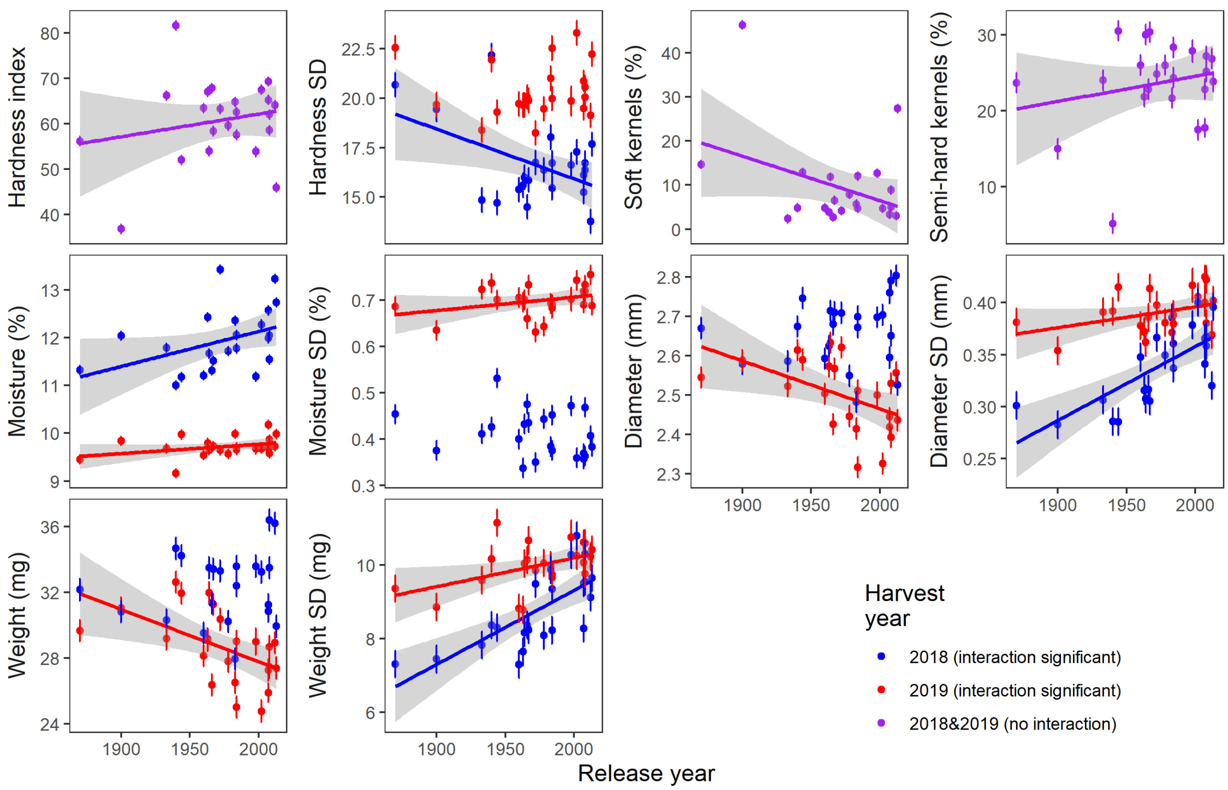

Figure 2.2. Kernel characteristics with significant trends across release year. For variables with a significant release year x harvest year interaction, least squares means are plotted by harvest year; for variables where only the main effect of release year is significant, least squares means are plotted across both harvest years; regression lines are plotted only for data with a significant trend across release year; the gray shaded area shows the 95% confidence interval of the regression line.)

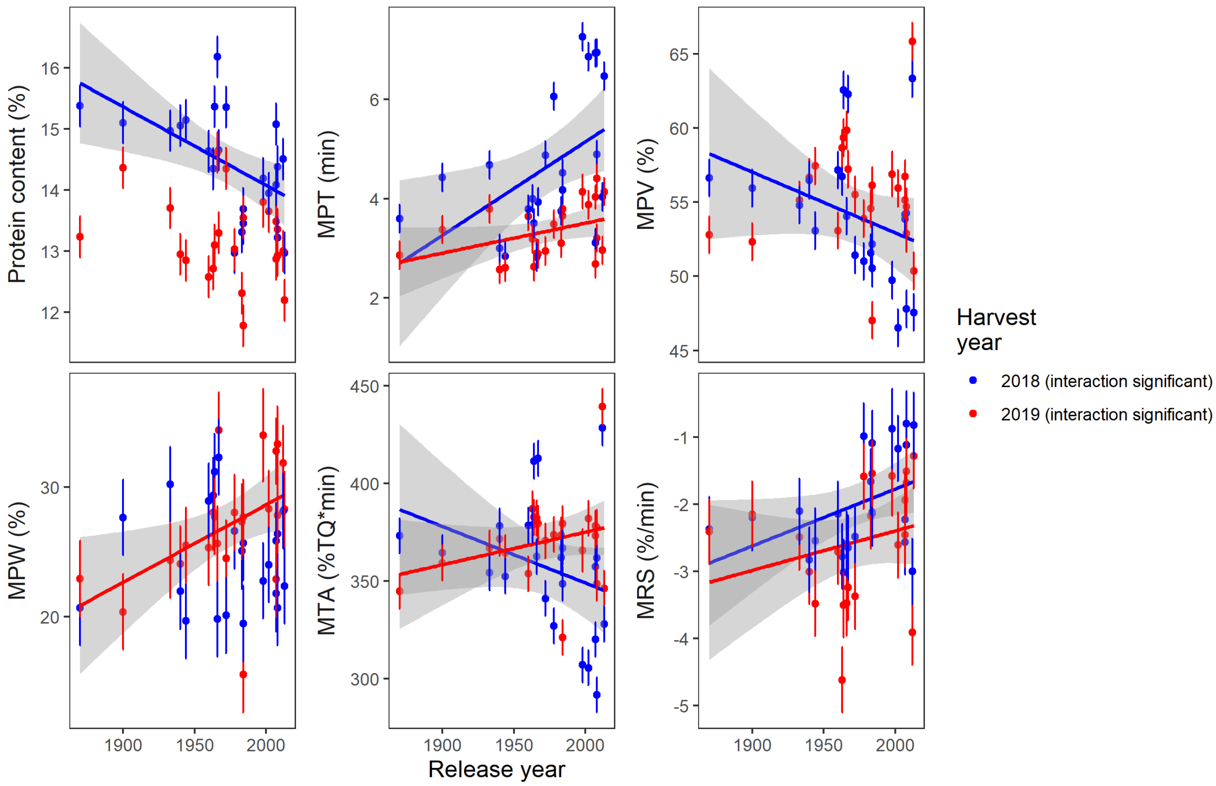

Figure 2.3. Protein content and mixing quality with significant trends across release year. For variables with a significant release year X harvest year interaction, least squares means are plotted by harvest year; for variables where only the main effect of release year is significant, least squares means are plotted across both harvest years; regression lines are plotted only for data with a significant trend across release year; the gray shaded area shows the 95% confidence interval of the regression line. (MPT: Midline peak time; MPV: Midline peak value; MPW: Midline peak width; MTA: Midline time max area; MRS: Midline right slope)

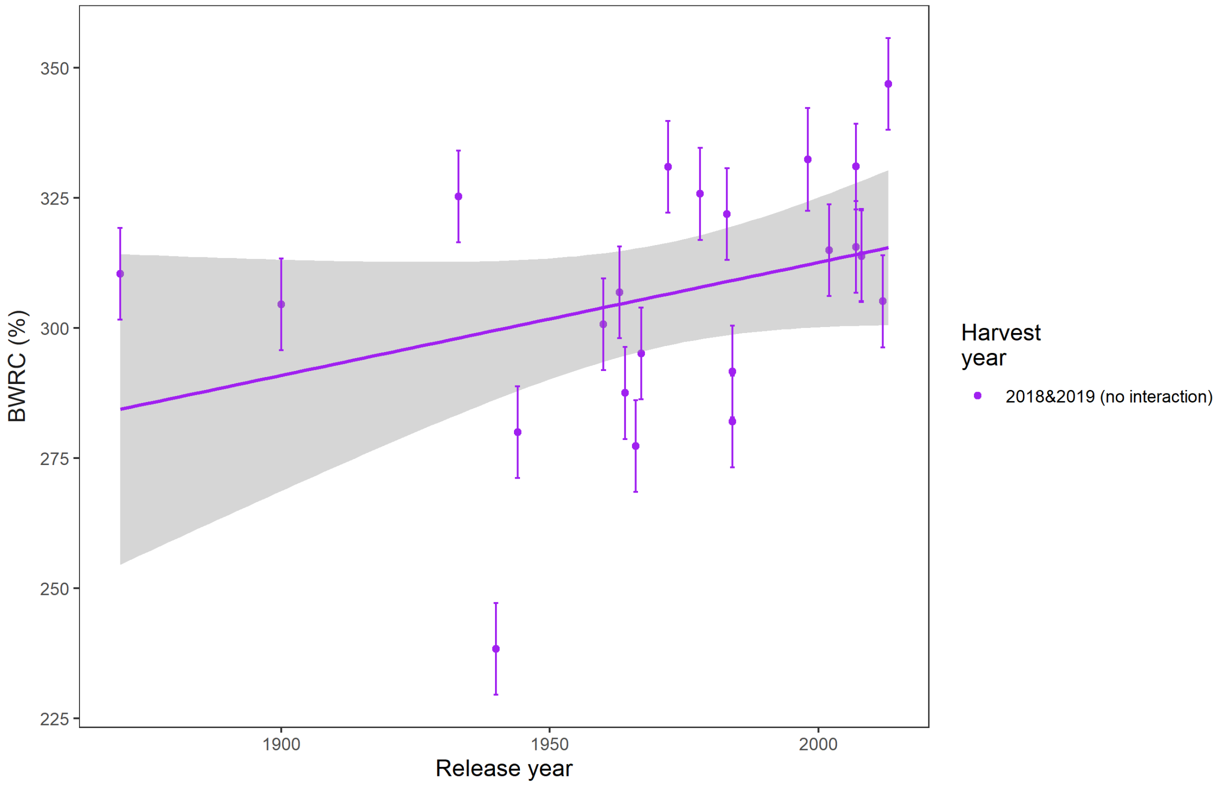

Figure 2.4. Bran water retention capacity (BWRC) with significant trends across release year. Least squares means are plotted across both harvest years; the gray shaded area shows the 95% confidence interval of the regression line.

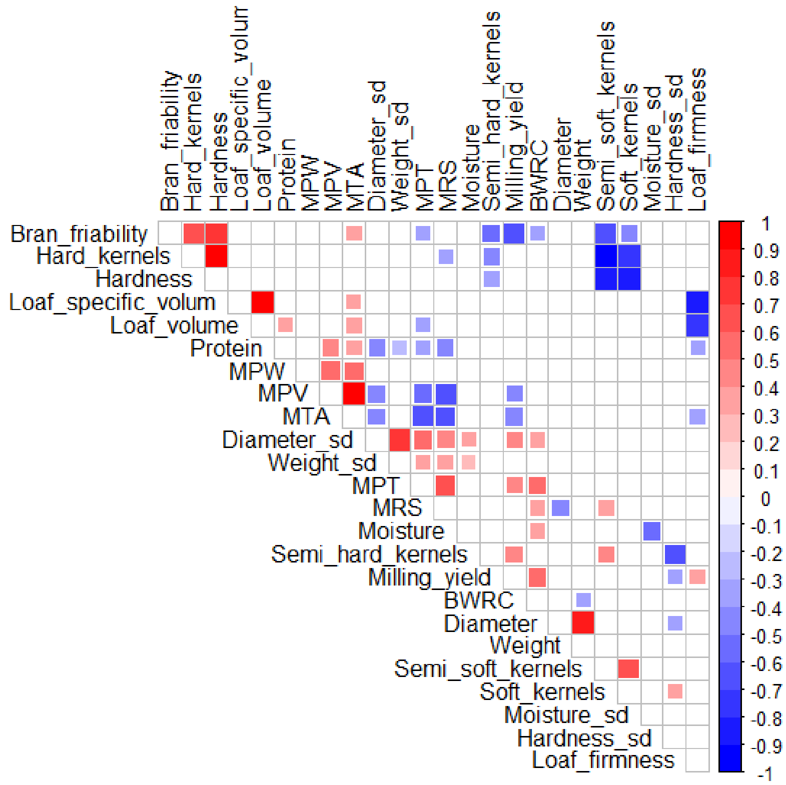

Figure 2.5. Partial correlation between each variable. Partial variable was harvest year and only significant correlations are plotted (p<0.05). (MPW: Midline peak width; MPV: Midline peak value; MTA: Midline time max area; MPT: Midline peak time; MRS: Midline right slope; BWRC: Bran water retention capacity)

2.6. REFERENCES

Baasandorj, T., Ohm, J., Manthey, F., & Simsek, S. (2015). Effect of kernel size and mill type on protein, milling yield, and baking quality of hard red spring wheat. *Cereal Chemistry, 92*(1), 81-87. 10.1094/CCHEM-12-13-0259-R

Bassignana, M., Arlian, D., Marti, A., Morandin, F., Zanoletti, M., & Pagani, M. (2015). Characterization of ancient wheat varieties and evaluation of their bread-making performances.

Bockus, W. W., De Wolf, E. D., Gill, B. S., Jardine, D. J., Stack, J. P., Bowden, R. L., Fritz, A. K., & Martin, T. J. (2011). Historical durability of resistance to wheat diseases in Kansas. *Plant Health Progress, 12*(1), 25. 10.1094/PHP-2011-0802-01-RV

Boxstael, F. V., Aerts, H., Linssen, S., Latré, J., Christiaens, A., Haesaert, G., Dierickx, I., Brusselle, J., & Keyzer, W. D. (2020). A comparison of the nutritional value of Einkorn, Emmer, Khorasan and modern wheat: whole grains, processed in bread, and population-level intake implications. *Journal of the Science of Food and Agriculture, 100*(11), 4108-4118. https://doi.org/10.1002/jsfa.10402

Breseghello, F., & Sorrells, M. E. (2006). Association mapping of kernel size and milling quality in wheat (Triticum aestivum L.) cultivars. *Genetics (Austin), 172*(2), 1165-1177. 10.1534/genetics.105.044586

Brorsen, B. W., Rayas-Duarte, P., & Ji, D. (2011). Predicting rheological properties of wheat dough based on wheat characteristics. *Journal of Agricultural Science (Toronto), 4*(3)10.5539/jas.v4n3p79

Call, L., Kapeller, M., Grausgruber, H., Reiter, E., Schoenlechner, R., & D'Amico, S. (2020). Effects of species and breeding on wheat protein composition. *Journal of Cereal Science, 93*, 102974. 10.1016/j.jcs.2020.102974

Campbell, G. M., Fang, C., & Muhamad, I. I. (2007). On predicting roller milling performance VI: Effect of kernel hardness and shape on the particle size distribution from first break milling of wheat. *Food and Bioproducts Processing, 85*(1), 7-23. 10.1205/fbp06005

Cereals & Grains Association. (1975). AACC Approved Methods of Analysis, 11th Ed. Method 44-15.02.Moisture—Air-Oven Methods. *Cereals & Grains Association,*

Cereals & Grains Association. (1995a). AACC Approved Methods of Analysis, 11th Ed. Method 10-10.03.Optimized Straight-Dough Bread-Making Method. *Cereals & Grains Association,* http://dx.doi.org/10.1094/AACCIntMethod-10-10.03

Cereals & Grains Association. (1995b). AACC Approved Methods of Analysis, 11th Ed. Method 54-40.02.Mixograph Method. *Cereals & Grains Association,* http://dx.doi.org/10.1094/AACCIntMethod-54-40.02

Cereals & Grains Association. (1999). AACC Approved Methods of Analysis, 11th Ed. Method 26-10.02.Experimental milling: Introduction, equipment, sample preparation, and tempering. *Cereals & Grains Association,*

Cereals & Grains Association. (2001). AACC Approved Methods of Analysis, 11th Ed. Method 10-05.01.Guidelines for Measurement of Volume by Rapeseed Displacement. *Cereals & Grains Association,*

Cereals & Grains Association. (2010). AACC Approved Methods of Analysis, 11th Ed. Method 46-30.01.Crude Protein -- Combustion Method. *Cereals & Grains Association,*

Cereals & Grains Association. (2012). AACC Approved Methods of Analysis, 11th Ed. Method 56-36.01.Firmness of Cooked Pulses. *Cereals & Grains Association,* 10.1094/CFW-57-5-0230

Claus, O. W. (2019). cowplot: Streamlined plot theme and plot annotations for 'ggplot2'. version 1.0.0.[computer software]

Dubcovsky, J., & Dvorak, J. (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science, 316*(5833), 1862-1866. 10.1126/science.1143986

Dziki, D., & Laskowski, J. (2004). Influence of kernel size on grinding process of wheat at respective grinding stages. *Polish Journal of Food and Nutrition Sciences, 13*(1), 29-33. https://www.cabdirect.org/cabdirect/abstract/20073138939

Dziki, D., & Laskowski, J. (2005). Wheat kernel physical properties and milling process Supplementation of food-nutritional and pro-health effects View project Food processing View project. *Acta Agrophysica, 6*

Gaines, C. S., Finney, P. L., & Andrews, L. C. (1997). Influence of kernel size and shriveling of soft wheat milling and baking quality. *Cereal Chemistry, 74*(6), 700-704. 10.1094/CCHEM.1997.74.6.700

Geisslitz, S., Longin, C. F. H., Scherf, K. A., & Koehler, P. (2019). Comparative study on gluten protein composition of ancient (Einkorn, Emmer and Spelt) and modern wheat species (Durum and Common Wheat). *Foods, 8*(9), 409. 10.3390/foods8090409

Guarda, G., Padovan, S., & Delogu, G. (2004). Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *European Journal of Agronomy, 21*(2), 181-192. 10.1016/j.eja.2003.08.001

Guzman, C., Peña, R. J., Singh, R., Autrique, E., Dreisigacker, S., Crossa, J., Rutkoski, J., Poland, J., & Battenfield, S. (2016). Wheat quality improvement at CIMMYT and the use of genomic selection on it. *Applied & Translational Genomics, 11*, 3-8. 10.1016/j.atg.2016.10.004

High Plains Regional Climate Center. (2020). https://hprcc.unl.edu/maps.php?map=ACISClimateMaps

Hrušková, M., Švec, I., & Jirsa, O. (2006). Correlation between milling and baking parameters of wheat varieties. *Journal of Food Engineering, 77*(3), 439-444. 10.1016/j.jfoodeng.2005.07.011

Hucl, P., Briggs, C., Graf, R. J., & Chibbar, R. N. (2015). Genetic gains in agronomic and selected end-use quality traits over a century of plant breeding of Canada Western Red Spring Wheat. *Cereal Chemistry, 92*(6), 537-543. 10.1094/CCHEM-02-15-0029-R

Kulathunga, Reuhs, Zwinger, & Simsek. (2021). *Comparative Study on Kernel Quality and Chemical Composition of Ancient and Modern Wheat Species: Einkorn, Emmer, Spelt and Hard Red Spring Wheat*. MDPI AG. 10.3390/foods10040761

Li, Y. Z., & Posner, E. S. (1987). *The Influence of Kernel Size on Wheat Millability (1987) Association of Operative Millers Technical Bulletin Nov:5089*

Malalgoda, M., Ohm, J., Meinhardt, S., & Simsek, S. (2018). Association between gluten protein composition and breadmaking quality characteristics in historical and modern spring wheat. *Cereal Chemistry, 95*(2), 226-238. https://doi.org/10.1002/cche.10014

Mefleh, M., Conte, P., Fadda, C., Giunta, F., Piga, A., Hassoun, G., & Motzo, R. (2019). From ancient to old and modern durum wheat varieties: interaction among cultivar traits, management, and technological quality. *Journal of the Science of Food and Agriculture, 99*(5), 2059-2067. https://doi.org/10.1002/jsfa.9388

Miles, C. W. (2018). *Relationship between mixsmart parameters and bread wheat quality characteristics in south African dry land cultivars*

Morgounov, A. I., Belan, I., Zelenskiy, Y., Roseeva, L., Tömösközi, S., Békés, F., Abugalieva, A., Cakmak, I., Vargas, M., & Crossa, J. (2013). Historical changes in grain yield and quality of spring wheat varieties cultivated in Siberia from 1900 to 2010. *Canadian Journal of Plant Science, 93*(3), 425-433. 10.4141/cjps2012-091

Muhamad, I. I., & Campbell, G. M. (2004). Effects of kernel hardness and moisture content on wheat breakage in the single kernel characterisation system. *Innovative Food Science & Emerging Technologies, 5*(1), 119-125. 10.1016/j.ifset.2003.10.003

Myoda, S. P., Gilbreth, S., Akins-Lewenthal, D., Davidson, S. K., & Samadpour, M. (2019). Occurrence and levels of Salmonella, Enterohemorrhagic Escherichia coli, and Listeria in raw wheat. *Journal of Food Protection, 82*(6), 1022-1027. 10.4315/0362-028X.JFP-18-345

Navrotskyi, S., Guo, G., Baenziger, P. S., Xu, L., & Rose, D. J. (2019). Impact of wheat bran physical properties and chemical composition on whole grain flour mixing and baking properties. *Journal of Cereal Science, 89*, 102790. https://www.sciencedirect.com/science/article/pii/S0733521019301985

Navrotskyi, Belamkar, Baenziger, & Rose. (2020). *Insights into the Genetic Architecture of Bran Friability and Water Retention Capacity, Two Important Traits for Whole Grain End-Use Quality in Winter Wheat*. MDPI AG. 10.3390/genes11080838

Ohm, J. B., Chung, O. K., & Deyoe, C. W. (1998). Single-Kernel characteristics of Hard Winter Wheats in relation to milling and baking quality. *Cereal Chemistry, 75*(1), 156-161. 10.1094/CCHEM.1998.75.1.156

Pollak, L. M., & Scott, M. P. (2005). Breeding for grain quality traits. *Maydica, 50*(3/4), 247-257. https://lib.dr.iastate.edu/agron_pubs/170

R Core Team. (2020). R: A language and environment for statistical computing. version 4.0.3.[computer software]

Rozo-Ortega, G. P., Serrago, R. A., Lo Valvo, P. J., Fleitas, M. C., Simón, M. R., & Miralles, D. J. (2021). Grain yield, milling and breadmaking quality responses to foliar diseases in old and modern Argentinean wheat cultivars. *Journal of Cereal Science, 99*, 103211. 10.1016/j.jcs.2021.103211

Sabillón, L., & Bianchini, A. (2016). From field to table: A review on the microbiological quality and safety of wheat-based products. *Cereal Chemistry, 93*(2), 105-115. 10.1094/CCHEM-06-15-0126-RW

Seyer, M., & Gélinas, P. (2009). Bran characteristics and wheat performance in whole wheat bread. *International Journal of Food Science & Technology, 44*(4), 688-693. 10.1111/j.1365-2621.2008.01819.x

Shewry, P. R., Pellny, T. K., & Lovegrove, A. (2016). Is modern wheat bad for health? *Nature Plants, 2*(7), 16097. 10.1038/nplants.2016.97

Sutton, K. H., Hay, R. L., & Mouat, C. H. (1992). The effect of kernel weight on the assessment of baking performance of wheats by RP-HPLC of glutenin subunits from single grains. *Journal of Cereal Science, 15*(3), 253-265. 10.1016/S0733-5210(09)80123-9

Underdahl, J. L., Mergoum, M., Ransom, J. K., & Schatz, B. G. (2008). Agronomic traits improvement and associations in Hard Red Spring Wheat cultivars released in North Dakota from 1968 to 2006. *Crop Science, 48*(1), 158-166. 10.2135/cropsci2007.01.0018

Wei, T., & Simko, V. (2017). R package "corrplot": Visualization of a correlation matrix (Version 0.84). [computer software]

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*

Yoon, B., Brorsen, B. W., & Lyford, C. P. (2002). Value of increasing kernel uniformity. *Journal of Agricultural and Resource Economics, 27*(2), 481-494. https://www.jstor.org/stable/40987848

CHAPTER 3 ASSOCIATION BETWEEN PROTEIN DIGESTIBILITY AND END-USE QUALITY OF HISTORICAL AND MODERN WHEATS

3.1. ABSTRACT

Previous research has suggested that in vitro protein digestibility increased over a century of wheat breeding but the possible correlation between protein digestibility and end-use quality variables has not been reported. Therefore, in vitro protein digestibility of flour and bread produced from 23 cultivars adapted to the Great Plains of the US and released between 1870 and 2013 were grown in triplicate in a single location over two crop years and their relationship with wheat end-use quality was evaluated. The proteins associated with high or low digestibility were also analyzed.

Flour protein digestibility did not change with release year of the cultivars and was not correlated with any end-use quality parameters. However, bread protein digestibility increased with release year and had positive correlations with kernel diameter standard deviation ($r = 0.41$, $p<0.05$), loaf firmness ($r=0.37$, $p<0.05$), white flour milling yield ($r=0.41$, $p<0.05$) and Mixograph midline peak time ($r=0.38$, $p<0.05$), and negative correlations with loaf volume ($r=-0.37$, $p<0.05$), Mixograph midline peak value ($r=-0.37$, $p<0.05$), and white flour protein content ($r=-0.87$, $p<0.0001$). High molecular weight protein increased while low molecular weight protein decreased as a function of release year. More gluten proteins associated with high digestibility which may need further study.

This study observed the relationship between protein digestibility and wheat end-use quality. Kernel diameter standard deviation, milling yield, loaf volume and Mixograph midline peak value were positive associated with protein digestibility which can be used to improve

protein digestibility in breeding as indicators. High protein digestibility associated with more gluten protein that may have important implications for human nutrition.

3.2. INTRODUCTION

Wheat is a widely grown crop in temperate countries and is used for both human food and livestock feed. Its popularity is based on its adaptability and high yield capacity, as well as the gluten protein fraction, which provides the viscoelastic properties that enable dough to be processed into bread, pasta, noodles, and other foods. Wheat breeders contribute to release new cultivars with improved agronomic and end-use quality traits. Accordingly, there are many studies that have shown that the milling and baking quality of wheat have changed dramatically from historical cultivars (Bassignana et al., 2015; Call et al., 2020; Guzman et al., 2016; Hucl et al., 2015; Kulathunga et al., 2021; Malalgoda, Ohm et al., 2018; Morgounov et al., 2013; Underdahl et al., 2008).

However, recently there has arisen much controversy around whether modern wheats are nutritionally inferior to historical wheats. Consumers, scientists, and the media put forth different claims about if modern wheat bad or good for health. In the last 50 years, the prevalence of celiac disease in the United States has increased by a factor of 4-5. Some specialists believe that this is because of changes in gluten protein content and composition between modern and historical wheats. To determine whether wheat breeding led to the rise of celiac disease prevalence, Broeck et al. compared the genetic diversity of gluten proteins for the existence of two celiac disease epitopes. They discovered that one of the celiac disease epitopes was higher in some modern wheats and the other one was lower, while other some modern cultivars had reduced levels of both epitopes (Broeck et al., 2010). Call et al. found that historical and modern wheats contained similar levels of immunotoxic components, which include gluten and amylase-

trypsin inhibitors (Call et al., 2020). Similar results can be found from many other studies (Malalgoda, Meinhardt et al., 2018; Prandi et al., 2017; Pronin et al., 2021). Taken together, these results provide convincing evidence that both historical and modern wheats are not appropriate for celiac disease patients.

However, there are few reports on changes in protein digestibility as a result of breeding. Only one such study reported that modern wheats tended to have higher protein digestibility than historical wheats (Gulati et al., 2020). This study, as well as one other, also showed that breeding efforts have increased high molecular weight protein content at the expense of low molecular weight proteins, which could have an additional impact on digestibility (Santis et al., 2017).

The purpose of this study was to confirm the relationship between release year and protein digestibility and then to relate changes in protein digestibility to end-use quality parameters.

3.3. MATERIALS AND METHODS

The *in vitro* protein digestibility of wheat flour and bread were studied using cultivars and environmental conditions as described in Chapter 2. The relationship between bread protein digestibility and wheat end-use quality was calculated based on the wheat end-use quality as described in Chapter 2.

The whole experimental design is shown in Figure 3.1. Overall, both flour and bread samples were digested. After centrifuge, supernatants from both flour and bread were used to measure the protein digestibility, the pellet of bread was saved for extracting protein by sonication as well as bread. After centrifuging the sonicated bread and digested bread, their supernatants were injected into HPLC while their pellet were injected into HPLC after reduced.

Then fractions collected from bread, reduced bread, digested bread, and reduced digested bread were used to mass spectrometry analysis. Details about each step is in next sections.

3.3.1. Materials

The flour and bread samples used in this study were from the milling and baking processes in Chapter 2 with the exception of 10 bread samples that were spoiled during freeze drying due to malfunctioning equipment (2018: Anton-replication-3, Clark's Cream-2, Red Chief-2, Sturdy-3, Triumph64-1&2; 2019: Centurk78-1&3, Clark's Cream-1, Lancer-1).

3.3.2. In-vitro protein digestion

Both bread and flour were digested as described by Versantvoort et al. with slight modifications (Versantvoort et al., 2005). Briefly, 120 mg of sample (flour or freeze-dried bread) were incubated with 4 mL of simulated gastric fluid (SGF; 0.5M NaCl adjusted to pH 2.5 with 1M HCl) and 30 mg of pepsin (5700U, SIGMA, Buchs, Switzerland) at 37 °C for 2 h, with pepsin activity measured according to (Anson, 1938). Gastric digestion was stopped by raising pH to 7 using 0.5 M sodium bicarbonate. The slurry was then mixed with 4 mL simulated intestinal fluid (SIF; 0.05 M potassium phosphate buffer, pH=7.0) containing 21 mg pancreatin (containing 2 p-Toluene-sulfonyl-L-arginine methyl ester Units of trypsin, SIGMA, Missouri, US) and 6 µL α-amylase (3000U/mL, E-AMGDF, Megazyme, Bray, Ireland) (Bernfeld, 1955). The trypsin activity in pancreatin was measured according to (Abita & Lazdunski, 1969). Then samples were incubated at 37 °C for another 4 h. The *in vitro* digestion was ended by plunging the tubes into a boiling water bath for 5 min followed by immediate cooling in cold water.

3.3.3. Digestibility

The protein digestibility was measured using ninhydrin (Plank & Valley, 2017). The digested samples were centrifuged at 17,000 x g for 15 min, and the supernatants were diluted 40

times in sodium acetate buffer (50 mM, pH=5.5). The diluted supernatant was then mixed with 2% ninhydrin reagent (sample: reagent, 2:1). Samples, blanks (sodium acetate buffer), and standards (0-1mM L-glycine) were then incubated at 70 °C for 35 min in the dark (by covering the tubes with aluminum foil). After cooling for 10 min, aqueous ethanol (50%, v/v) was added (alcohol: sample & reagent, 1:1). The absorbances were read at 570 nm against the blank sample. Protein digestibility was calculated as the molar percentage of free α-amino nitrogen measured after digestion from the total nitrogen prior to digestion (measured by combustion in Chapter 2).

3.3.4. SE-HPLC

3.3.4.1. Protein extraction and SE-HPLC

Bread proteins were extracted as described by Ohm et al (2009) with some modification. Ten milligrams of freeze-dried bread (dry base) were weighed into a 2 mL microcentrifuge tube then 1 mL of 0.1 M phosphate buffer (pH 6.9, containing 1% sodium dodecyl sulfate) was added. Proteins were extracted using a sonicating probe (VC50T, Connecticut, US) adjusted to 10 W output (20% power) for 45 s. The samples were centrifuged at 17,000 x g for 15 min and the supernatants were filtered through a 0.45 μm syringe filter. The filtered samples were heated for 2 min at 80 °C and then injected into a HPLC (Model 1260, Agilent, German) equipped with a size-exclusion column (300 × 4.6 mm, BIOSEP-SEC-S4000, Phenomenex, with guard cartridge) according to Ohm et al. (2009). Injection volume was 10 µL and the eluting solution was 50% acetonitrile in water with 0.1% (v/v) trifluoracetic acid. The flow rate was 0.5 mL/min and solutes were detected at 214 nm. The pellet remaining after protein extraction was retained for analysis after reduction.

Digested bread proteins were extracted by centrifuging the slurry after digestion at 17000 x g for 15 min and discarding the supernatant (containing digestible amino acids and peptides).

Then, 1 mL of 0.1 M phosphate buffer (pH 6.9) containing 1% SDS was added to the pellet and sonicated at 20 W output for 30 s. The protein extract was centrifuged at 17000 x g for 15 min and then treated and analyzed as described for bread proteins. As with bread samples, the pellet remaining after protein extraction was retained for analysis after reduction.

3.3.4.2. Reduction studies

To analyze proteins that were not extractable without reduction, 1 mL SDS and 5 µL of 5% β-Mercaptoethanol were added to the pellets of bread and digested bread after protein extraction (Singh, 2005). The mixture was vortex mixed for 10 s and then incubated at 80 °C for 30 min. The sample was centrifuged at 17000 x g for 15 min and filtered then analyzed as described above.

3.3.5. Mass spectrometry (LC-MS/MS)

Selected two cultivars, one has low digestibility (Kharkof) and one has high digestibility (Freeman) in both harvest years. Freeze-dried bread (undigested) from these two lines were re-run on the HPLC under reducing and non-reducing conditions as described above and the eluents corresponding to reduced bread fractions 1-4 and bread fraction were collected by running samples 10 times to get enough protein for MS analysis. The details about how the fractions were determined is in the next section.

Eluents were dried down and resuspended in 1X NuPAGE LDS Sample buffer (Thermo Fisher Scientific), and then heated at 95°C for 5 min prior to loading 50 µL of the sample onto a Bolt™ 12% Bis-Tris-Plus gel (Thermo Fisher Scientific) run with 1X Bolt MES SDS running buffer (Thermo Fisher Scientific). The proteins were run into the top of the gel for about 10 mm. The gel was fixed for 1 h in methanol: acetic acid: water (40:10:50), washed with water briefly

and stained with colloidal coomassie blue G250 (Sigma B2025) overnight. The samples were then destained before excising the whole lane for further processing.

Excised gel pieces were washed with water and then with 50 mM ammonium bicarbonate/50% acetonitrile to remove SDS and coomassie blue stain. Samples were then reduced with 10 mM dithiothreitol and alkylated with iodoacetamide. Trypsin (200 ng) was added and digestion was carried out overnight at 37°C. Peptides were extracted from the gel pieces and dried down in a Speed-Vac. The digests were redissolved in 2.5% acetonitrile, 0.1% formic acid. Analysis was carried out using a HPLC equipped with a C18 column (0.075 mm x250mm C18 Waters CSH column) feeding into an Orbitrap Eclipse mass spectrometer run in OT-IT-HCD mode for 2 h. The LC aqueous mobile phase contained 0.1% (v/v) formic acid in water and the organic mobile phase contained 0.1% (v/v) formic acid in 80% (v/v) acetonitrile. Mass spectra for the eluted peptides were acquired on a Thermo Orbitrap Eclipse Tribrid mass spectrometer in data-dependent mode using a mass range of m/z 250–1500, resolution 120,000, AGC target 4 x 106, maximum injection time 50 ms for the MS1 peptide measurements. Data-dependent MS2 spectra were acquired by HCD with a collision energy set at 30%, AGC target set to 5 x 105, 15,000 resolution, intensity threshold 1 x 106 and a maximum injection time of 54 ms. Dynamic exclusion was set at 10 sec and the isolation window set to 1.6 m/z.

Data were analyzed using Mascot (Matrix Science, London, UK; version 2.7.0). Mascot was set up to search the cRAP_20150130.fasta (124 entries); uniprot-refprot_UP000019116_Triticum_aestivum_ 20210614 database (130,673 entries) assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 0.6 Da and a parent ion tolerance of 10.0 PPM. Carbamidomethyl of cysteine was set as a fixed

modification. Deamidation of asparagine and glutamine and oxidation of methionine were specified in Mascot as amino acid modifications.

Scaffold (version 4.8.9, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 80.0% probability by the Peptide Prophet algorithm (Keller et al., 2002) with Scaffold delta-mass correction. Protein identifications were accepted if they could be established at greater than 99.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii et al., 2003). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins sharing significant peptide evidence were grouped into clusters.

3.3.6. Statistical analysis

3.3.6.1. SE-HPLC data analysis

Data analysis followed Ohm et al (2009) with some modifications. Before analysis, all the data was fixed by putting the highest point at 8 min to make sure there was no shift. SE-HPLC data were analyzed in two ways. First, the absorbance area values of major bread protein sections A to E (A to H for digested bread because it had more peaks in low molecular weight region) were calculated to analyze the relationship between release year (Figure 3.4). Area under the curve from SE-HPLC chromatograms of protein extracts were calculated for each 0.01 min interval between 5 min and 10 min (bread and reduced bread) or 13 min (digested bread and reduced digested bread) (Ohm et al., 2009).

In another analysis, the area of each 0.01 min interval was correlated with protein digestibility results from each harvest year. The fractions in reduced bread that had significant

correlations with protein digestibility in both harvest years were termed fractions 1-4 (Figure 3.6). Proteins from fractions 1-4 and bread fraction were analyzed for composition by LC-MS/MS as described in the previous section.

3.3.6.2. Other analysis

The relationships between in vitro protein digestibility, HPLC fractions and release year were analyzed using a two-factor ANOVA. Partial correlation used harvest year as the partial variable to determine the relationships between least-squares means of in vitro protein digestibility and wheat end-use qualities. All statistics were performed using SAS software (version 9.4, Cary, NC USA). LC-MS/MS results were analyzed using 'DESeq2' package in R (version 4.0.3) (Love et al., 2014). Protein cluster were performed using Molecular Evolutionary Genetics Analysis (MEGA) software (version X). Data were plotted using the 'ggplot2', 'cowplot', and 'corrplot' packages in R (version 4.0.3). (Claus, 2019; R Core Team., 2020; Wei & Simko, 2017; Wickham, 2016).

3.4. RESULTS AND DISCUSSION

3.4.1. Protein digestibility

In vitro protein digestibility was analyzed on both wheat white flour and bread. Only bread protein digestibility had a significant relationship with release year; no significant relationship was found between flour protein digestibility and release year (Table 3.1). The bread protein digestibility increased across release year (Figure 3.2). The results for bread digestibility are similar to a previous study which also showed that historical wheat tended to have lower protein digestibility than modern cultivars, although there were some historical cultivars that also had high digestibility (Gulati et al., 2020). Our intent in analyzing flour protein digestibility was to determine if results from bread and flour were similar, enabling the analysis of flour

digestibility, which is more convenient, in the future. However, the results indicate that flour protein digestibility is not an appropriate surrogate for bread protein digestibility. Since bread is the form that the wheat is consumed, it is a better sample than flour for studying wheat protein digestibility.

Similar to the ANOVA results, there were no significant correlations between flour protein digestibility and end-use quality variables (Table 3.2). However, bread protein digestibility was correlated with several end-use quality variables (Table 3.2). The significant correlations between wheat end-use quality and protein digestibility were plotted (Figure 3.3). Bread protein digestibility had positive correlations with kernel diameter standard deviation, loaf firmness, white flour milling yield and Mixograph midline peak time (MPT), and negative correlations with loaf volume, Mixograph midline peak value (MPV), and white flour protein content. The kernel diameter standard deviation, flour milling yield, and MPT were showed to increase with release year in other studies (Bassignana et al., 2015; Malalgoda et al., 2018), while the protein content was shown to decrease (Shewry et al., 2016).

Those significant correlations can be used as indicators for breeding choice. To breed high protein digestibility wheat, breeders can select wheat with high diameter standard deviation, milling yield, MPT, and loaf firmness, and low protein content, MPV, and loaf volume.

3.4.2. Protein component

To determine specific proteins associated with high and low digestibility, SE-HPLC and LC-MS/MS was used to analyze the protein component of bread and digested bread. The major protein peaks from SE-HPLC of non-reduced and reduced bread and digested bread were termed A-E in order of decreasing molecular weight. These fractions were analyzed for changes across release year of wheat cultivars (Table 3.1). In bread protein, sections B, C, and E increased with

release year, while section D decreased. Reduced bread and the sum of non-reduced bread protein had the same changing trend with bread protein increases for sections B, C, E, and decreases for section D (Figure 3.5).

Because higher molecular weight protein elutes earlier than lower molecular weight components in the SEC, sections A-F presented decreasing molecular weight components. Specially, in bread HPLC results (Figure 3.4), fraction C and D represent the high molecular weight protein and low molecular protein respectively. Thus, the high molecular weight extractable protein (peak C) increased slightly, and low molecular weight extractable protein (peak D) decreased dramatically during breeding (Figure 3.5).

As gluten proteins form many disulfide bonds during bread making that render the majority of proteins unextractable with sonication, the reduced bread results showed the majority of bread proteins that were extractable after reduction of disulfide bonds (Singh, 2005). The sum of unreduced bread and reduced bread showed the total protein in bread. Both unextractable protein and total protein had a similar trend with unextractable protein which was increased in high molecular weight protein and decreased in low molecular weight protein (Figure 3.5).

No relationships were found between protein fractions remaining after digestion and release year (Table 3.1 & Figure 3.5). The main peaks shown in bread HPLC (7 min and 8 min) disappeared in digested HPLC showed the proteins in bread had been digested. Under reducing conditions, the reduced bread chromatograph is higher than the bread chromatograph, but the reduced digested bread chromatograph is at the bottom around zero, which means the unextractable protein had been digested efficiently.

A previous study analyzed the changes in flour protein molecular weight profile with release year which is consisted with our results (Gulati et al., 2020). They found that in white

flour, high molecular weight protein was increased, and low molecular weight protein was decreased. Thus, the protein difference between historical and modern wheat is consistent after being processed into bread which the polymeric glutenin is increased during breeding.

3.4.3. Mass spectrometry

In LC-MS/MS, 1317 proteins were identified. For non-reduced bread protein extraction, there were 106 proteins have been found from the low digestibility cultivar, Kharkof, and 24 proteins have been found from high digestibility cultivar, Freeman. In reduced bread protein, fractions 1-4 had 72, 83, 112, and 97 proteins in the low digestibility cultivar, respectively, and 152, 135, 74, and 81 proteins in the high digestibility cultivars, respectively (Figure 3.7). More gluten proteins have been found in the high digestibility cultivar.

The gluten proteins associated with digestibility in bread and reduced bread were selected to do cluster analysis by MEGA (Figure 3.8). For the gluten proteins in bread, GLT3 WHEAT protein (Glutenin, high molecular weight subunit 12 OS=Triticum aestivum OX=4565 PE=3 SV=1) (OS=Organism Name, OX=Organism Identifier, PE=Protein Existence, SV=Sequence Version) was different from the other four, indicating that this gluten protein, which was associated with the low digestibility cultivar, was unique compared with the high digestibility gluten proteins. However, for the gluten proteins in reduced bread, the proteins associated with the low digestibility cultivar, which were J7HT09 WHEAT (Alpha-gliadin OS=Triticum aestivum OX=4565 GN=Gli-CS-1 PE=4 SV=1) and GDA3 WHEAT (+1) (Alpha/beta-gliadin A-III OS=Triticum aestivum OX=4565 PE=2 SV=1) proteins, were in the middle of the tree plot, which meant that they were not different from the proteins associated with high digestibility. Thus, the reason behind the difference between low and high digestibility proteins needs further research.

3.5. CONCLUSION

The wheat protein digestibility increased with cultivar release year. The correlation between protein digestibility and end-use quality has been found, which can be used in breeding to improve the wheat protein digestibility in the future. High molecular weight protein increased, and low molecular weight protein decreased with release year. More gluten proteins had been found in high digestibility samples. This shows that modern wheats have increased in digestibility and the gluten proteins that are elevated in modern wheats do not have poorer digestibility than historical cultivars. The reason behind the digestibility difference is still not clear; thus, further studies need to work on the details of the unique proteins in low and high digestibility samples.

Table 3.1. ANOVA (mean squares) among protein digestibility and HPLC fractions, and release year (RY), harvest year (HY) and their interactions

Variable/ Sample	HY			RY	RY*HY
	2018	2019	Both		
Flour dig (NH_2/N)	$2.9x10^{-3}$	$4.65x10^{-5}$	$1.8x10^{-3}$	$1.5x10^{-3}$	$1.1x10^{-3}$
Bread dig (NH_2/N)	0.026**	$4.0x10^{-3}$	0.026**	$4.7x10^{-3}$	$4.4 x10^{-3}$
Bread A	$2.8x10^{-5}$	$6.8x10^{-6}$	$2.0x10^{-5}$	$2.5x10^{-4}$**	$1.6x10^{-5}$
Bread B	$7.4x10^{-5}$	$5.2x10^{-5}$*	$9.0x10^{-5}$*	$8.0x10^{-6}$	$3.9x10^{-5}$
Bread C	$4.9x10^{-4}$**	$5.6x10^{-4}$**	$9.8x10^{-4}$**	$1.0x10^{-3}$**	$5.9x10^{-5}$
Bread D	$1.0x10^{-3}$**	$1.0x10^{-3}$**	$2.0x10^{-3}$**	0.02**	$1.7x10^{-4}$
Bread E	$4.7x10^{-4}$*	$8.3x10^{-4}$**	$1.0x10^{-3}$**	0.017**	$2.2x10^{-4}$
Reduced Bread A	$1.5x10^{-5}$	$5.5x10^{-5}$**	$1.2x10^{-5}$	$2.0x10^{-4}$**	$8.6x10^{-6}$
Reduced Bread B	$8.8x10^{-5}$	$7.5x10^{-5}$**	$1.2x10^{-4}$**	$4.2x10^{-6}$	$4.6x10^{-5}$
Reduced Bread C	$5.7x10^{-5}$**	$6.9x10^{-5}$**	$1.1x10^{-4}$**	$3.0x10^{-3}$**	$1.5x10^{-5}$
Reduced Bread D	$4.4x10^{-4}$	$5.2x10^{-4}$**	$7.8x10^{-4}$**	$4.0x10^{-3}$**	$1.7x10^{-4}$
Reduced Bread E	$3.6x10^{-5}$**	$1.0x10^{-4}$**	$1.1x10^{-4}$**	0.013**	$2.4x10^{-5}$
SBR A	$3.5x10^{-5}$	$1.3x10^{-5}$**	$2.6x10^{-5}$	$9.2x10^{-4}$**	$2.0x10^{-5}$
SBR B	$1.8x10^{-5}$*	$1.4x10^{-5}$**	$2.3x10^{-4}$**	$4.6x10^{-5}$	$8.7x10^{-5}$
SBR C	$5.4x10^{-4}$**	$5.7x10^{-5}$**	$1.0x10^{-3}$**	$5.6x10^{-4}$**	$7.9x10^{-5}$
SBR D	$1.0x10^{-3}$**	$2.0x10^{-3}$**	$3.0x10^{-3}$**	0.04**	$4.2x10^{-4}$
SBR E	$5.7x10^{-4}$**	$1.0x10^{-3}$**	$1.0x10^{-3}$**	0.06**	$3.2x10^{-4}$*
Digested Bread A	$5.6x10^{-4}$	$1.1x10^{-4}$	$4.3x10^{-4}$	$5.6x10^{-3}$**	$2.7x10^{-4}$
Digested Bread B	$1.2x10^{-4}$	$2.5x10^{-5}$	$9.5x10^{-5}$	$1.1x10^{-3}$**	$5.6x10^{-5}$
Digested Bread C	$1.8x10^{-4}$	$4.5x10^{-5}$	$1.4x10^{-4}$	$1.6x10^{-4}$**	$7.2x10^{-5}$
Digested Bread D	$1.8x10^{-4}$	$4.5x10^{-5}$	$1.3x10^{-4}$	$1.2x10^{-3}$**	$7.8x10^{-5}$
Digested Bread E	$2.4x10^{-5}$	$7.3x10^{-6}$	$1.6x10^{-5}$	$3.0x10^{-4}$**	$1.5x10^{-5}$
Digested Bread F	$1.8x10^{-3}$	$4.9x10^{-4}$	$1.3x10^{-3}$	0.018**	$7.8x10^{-4}$
Digested Bread G	$2.6x10^{-4}$	$3.3x10^{-5}$	$1.7x10^{-4}$	$3.1x10^{-4}$	$1.3x10^{-4}$
Digested Bread H	$3.9x10^{-4}$	$3.8x10^{-5}$	$3.0x10^{-4}$	$3.6x10^{-4}$	$4.0x10^{-4}$
RDB A	$1.8x10^{-3}$	$1.9x10^{-4}$	$1.3x10^{-3}$	$1.1x10^{-3}$	$7.3x10^{-4}$
RDB B	$1.2x10^{-4}$	$1.7x10^{-5}$	$9.3x10^{-5}$	$1.4x10^{-3}$**	$5.7x10^{-5}$
RDB C	$1.6x10^{-5}$	$1.4x10^{-5}$**	$1.5x10^{-5}$	$4.4x10^{-6}$	$1.5x10^{-5}$
RDB D	$9.6x10^{-5}$	$3.4x10^{-5}$	$6.8x10^{-5}$	$1.2x10^{-3}$**	$5.3x10^{-5}$
RDB E	$2.7x10^{-5}$	$1.0x10^{-5}$	$2.0x10^{-5}$	$3.6x10^{-4}$**	$1.9x10^{-5}$
RDB F	$1.1x10^{-3}$	$2.1x10^{-4}$	$8.5x10^{-4}$	$2.1x10^{-3}$*	$4.3x10^{-4}$
RDB G	$2.9x10^{-4}$	$6.9x10^{-5}$	$2.2x10^{-4}$	$7.6x10^{-5}$	$1.3x10^{-4}$
RDB H	$4.0x10^{-4}$	$2.8x10^{-4}$**	$3.0x10^{-4}$	$3.6x10^{-4}$	$4.0x10^{-4}$
SDBR A	$3.0x10^{-3}$	$2.5x10^{-4}$	$2.0x10^{-3}$	$1.6x10^{-3}$	$1.4x10^{-3}$
SDBR B	$2.9x10^{-4}$	$4.0x10^{-5}$	$2.0x10^{-4}$	$1.4x10^{-5}$	$1.6x10^{-4}$
SDBR C	$2.4x10^{-4}$	$9.2x10^{-5}$	$1.9x10^{-4}$	$1.5x10^{-3}$**	$1.3x10^{-4}$
SDBR D	$4.3x10^{-4}$	$1.4x10^{-4}$*	$3.3x10^{-4}$	$5.4x10^{-3}$**	$1.9x10^{-4}$
SDBR E	$6.4x10^{-5}$	$1.0x10^{-5}$	$3.6x10^{-5}$	$5.1x10^{-6}$	$4.4x10^{-5}$
SDBR F	$2.5x10^{-3}$	$1.1x10^{-3}$**	$2.1x10^{-3}$	$8.9x10^{-3}$**	$1.3x10^{-3}$
SDBR G	$6.0x10^{-4}$	$1.2x10^{-4}$**	$4.7x10^{-4}$	$9.4x10^{-4}$*	$2.2x10^{-4}$
SDBR H	$6.8x10^{-4}$	$3.2x10^{-4}$*	$4.0x10^{-4}$	$6.7x10^{-4}$	$6.4x10^{-4}$

ns, *, ** non-significant and significant at the 0.05 and 0.01 probability levels, respectively.

SBR: The sum of bread and reduced bread; RDB: Reduced digested bread; SDBR: The sum of digested bread and reduced digested bread.

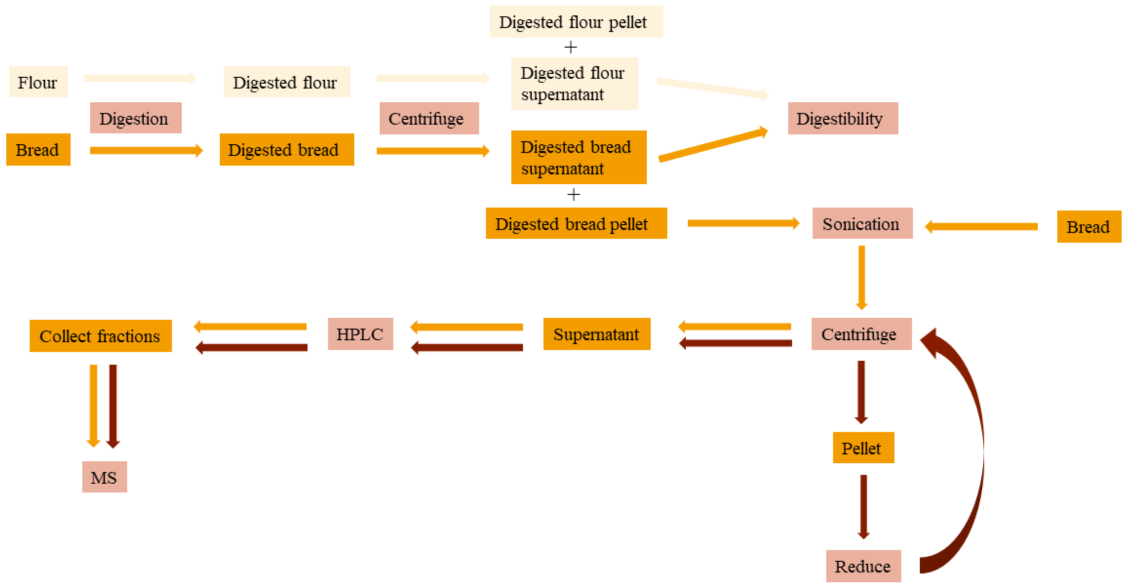

Figure 3.1. Experimental design flow chart.

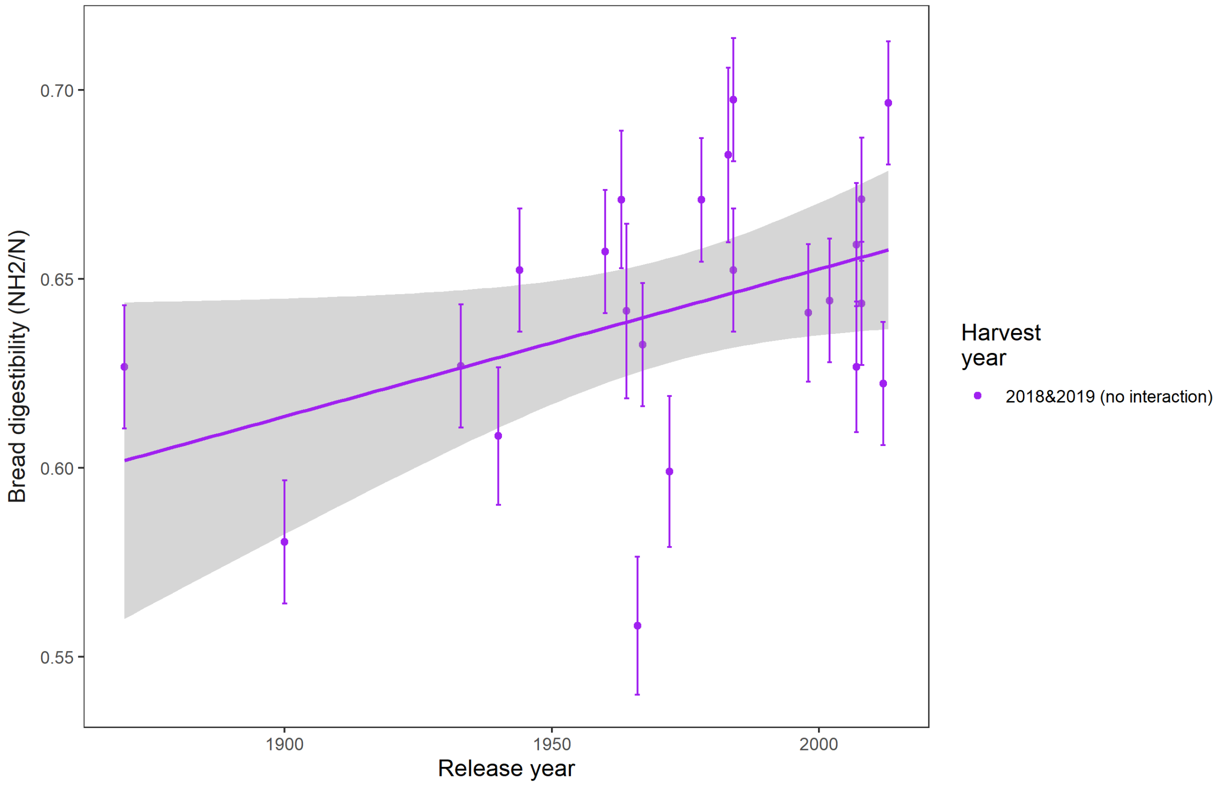

Figure 3.2. Bread protein digestibility with significant trends across release year.

Table 3.2. Correlation between protein digestibility and end-use quality.

Variable	Flour protein digestibility	Bread protein digestibility
Hardness	0.17	-0.10
Hardness standard deviation	-0.0037	-0.032
Moisture	-0.22	0.15
Moisture standard deviation	0.23	-0.032
Diameter	0.033	-0.19
Diameter standard deviation	0.26	0.41*
Weight	0.094	-0.13
Weight standard deviation	0.13	0.24
%Soft kernels	-0.26	-0.071
%Semi-soft kernels	-0.047	0.22
%Semi-hard kernels	0.18	0.31
%Hard kernels	0.10	-0.16
Milling yield	0.15	0.41*
Bran friability	-0.095	-0.27
BWRC	-0.17	0.19
Protein content	-0.14	-0.87**
MPT	0.12	0.38*
MPV	-0.12	-0.37*
MPW	0.0048	-0.016
MRS	0.14	0.29
MTA	-0.18	-0.34
Loaf volume	-0.17	-0.37*
Loaf firmness	0.33	0.37*
Loaf specific volume	-0.19	-0.28

ns, *, ** non-significant and significant at the 0.05 and 0.01 probability levels, respectively.
BWRC: bran water retention capacity; MPT: Midline peak time; MPV: Midline peak value; MPW: Midline peak width; MTA: Midline time max area; MRS: Midline right slope.

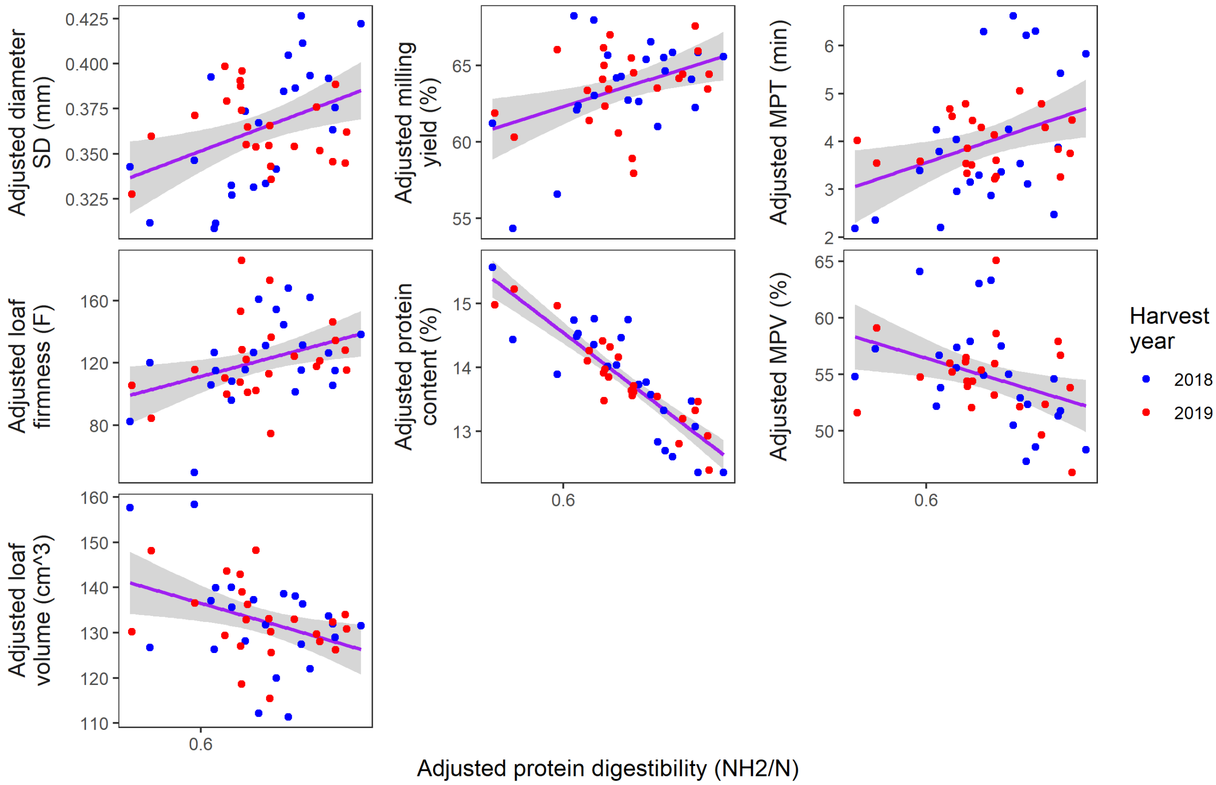

Figure 3.3. Correlations between wheat end-use quality and bread protein digestibility. MPT: midline peak time, MPV: midline peak value.

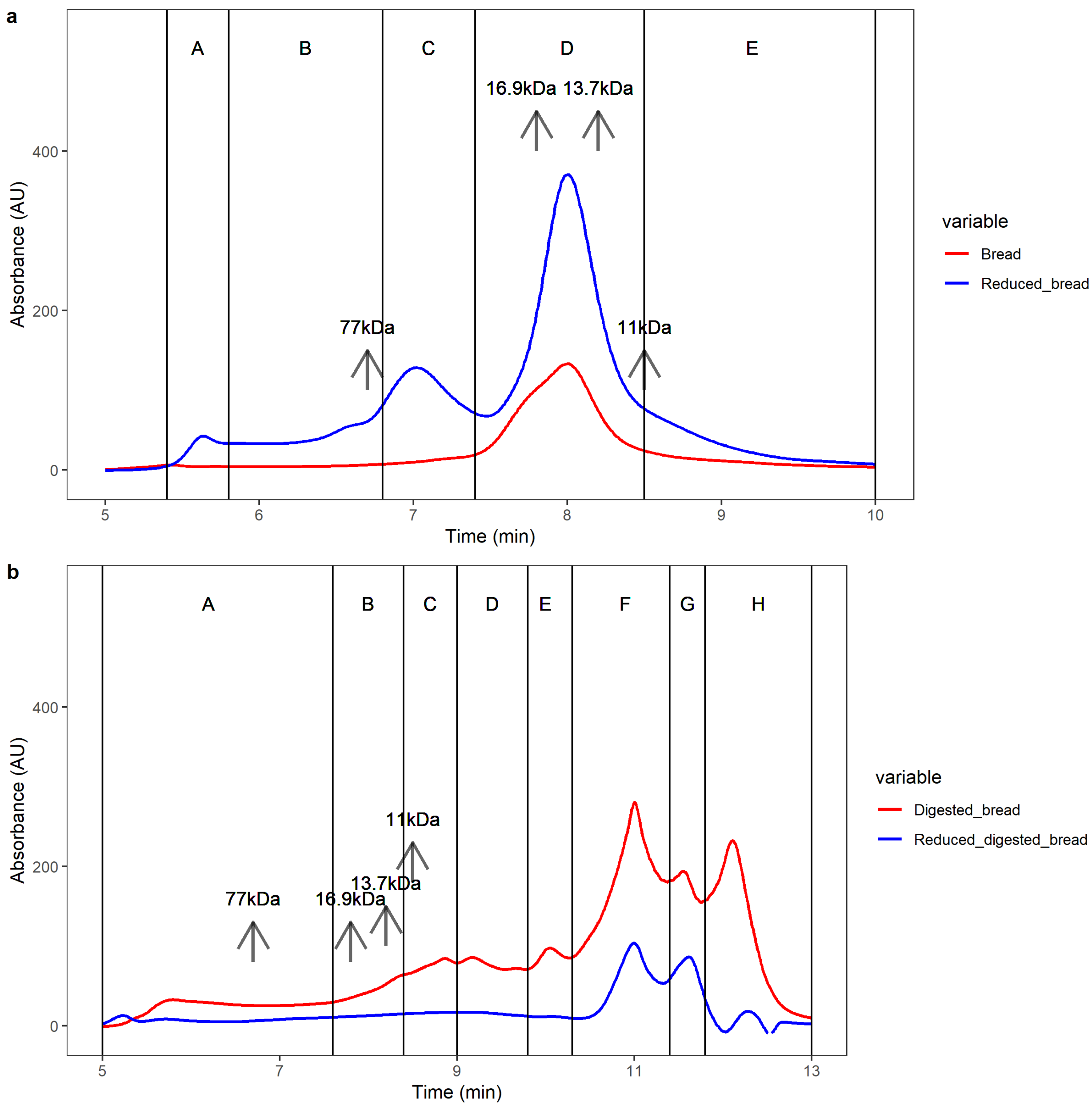

Figure 3.4. SE-HPLC separation of bread and reduced bread protein (a), and digested bread and reduced digested bread protein (b). Arrows show the molecular weight range at different retention times.

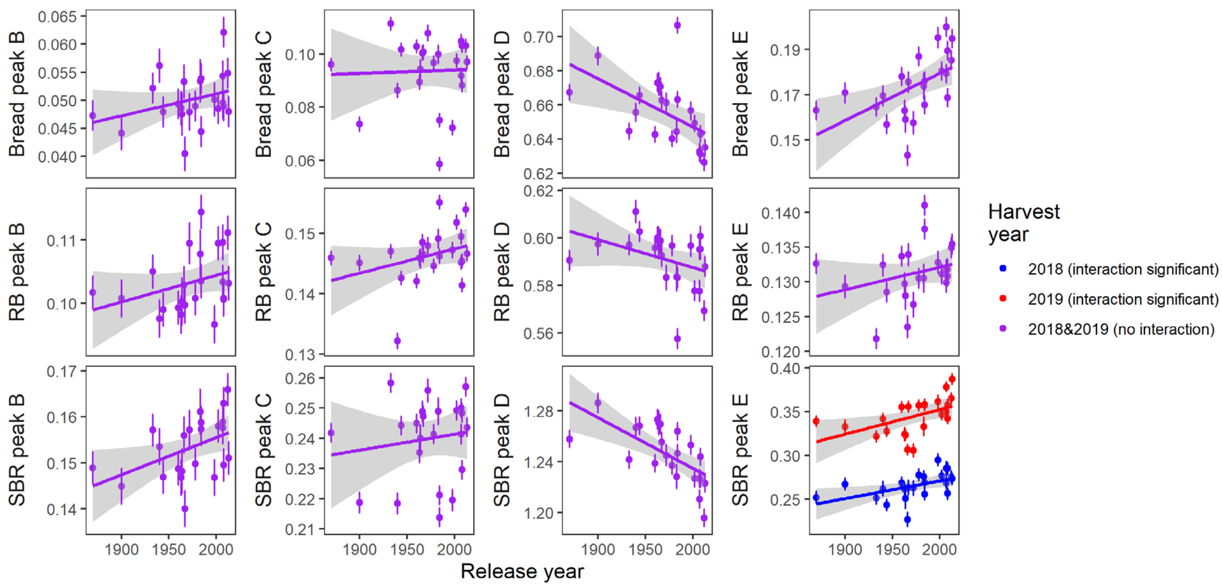

Figure 3.5. HPLC fractions with significant trends across release year. For variables with a significant release year x harvest year interaction, least squares means are plotted by harvest year; for variables where only the main effect of release year is significant, least squares means are plotted across both harvest years; regression lines are plotted only for data with a significant trend across release year; the gray shaded area shows the 95% confidence interval of the regression line.) RB: reduced bread; SBR: The sum of bread and reduced bread.

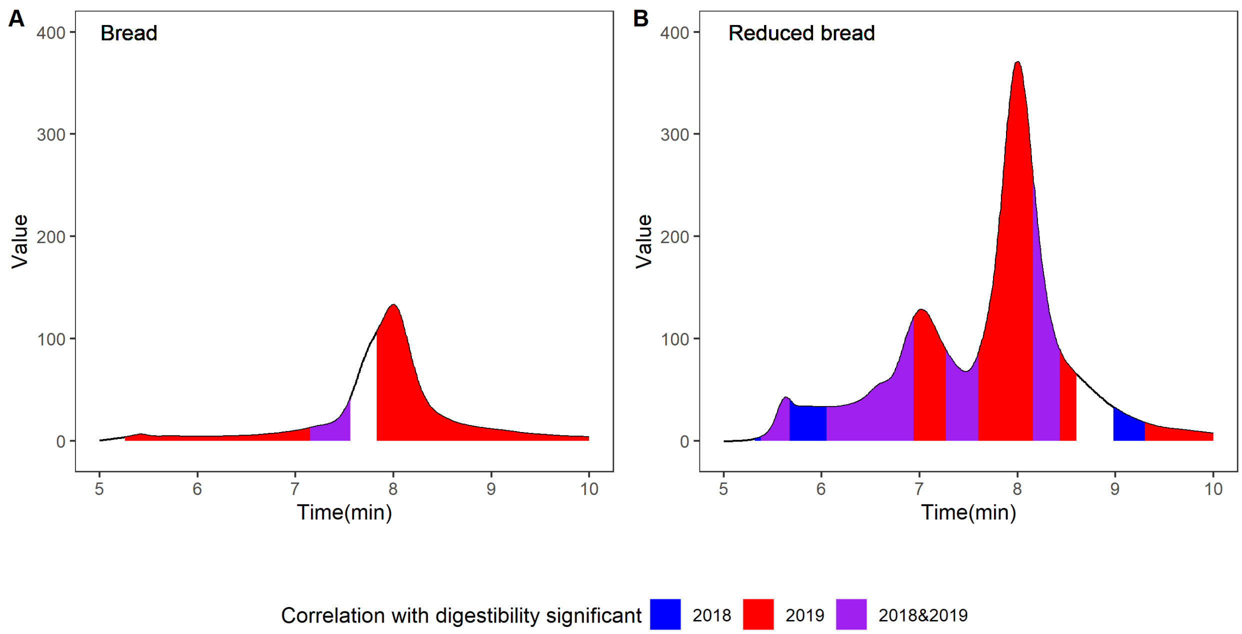

Figure 3.6. The bread (A) and reduced bread (B) HPLC fractions have significant correlation with bread protein digestibility.

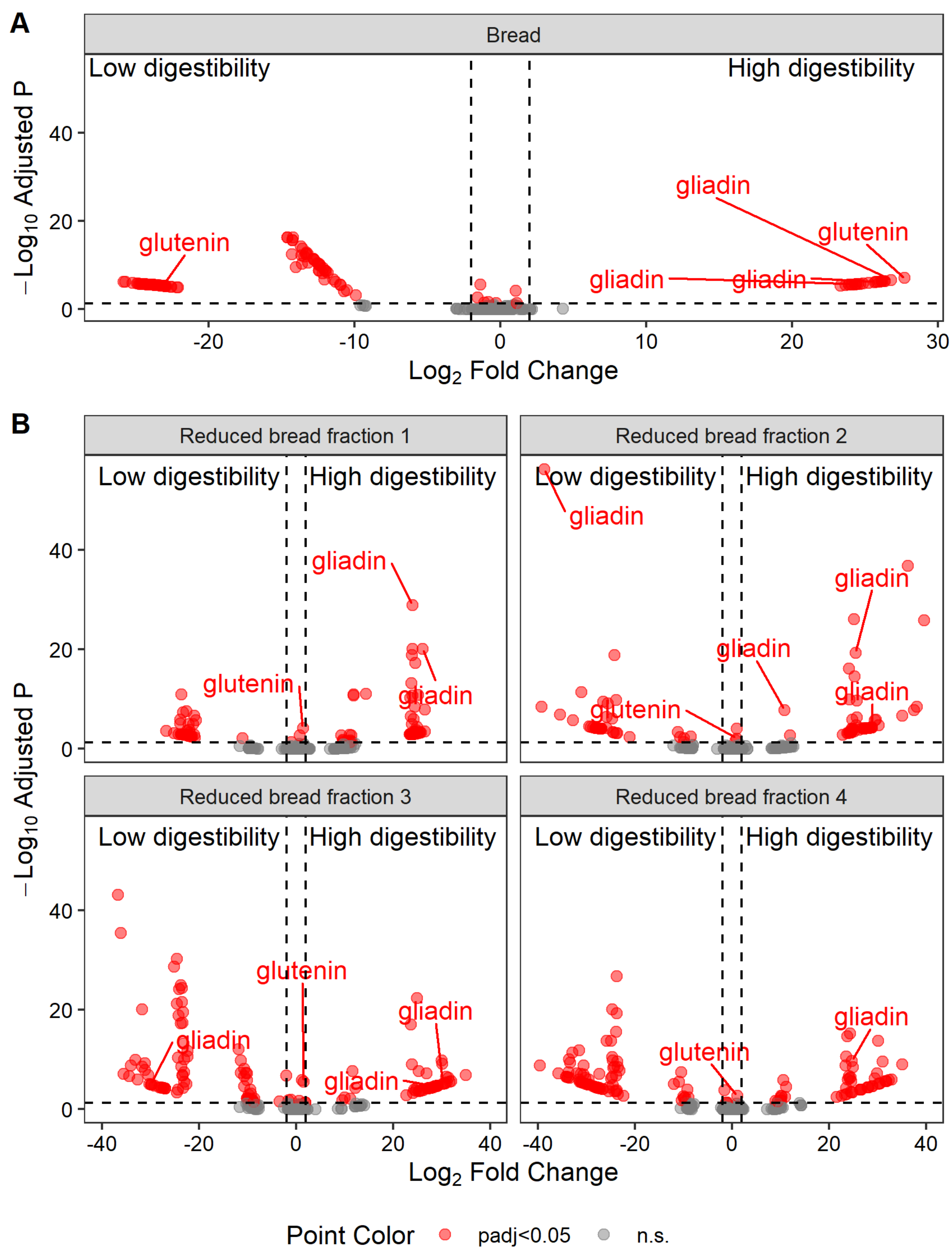

Figure 3.7. Volcano plots of bread (A) and reduced bread (B) showing statistical significance of log2 fold-change in quantitative normalized spectral abundance factor between low and high digestibility.

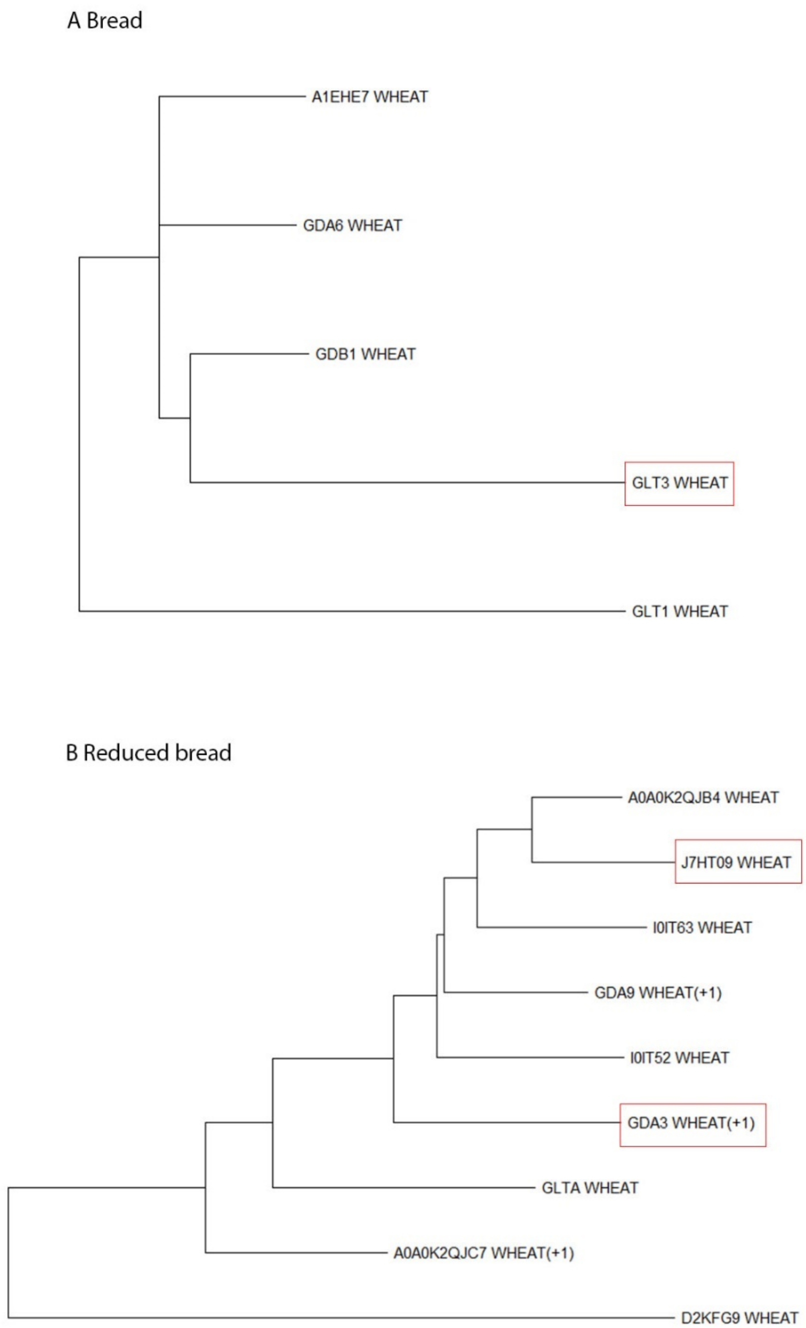

Figure 3.8. Low and high digestibility protein cluster for bread (A) and reduced bread (B). Proteins in red rectangle are low digestibility proteins.

3.6. REFERENCES

Abita, J. P., & Lazdunski, M. (1969). On the structural and functional role of carboxylates in chymotrypsinogen A: a comparison with chymotrypsin, trypsinogen and trypsin. *Biochemical and Biophysical Research Communications, 35*(5), 707-712. 10.1016/0006-291x(69)90463-x

Anson, M. L. (1938). The Estimation of Pepsin, Trypsin, Papin, and Cathepsin with Hemoglobin. *Journal of General Physiology, 22*(1), 79-89. 10.1085/jgp.22.1.79

Bassignana, M., Arlian, D., Marti, A., Morandin, F., Zanoletti, M., & Pagani, M. (2015). Characterization of ancient wheat varieties and evaluation of their bread-making performances.

Bernfeld, P. (1955). Amylases, α and β. *Methods in Enzymology* (pp. 149-158). Academic Press.

Broeck, H., Jong, H., Salentijn, E., Dekking, L., Bosch, D., Hamer, R., Gilissen, L., Meer, I., & Smulders, M. (2010). Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of celiac disease. *Theoretical and Applied Genetics, 121*(8), 1527-1539. 10.1007/s00122-010-1408-4

Call, L., Kapeller, M., Grausgruber, H., Reiter, E., Schoenlechner, R., & D'Amico, S. (2020). Effects of species and breeding on wheat protein composition. *Journal of Cereal Science, 93*, 102974. 10.1016/j.jcs.2020.102974

Claus, O. W. (2019). cowplot: Streamlined plot theme and plot annotations for ‘ggplot2’. version 1.0.0.[computer software]

Gulati, P., Brahma, S., Graybosch, R. A., Chen, Y., & Rose, D. J. (2020). In vitro digestibility of proteins from historical and modern wheat cultivars. *Journal of the Science of Food and Agriculture, 100*(6), 2579-2584. https://doi.org/10.1002/jsfa.10283

Guzman, C., Peña, R. J., Singh, R., Autrique, E., Dreisigacker, S., Crossa, J., Rutkoski, J., Poland, J., & Battenfield, S. (2016). Wheat quality improvement at CIMMYT and the use of genomic selection on it. *Applied & Translational Genomics, 11*, 3-8. 10.1016/j.atg.2016.10.004

Hucl, P., Briggs, C., Graf, R. J., & Chibbar, R. N. (2015). Genetic Gains in Agronomic and Selected End-Use Quality Traits over a Century of Plant Breeding of Canada Western Red Spring Wheat. *Cereal Chemistry, 92*(6), 537-543. 10.1094/CCHEM-02-15-0029-R

Keller, A., Nesvizhskii, A. I., Kolker, E., & Aebersold, R. (2002). Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. *Analytical Chemistry, 74*(20), 5383-5392. 10.1021/ac025747h

Kulathunga, Reuhs, Zwinger, & Simsek. (2021). *Comparative Study on Kernel Quality and Chemical Composition of Ancient and Modern Wheat Species: Einkorn, Emmer, Spelt and Hard Red Spring Wheat*. MDPI AG. 10.3390/foods10040761

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biology,* 10.1186/s13059-014-0550-8

Malalgoda, M., Meinhardt, S. W., & Simsek, S. (2018). Detection and quantitation of immunogenic epitopes related to celiac disease in historical and modern hard red spring wheat cultivars. *Food Chemistry, 264*, 101-107. https://www.sciencedirect.com/science/article/pii/S0308814618307799

Malalgoda, M., Ohm, J., Meinhardt, S., & Simsek, S. (2018). Association between gluten protein composition and breadmaking quality characteristics in historical and modern spring wheat. *Cereal Chemistry, 95*(2), 226-238. https://doi.org/10.1002/cche.10014

Morgounov, A. I., Belan, I., Zelenskiy, Y., Roseeva, L., Tömösközi, S., Békés, F., Abugalieva, A., Cakmak, I., Vargas, M., & Crossa, J. (2013). Historical changes in grain yield and quality of spring wheat varieties cultivated in Siberia from 1900 to 2010. *Canadian Journal of Plant Science, 93*(3), 425-433. 10.4141/cjps2012-091

Nesvizhskii, A., Keller, A., Kolker, E., & Aebersold, R. (2003). A statistical model for identifying proteins by tandem mass spectrometry. *Analytical Chemistry, 75*(17), 4646-4658. 10.1021/ac0341261

Ohm, J., Hareland, G., Simsek, S., & Seabourn, B. (2009). Size-Exclusion HPLC of Protein Using a Narrow-Bore Column for Evaluation of Breadmaking Quality of Hard Spring Wheat Flours. *Cereal Chemistry, 86*(4), 463-469. 10.1094/CCHEM-86-4-0463

Plank, D. W., & Valley, G. (2017). In General Mills Inc (Ed.), *In vitro method for estimating in vivo protein digestibility*

Prandi, B., Tedeschi, T., Folloni, S., Galaverna, G., & Sforza, S. (2017). Peptides from gluten digestion: A comparison between old and modern wheat varieties. *Food Research International, 91*, 92-102. 10.1016/j.foodres.2016.11.034

Pronin, D., Börner, A., & Scherf, K. A. (2021). Old and modern wheat (Triticum aestivum L.) cultivars and their potential to elicit celiac disease. *Food Chemistry, 339*, 127952. 10.1016/j.foodchem.2020.127952

R Core Team. (2020). R: A language and environment for statistical computing. version 4.0.3.[computer software]

Santis, M. A. D., Giuliani, M. M., Giuzio, L., Vita, P. D., Lovegrove, A., Shewry, P. R., & Flagella, Z. (2017). Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in Italy. *European Journal of Agronomy, 87*, 19-29. 10.1016/j.eja.2017.04.003

Shewry, P. R., Pellny, T. K., & Lovegrove, A. (2016). Is modern wheat bad for health? *Nature Plants, 2*(7), 16097. 10.1038/nplants.2016.97

Singh, H. (2005). A study of changes in wheat protein during bread baking using SE-HPLC. *Food Chemistry, 90*(1-2), 247-250. 10.1016/j.foodchem.2004.03.047

Underdahl, J. L., Mergoum, M., Ransom, J. K., & Schatz, B. G. (2008). Agronomic Traits Improvement and Associations in Hard Red Spring Wheat Cultivars Released in North Dakota from 1968 to 2006. *Crop Science, 48*(1), 158-166. 10.2135/cropsci2007.01.0018

Versantvoort, C. H. M., Oomen, A. G., Van de Kamp, E., Rompelberg, C. J. M., & Sips, Adriënne J. A. M. (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 43*(1), 31-40. 10.1016/j.fct.2004.08.007

Wei, T., & Simko, V. (2017). R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). [computer software]

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*

4. OVERALL CONCLUSIONS

Over the years, plant breeders continue to release wheat cultivars that are better adapted to conditions in specific regions and with other advantageous traits. Many researchers are arguing about whether historical wheats are better than modern wheat on human health. This study addressed how wheat quality traits changed during breeding and how they relate to certain nutritional characteristics such as protein digestibility and protein composition. Therefore, we analyzed 24 traits related to milling and baking quality along with in vitro protein digestibility of both flour and bread in 23 hard winter wheat cultivars.

Several quality characteristics improved across release year which is evidence of plant breeding efforts over the years. The hardness and moisture content of kernels increased by release year. The observed increase in hardness was accompanied by a decrease in %soft kernels and an increase in %semi-hard kernels. A decreasing trend was observed for hardness standard deviation in 2018. Although diameter and weight did not change with release year, their standard deviation increased with release year. Flour protein content decreased with release year and mixing time increased. No significant relationship was found for baking property variables, but bran water retention capacity (BWRC), which is correlated with whole wheat bread quality, increased with release year.

Digestibility of bread increased with release year and was significantly positively correlated with kernel diameter standard deviation, milling yield, Mixograph mixing peak time, and loaf firmness while negatively correlated with white flour protein content, Mixograph mixing peak value, and loaf volume. Flour protein digestibility has no relationship with release year and no correlation with end-use quality characteristics. As the reason of digestibility difference, high molecular weight protein increased while low molecular weight protein

decreased as a function of release year. More gluten proteins associated with high digestibility which may need further study.

In conclusion, wheat kernels have become harder, moister, more uniform in hardness but more variable in shape over a century of breeding; bran quality has decreased, which may have implications for grain quality and milling productivity. The baking quality remained constant despite a strong decrease in protein concentration. The digestibility of proteins increased during breeding and related to several quality characteristics, but this is only evident in proteins after being processed into bread. High molecular weight protein increased, and low molecular weight protein decreased in modern wheat. More gluten proteins present in high digestibility cultivar. This study does not support that modern wheat is harmful for human health. In fact, modern wheat is better than historical wheat in many different ways.