

Functionality and characterization of intermediate wheatgrass in spontaneous fermentation and
end product quality

by

Brianna Iorga

B.S., Oklahoma State University, 2022

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

Approved by:

Major Professor
Elisa Karkle

Copyright

© Brianna Iorga 2024.

Abstract

Intermediate wheatgrass (IWG) is a perennial wheat and found to have better environmental effects compared to annual plants. Soil nutrition is increased, the plant is drought and disease tolerant, and there is a decrease in labor input. There are also nutritional benefits found in IWG, such as a higher protein, fiber, and mineral content than that of wheat. IWG yield and kernel size is smaller, when compared to wheat though. But the biggest drawback is the decreased amount of high-molecular-weight glutenin subunits (HMW-GS). Throughout various research IWG has been tested in bread products and due to lack of HMW-GS has a poor performance. The Land Institute has been spearheading breeding efforts however and has made improvements to the mixing quality. This study evaluated the breeding efforts and measured the performance of four IWG cultivars in breadmaking, where one cultivar was specifically bred for mixing quality (MQ). Mixograph parameters and baking trial results were found to be significantly different for MQ. The breeding efforts were found to be successful as dough quality, mixing tolerance, bread volume, and number of cells all increased.

The high protein, ash, and starch content indicated IWG would be a suitable candidate in sourdough fermentation. The objective of this study was to determine the performance of IWG in a spontaneous fermentation process, compared to a whole wheat (WW) flour. Three fermentation trials were conducted to determine processing, acidification kinetics, lactic acid bacteria (LAB) and metabolite evolution. IWG was found to have a slightly higher $V_{\max}$ than WW but achieved higher final TTA. The fermentation quotient (FQ) and lactic acid production were also found to be higher for IWG, in agreement with high LAB enumeration. The sourdough was also utilized in a wheat-based bread trial and were analyzed for specific volume, firmness, acidity, and a first

day to visual mold analysis. The IWG bread was found to be more acidic but had no negative effects to the product quality including volume and post-bake firmness.

The spontaneously fermented sourdoughs were also evaluated in a sensory analysis. IWG sourdough or whole wheat sourdough was added to a wheat-based formulation and evaluated with a degree of difference (DOD) test and Just About Right scale (JAR) (N= 50 responses in 2 sessions). This was to determine how similar or different IWG was from WW and determine how the breads do or do not meet consumer expectations. Four attributes (appearance, aroma, flavor, and texture) and overall questions were analyzed for both DOD and JAR. An action criteria of 2 was chosen for the DOD, signifying that below 2 there was low risk consumers would notice a difference. IWG had a DOD average of 2.4. However, panelists also found differences in a WW blind control (1.8). This is an indication that panelists were looking for a difference, thus actual difference can be assumed to be smaller. Both IWG and WW had an average JAR score above 3 (“meets expectations”), indicating that despite the differences noticed IWG still met consumer’s expectations. With formula optimization the samples could align more, allowing for the potential use of IWG in a sourdough process and wheat-based bread.

Table of Contents

List of Figures	viii
List of Tables	x
Acknowledgements	xi
Dedication	xii
Chapter 1 - Background	1
1.1 Historical Overview of Intermediate Wheat Grass	1
1.2 IWG Composition	3
1.3 IWG Protein Structure and Functionality	4
1.4 Mixing and Gluten Formation Overview	5
1.5 Sourdough Overview	6
1.5.1 Stable Sourdough Establishment and Properties	8
1.5.2 Microflora of Sourdough	11
1.6 Sensory Analysis Overview	12
1.7 Overall Project Aim	13
1.8 References	15
Chapter 2 - Functional Evaluation of Intermediate Wheatgrass Cultivars Bred for Mixing Quality as Compared to Parent Lines	19
2.1 Introduction	19
2.2 Materials and methods	20
2.2.1 Ingredients	20
2.2.2 Milling	21
2.2.3 Mixograph	21
2.2.4 Dough Preparation and Baking	22
2.2.5 Physical Measurements of Breads	23
2.2.6 Crumb Grain Analysis	23
2.2.7 Statistical Analysis	23
2.3 Results and Discussion	24
2.3.1 Variation in Mixing Properties	24
2.3.2 Variation in Final Product Properties	26

2.4 Conclusion	29
2.5 References.....	30
Chapter 3 - Functionality of Intermediate Wheatgrass in Spontaneous Sourdough Fermentation and Bread Quality	
3.1 Introduction.....	32
3.2 Materials and Methods.....	33
3.2.1 Ingredients.....	33
3.2.2 Milling.....	34
3.2.3 Raw Material Characterization	34
3.2.4 Sourdough Establishment and Characterization	35
3.2.4.1 Acidification Kinetics and LAB Evolution.....	36
3.2.5 Baking Trial	37
3.2.5.1 Physical Measurements of Breads	38
3.2.6 Statistical Analysis.....	39
3.3 Results and Discussion	39
3.3.1 Raw Material.....	39
3.3.2 Sourdough	43
3.3.2.1 Sourdough Establishment—11-day Fermentation Trial	43
3.3.2.2 Acidification Kinetics Fermentation Trial	46
3.3.2.3 LAB and Organic Acid Evolution Fermentation Trial	50
3.3.3 Baking Trial	58
3.5 Conclusion	58
3.6 References.....	60
Chapter 4 - Degree of Difference Sensory Testing of Breads Made with Intermediate Wheatgrass Versus Whole Wheat Spontaneously Fermented Sourdoughs	
4.1 Introduction.....	63
4.2 Materials and Methods.....	64
4.2.1 Sourdough Fermentation.....	64
4.2.2 Baking	65
4.2.3 Physical Measurement of Breads	66
4.2.4 Sensory Evaluation by the Degree of Difference (DOD) Test	67

4.2.5 Statistical Analysis	69
4.3 Results and Discussion	69
4.4 Conclusion	84
4.5 References	86
Chapter 5 - Overall Conclusions and Future Work	88
Appendix A - Chapter 2: Multivariate Analysis Tables	90
Appendix B - Chapter 4: Supplemental Information.....	92

List of Figures

Figure 1.1 Kernza kernels compared to hard red winter wheat kernels.....	2
Figure 1.2 Substrate and process factors impacting sourdough characteristics performances and the final products overall quality (adapted from Corsetti, 2013).....	8
Figure 2.1 Mixograph profiles of IWG cultivars.	25
Figure 2.2 Cross-section of finished IWG bread from each cultivar. Left to right is block 1, 2, 3, and 4 of each cultivar.	28
Figure 2.3 Cell images from C-Cell indicate how cells and cell wall are segmented.	29
Figure 3.1 Particle size distribution curve of WW, K6, and K7. Sieve opening sizes of 850 μm , 425 μm , 212 μm , 150 μm , 75 μm , 45 μm , and 25 μm were used.	40
Figure 3.2 Select Morphologi photos of WW, K6, and K7 flour particles representing the various size and shapes.	41
Figure 3.3 (a) 11-day fermentation trial pH progression and (b) 11-day fermentation trial TTA progression.	44
Figure 3.4 11-day fermentation trial final day metabolites production.	45
Figure 3.5 Acidification kinetics over 7 days, measured every 8 hours for the first 96 hours and then measured every 24 hours for the remainder. A table with V_{max} at a T_{max} of 40 hours is included, where different letters signify significant statistical difference at $p < 0.05$	47
Figure 3.6 Acidification kinetics fermentation trial TTA progression over 7 days, measured every 8 hours for the first 96 hours and then measured every 24 hours for the remainder.	48
Figure 3.7 Photo of WW, K6, and K7 sourdoughs on final day.....	49
Figure 3.8 (a) LAB evolution fermentation trial WW pH, TTA, and LAB evolution and (b) LAB evolution fermentation trial K6 pH, TTA, and LAB evolution and (c) LAB evolution fermentation trial K7 pH, TTA, and LAB evolution.	52
Figure 3.9 LAB evolution fermentation trial yeast and mold evolution for WW, K6, and K7.	53
Figure 3.10 (a) LAB evolution fermentation trial organic acid, ethanol, and glucose evolution for WW and (b) LAB evolution fermentation trial organic acid, ethanol, and glucose evolution for K6 and (c) LAB evolution fermentation trial organic acid, ethanol, and glucose evolution for K7.	56

Figure 3.11 LAB evolution fermentation trial lactic acid vs acetic acid evolution for WW, K6, and K7, along with a table of the fermentation quotient (FQ) from Day 7.	57
Figure 4.1 DOD scale ranging from 0 to 7.	69
Figure 4.2 JAR scale ranging from 1 to 5.	69
Figure 4.3 Degree of difference histograms (a) overall (b) appearance (c) aroma (d) flavor and (e) texture with each attributes average scores for K and WW. Black line represents action criteria chosen at 2. Signifying at the action criteria and below there is a low risk of consumers being able to notice a difference in the product. Above the action criteria it is assumed that there is a risk that consumers would notice a difference in the product, and as the score increases, the intensity of the difference also increases.	73
Figure 4.4 Just About Right histograms (a) overall (b) appearance (c) aroma (d) flavor and (e) texture with each attributes average scores for K and WW.	77
Figure 4.5 (a) Word cloud of open-ended question for liking K sample and (b) Word cloud of open ended question for disliking K sample. The larger, bolder words signify a higher association of characteristics among various panelists.	78
Figure 4.6 (a) Word cloud of open-ended question for liking WW sample and (b) Word cloud of open-ended question for disliking WW sample. The larger, bolder words signify a higher association of characteristics among various panelists.	79
Figure 4.7 C-Cell images for brightness of the final breads from each session.	82
Figure 4.8 Metabolites production result of WW and K sourdough from final day of establishment.	84

List of Tables

Table 2.1 Mixograph properties of the 4 IWG cultivars.....	26
Table 2.2 Baking properties and internal characteristics of the 4 IWG cultivars.	27
Table 3.1 (a) Volume based d10, d50, and d90 analysis and (b) WW, K6, and K7 flour particle size and shape analysis.....	41
Table 3.2 Raw material characterization: dietary fiber, α - and β -amylase, protein content, and mineral analysis.	42
Table 3.3 11-day fermentation trial final day fermentation quotient.....	46
Table 3.4 Acidification Kinetics Fermentation Trial Δ pH (final minus initial pH) and $V_{\max}$	47
Table 3.5 Color analysis of K6 vs. WW and K7 vs WW.	50
Table 3.6 Final product analysis: pH, TTA, 1-day post-bake firmness, 5-days post-bake firmness, and specific volume.	58
Table 4.1 Analytical results on the sourdough and final product.	80
Table 4.2 ΔL^* , Δa^* , Δb^* , and ΔE^*_{ab} averages of crust color K vs. WW.....	82

Acknowledgements

Firstly, I would like to acknowledge my adviser and my committee members who have helped me tremendously on this journey: Dr. Elisa Karkle, Dr. Valentina Trinetta, and Dr. Hikmet Boyacioglu.

Secondly, I would like to acknowledge The Land Institute, Sustain-A-Grain, and Addie Clary along with the KSU Agronomy Farm for the donations of grain utilized.

Thirdly, I would like to acknowledge everyone who have taught and helped me with experiments and/or permission to use their labs and equipment: Dr. Valentina Trinetta and her students, Dr. Kaliramesh Siliveru and his students, Dr. Yi Zheng and Meicen Liu, Aaron Clanton, Dr. Gengjun Chen and the Wheat Quality Lab, Valerie Rexroth, Jason Watt, the USDA (Mayra Perez-Fajardo), Dr. Hulya Dogan, Andrew Dorsch, Riley Smith, my lab mates, and the Pet Food Program Lab.

Fourthly, I would like to give an extra acknowledgment to Aaron Clanton, Andrew Dorsch, Riley Smith, and the Bakery Science Undergraduates (you know who you are) for all of the answered questions, wisdom, memories and the plethora of laughs shared. :)

Lastly, I would like to give a special acknowledgement to the panelists in my sensory evaluation, it wouldn't have been the same without all of you.

Dedication

I also wanted to make another important acknowledgment and a dedication to the Lord. This experience would have been entirely different without Him, and I am grateful for all of the opportunities and lessons I've had during my time here. All the glory is yours. "I can do all things through Him who gives me strength." -Philippians 4:13

I wanted to dedicate this to my adviser, Dr. Elisa Karkle. Thank you for all of the laughs, encouragement, and opportunities you have given me throughout my time here. We were able to visit Taiwan and Europe together! Writing this thesis has been a rollercoaster and I'm grateful for the support and sticking with me to the end. Thank you for taking a chance on me as your first student (and letting me be a Guinea pig) and expanding my knowledge and passion for the bakery world.

Another dedication is to Aaron Clanton, an unofficial adviser and part-time Peanuts therapist. I have enjoyed learning from you, asking you all of my crazy questions, and all of the silly jokes shared. Thank you for always lending a listening ear, and also sparking my passion for the bakery industry.

I also wanted to dedicate this to Renee Albers-Nelson and Dr. Bailey Norwood from Oklahoma State University. You both had such a strong impact in directing my path to get here and find my love for the bakery industry. So, thank you and Go Pokes!

Kristina and Edma, you have both helped keep me sane during some crazy times. I know I can come to you guys with anything and I'm so grateful to have both of you in my life. Here is to more laughs, memories, and crazy stories to share!

A final dedication is to my parents for the constant support and advice. Thank you both for allowing me to follow my crazy ideas and always being there when I need you. Vă iubesc!

Chapter 1 - Background

1.1 Historical Overview of Intermediate Wheat Grass

Cereal grains are an important food source around the world. Wheat, rice and maize alone contribute approximately half of the global caloric intake and about 40% of protein intake (Erenstein et al., 2022). As annual crops, production has been associated with high fertilizer input, soil erosion, reduced microbial biomass, among other environmental concerns (Taylor et al., 2023, Bharathi et al., 2022, Jungers et al., 2019). Perennial crops can address many of these concerns, thus there is interest in identifying and breeding perennial cereals for food. Annual crops require planting every year, but perennials can be planted once and have multiple yields over multiple years. Intermediate wheatgrass (IWG, *Thinopyrum intermedium*) has been under investigation and development as a potential option.

IWG, native of Western Asia, was introduced as a forage crop in the United States in 1932 (Bharathi et al., 2022). Since the 1980's, various groups began reporting IWG's nutritional composition and functionality as a food source (Becker et al., 1991). While gluten formation is lacking, the grain's nutritional profile often exceeds wheat and other cereal grains, creating numerous possibilities to explore where IWG may be most beneficial in the food chain.

One of the institutions spearheading the breeding effort is The Land Institute in Kansas, USA, also giving IWG the trademark name Kernza. However, for the purpose of this thesis, it will be referred to as intermediate wheatgrass (IWG). The Land Institute has been responsible for many of the breeding adaptations made to improve IWG since the mid-2000s (Bharathi et al., 2022). According to DeHaan et al. (2023), five geographically diverse breeding programs are currently in place, addressing yield, seed size and other traits.

The Land Institute's breeding program initially relied on phenotypic selection, but as high-efficiency, low-cost DNA sequencing advanced, the breeding cycle was reduced from three years to one year per cycle. The narrow endosperm reflects the short history of domestication; however, breeding efforts have successfully increased the endosperm (Tyl et al., 2019). Kernel weight on a wet basis is about 10 mg, as compared to 40 mg for annual wheat (Marcus and Fox, 2022). The first official release of IWG was the MN 1504 cultivar, now referred to as MN Clearwater, in the Fall of 2019 for commercial usage (Bharathi et al., 2022).



Figure 1.1 Kernza kernels compared to hard red winter wheat kernels.

1.2 IWG Composition

IWG has an extensive root system, which can measure upwards of 3 meters, compared to wheat's 50–300 centimeter root system (Marcus and Fox, 2022). Such root system has been associated with retention of organic carbon in the soil, helping improve soil health, and increasing the efficiency of water usage. IWG has shown 96-99% less nitrate leaching as compared to maize (Jungers et al., 2019). Soil fungal richness for IWG was increased as compared to annual wheat (Taylor et al., 2023). The root system associated with no annual disturbance is responsible for reduced erosion. With IWG's increased resistance to drought, frost, and common wheat diseases, pesticide usage is also potentially reduced (Bharathi et al., 2022; Marcus and Fox, 2022; Marti et al., 2015). These environmental benefits place IWG as an important player in the search for more sustainable crops and practices.

Whole-milled IWG has a higher protein content (20% versus 15%), high ash (2.6%), and dietary fiber (16.8% versus 2.5%), but adequate starch (46.7 versus 73.9) when compared to wheat (Marti et al., 2015). The breeding process does affect the chemical composition of the crop, specifically ash and protein content. These changes are associated with the breeding program and efforts made to increase the grain size (Bharathi et al., 2022). However, with the breeding efforts successfully increasing the endosperm, breeders were able to retain most of the protein while decreasing the insoluble fiber portion.

There is an indication of higher quantities of arabinoxylans and cellulose within the high insoluble fiber of IWG. The arabinoxylans had little branching, sharing some structural features with wheat and rye (Bharathi et al., 2022). According to Dai et al. (2021) cereal fiber intake has been shown to decrease the risk of certain types of cancer, related mostly to the digestive tract, along with cardiovascular diseases. Increasing cereal bran in products has been one way to

address this, but consumer acceptance is often reduced. This may be partially because of the structural and textural differences when adding cereal bran. Many studies have looked at ways to combat low acceptance by reducing particle size or employing fermentation. Such strategies are relevant to add value to grains high in insoluble dietary fiber, such as IWG (Dai et al., 2021). Whole grains are seen as “healthy” to consumers and high fiber containing foods, particularly IWG can help fulfill this demand due to the high bran to endosperm ratio (Bharathi et al., 2022).

The starch gelatinization temperature of IWG is 62-68°C, compared to wheat’s range of 58-64°C (Becker et al., 1991). The setback and final viscosities are decreased, compared to wheat, which could indicate a low retrogradation tendency. This can be relevant for bread shelf life, as setback is correlated to bread staling during storage (Bharathi et al., 2022; Marti et al., 2015). On the other hand, IWG is more susceptible than wheat to rancidity during storage due to the higher lipid content (Bharathi et al., 2022). The polyphenol oxidase (PPO) activity of IWG was similar to wheat (Tyl et al., 2019).

According to Becker et al., (1991), lysine is the limiting amino acid for IWG, as it is for wheat. However, IWG has substantially higher content of the other essential amino acids compared to wheat. Uncooked IWG flour trypsin inhibitor and hemagglutinin activity levels are significantly lower compared to wheat and triticale (Becker et al., 1991). Therefore, after consuming IWG is less likely to have opposing effects (Bharathi et al., 2022).

1.3 IWG Protein Structure and Functionality

Despite the high protein content of IWG, gluten formation is considered minimal. Gluten is a complex structure made up of two main proteins, gliadin and glutenin, which are mixed in the presence of water. High molecular weight glutenin subunits (HMW-GS) are the protein

fractions related to the dough-forming capacity of wheat. IWG contains HMW-GS in insufficient amounts as compared to wheat. However, low molecular weight glutenin subunits (LMW-GS) along with α -, β - and γ - gliadins were abundant (Rahardjo et al., 2018). Gliadins plasticize and provide mobility to polymeric HMW-GS (Van Der Borgh et al., 2005). In wheat, the appropriate amounts result in the viscoelasticity of doughs, whereas the elastic component is mostly deficient in IWG. HMW-GS plays a key role in improving dough strength through the facilitation of protein-protein interactions (Bharathi et al., 2022). The lack of HMW-GS suggests a weak gluten structure and gas holding capacity, thus impacting dough strength, elasticity, and final bread quality (Rahardjo et al., 2018). It is important to point out that in an evaluation of 60 genotypes, Zhang et al. (2015) identified select genotypes with HMW-GS and mixing parameters comparable to wheat. Thus, targeted breeding may improve the dough-forming properties of IWG.

1.4 Mixing and Gluten Formation Overview

Mixing is one of the ways gluten development is established. Covalent and non-covalent forces stabilize a continuous three-dimensional form of gluten proteins (Shewry, 2023). The HMW-GS play an important role in the mixing quality as they are largely responsible for the formation of gluten. Gliadin is responsible for the extensibility of a dough, while glutenin is responsible for the elasticity and strength of the dough (Bharathi et al., 2022). Different flour strengths have different ratios of extensibility and elasticity, indicating certain products where they would perform better. According to Rahardjo et al. (2018) the stronger wheat flours have a balance between a higher resistance and extensibility, which is important for gas retention during fermentation for bread and other yeast-leavened products. On the other hand, soft wheat flours

have a higher extensibility and lower resistance to extension, better suited for cakes and pastries (Rahardjo et al., 2018).

Along with developing gluten, mixing has a few other main functions, including producing a homogenous structure, incorporating air into the dough, and hydrating the flour particles and other dry ingredients (Yazar, 2023; Sun et al., 2022; Dobraszczyk and Morgenstern, 2003). Hydration of the flour is important in proper development. The mixer's blades wear down the flour particles as the water is incorporated with the proteins causing more water-protein interaction (Yazar, 2023), thus creating a continuous three-dimensional gluten matrix.

Water absorption is an important aspect and shows the amount of water in farinograph testing, that is required to reach 500 BU dough consistency, which can be affected by various factors such as damaged starch, fiber, and the quality of gluten proteins (Rahardjo et al., 2018). Fiber-rich samples have been studied to absorb copious amounts of water, mostly determined by the large presence of hydroxyl groups, and interacting with the water through hydrogen bonding, thus also increasing the mixing torque (Rahardjo et al., 2018; Marti et al., 2015). However, such torque increase does not translate into better protein polymerization and gas retention capacity. Marti et al. (2015) found as the percentage of IWG is increased in a wheat-IWG flour blend, the number of accessible thiols per gram also increased. The high levels of thiols did not seem to be as available for aggregation as seen in wheat (Marti et al., 2015).

1.5 Sourdough Overview

In the United States, there is no regulation for what constitutes a sourdough. There is sourdough bread, but sourdough at its core is a process and can be utilized as an ingredient. However, at its base it is a mixture of flour and water, and possibly salt, which is allowed to ferment for a certain time. Sourdough made this way is referred to as spontaneous fermentation

because the fermentation is spontaneous lactic acid bacteria and yeasts sourced from the flour and environment (Corsetti, 2013), rather than adding a specific culture. Typically, one or two specialized bacteria can be used to inoculate a sourdough, which is commonly used in the large-scale baking industry. The acidification process is the lactic acid bacteria's effect, whereas yeasts are responsible for the leavening of the dough through releasing CO₂ (Corsetti, 2013). After the first initial mixture of flour and water ferments for the specified time, it now becomes referred to as a starter. In spontaneous fermentation, consecutive refreshments, more commonly referred to as backslopping, occur to obtain and optimize the microbial community's metabolic activity. Backslopping is when a portion, or all, of the starter is kept and used to inoculate a fresh flour and water mixture and undergoes fermentation for the same specified time, typically 24 hours. If a sourdough mixture and consecutive backslopping is started to be used as a leavening agent, the term "mother sponge" is given (Corsetti, 2013). A mature sourdough is when the technological properties such as acidification or leavening capacity are constant (Ercolini et al., 2013).

Sourdough has been categorized into three main types: I, II, and III (Calvert et al., 2021). Type I sourdough is the traditional sourdough method, where the microorganism's metabolic activity is maintained through daily backslopping. This type of sourdough typically accomplishes dough leavening without the supplementation of baker's yeast (Corsetti, 2013). Type II sourdough is typically a pumpable liquid sourdough. This sourdough type is kept in a temperature controlled industrial level bioreactor or tank with the main goal being to shorten the fermentation process (Corsetti 2013). Baker's yeast can be added to decrease the processing time; although, Type II sourdough is not ideal for leavening, but rather utilized for dough acidification and a dough improver (Arora et al., 2021; Corsetti 2013). Type III sourdough are simply Type II sourdoughs which have been dried and stabilized after development. These are

typically used on the industrial level as they resemble a type I sourdough's quality but are easier to manage and require less time (Corsetti, 2013).

1.5.1 Stable Sourdough Establishment and Properties

There are many factors that influence the microflora of sourdough, thus impacting the final product quality as seen in Figure 1.2. These characteristics relate to a sourdough's performance and can be determined through parameters such as pH, TTA, and absorption. The final pH of a sourdough starter is typically around 3.5-4.3 and is used to measure a mature sourdough (Corsetti, 2013). However, the industry standard is to measure the total titratable acidity (TTA) as it measures the total organic acids, with direct influence on product flavor.

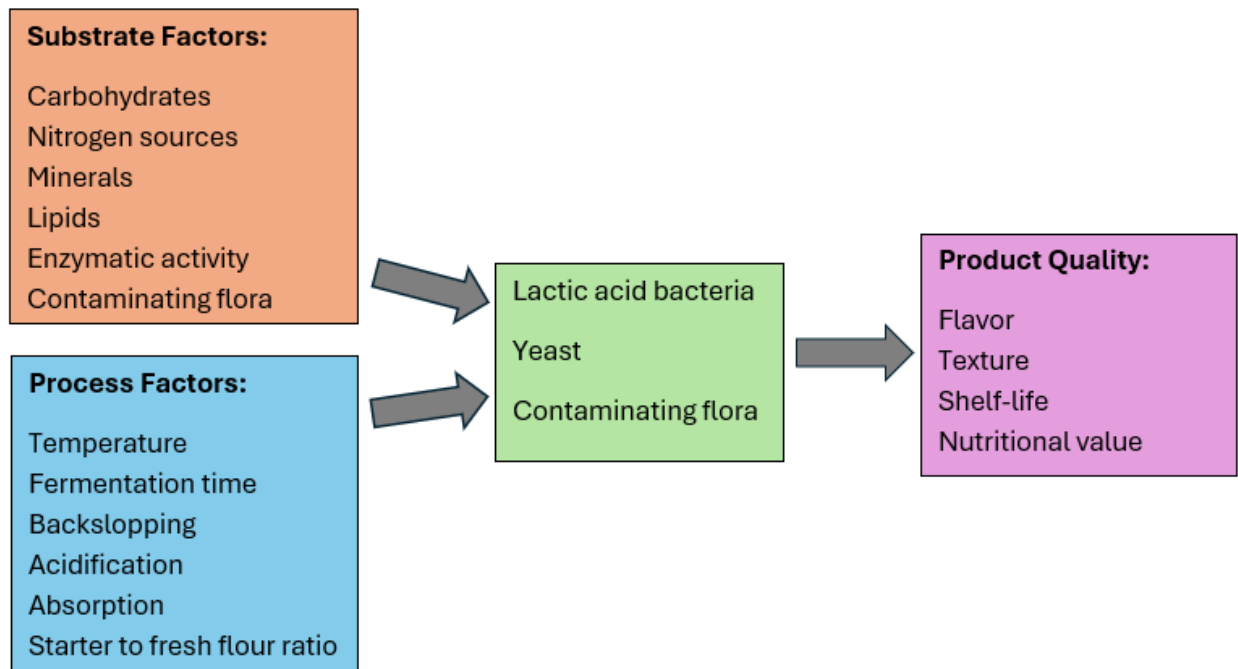


Figure 1.2 Substrate and process factors impacting sourdough characteristics performances and the final products overall quality (adapted from Corsetti, 2013).

Process factors and interactions between microorganisms influence the formation of a stable microflora as well as the final characteristics of a sourdough (Galli et al., 2019).

Establishing a stable sourdough allows for a microorganism, or several, to become the dominant species and have a constant presence and behavior within the ever-changing sourdough.

Spontaneous fermentation relies on the flour and environment to introduce microorganisms. The contaminating microorganisms will become metabolically active upon the addition of water, and at the end of the process the most competitive microorganism(s) will dominate (Hammes et al., 2005). In a study by Galli et al. (2019), some strains of lactobacilli and yeasts were affected by particular fermentation conditions, despite the absence of competition for the microorganisms present in the sourdough. Due to substrate and processing factors, a mature sourdough's microbial community is vastly diverse and complex.

To establish a controlled sourdough process, continuous backslopping will provide a stable consortium of a few dominant lactic acid bacteria (LAB) and yeast species. During backslopping, the sourdough LAB metabolic profiles tend to vary due to changes in the bacterial population. Continuously backslopped sourdoughs over a period provides an opportunity for improbable sourced contaminants to set themselves up in the sourdough microbiota as a stable member (Ganzle and Zheng, 2019). Although between the artisan and laboratory stages other non-dominant LAB species could temporarily contaminate sourdoughs.

Throughout various literature, there was a consensus that with spontaneous fermentation and continuous feedings a stable, mature sourdough was established in about 5-7 days (Galli et al., 2019; Ercolini et al., 2013). After about five days, microbial diversity was shown to be simplified and has continually persisted (Ercolini et al., 2013). All sourdoughs contained specific species and strains which dominated with time and were influenced by the environment; however, cell density and other related biochemical attributes were not affected by the environment (Corsetti, 2013).

Biochemical changes influence the sourdough properties, thus the quality and structure of the bread or other final product. During fermentation LAB and yeast produce metabolites such as organic acids, exopolysaccharides (EPS), enzymes, and CO₂ which contribute to these effects.

The drop in pH due to the production of organic acids causes significant changes in a dough's structure forming constituent. The acidic environment causes a positive net charge for the gluten proteins, increasing the swelling and solubility capacity, which is the acids primary impact (Galle, 2013). Doughs with a pH of 4 have increased proteolysis, thus deteriorating the gluten structure and reducing the sourdough's firmness and elasticity (Arendt et al., 2007), which allows for a softer, more extensible dough with increased gas retention. This is associated with an increase in the volume of the bread, and therefore a decreased staling rate. However, as sourdough acidity increases, bread volume will decrease. The pH drop also affects amylase activity by partially inactivating them. LAB-produced enzymes primary effect has been particularly associated with explaining the staling of sourdough breads (Galle, 2013; Arendt et al., 2007). Staling rates of sourdough breads are varied, despite similar acidity levels. The most effective LAB strains in delaying staling are contributed to their proteolytic and amylolytic activity (Galle, 2013).

Exopolysaccharides are bacterial polysaccharides which have leached into the environment. They can be separated into two classes, homopolysaccharides and heteropolysaccharides, which contains one type of monosaccharide or several monosaccharides, respectively. EPS can be synthesized by some LAB strains from sucrose and has a few main functions in sourdough production: improving the bread's nutritional value, texture, and shelf-life (Galle, 2013). The viscosity of a sourdough is influenced by a LAB's ability to form EPS. The dough and bread structure can also be further improved from LAB-produced EPS. The

dough's water absorption, rheology, bread's softness, crumb freshness, and specific volume are beneficially impacted (Galle, 2013; Tieking and Ganzle, 2005).

1.5.2 Microflora of Sourdough

The cell density of yeasts and LAB are typically estimated with a standard plate count (Corsetti, 2013). The specific yeasts and LAB present in a sample are able to be profiled through untargeted sequencing, also known as shotgun metagenomics (Quince et al., 2017). Shotgun metagenomics can read the whole genome sequence, compared to 16S rRNA gene sequencing, which can only read one specific DNA region. Thus, allowing microbial communities to be profiled for composition and functional potential. There are five steps that comprise a shotgun metagenomic study: experimental pipeline, pre-processing, sequence analysis, post-processing, and validation (Quince et al., 2017).

The most common microflora in sourdough comes from the lactic acid bacteria (LAB) family along with yeasts. For wheat specifically, the most common LAB in a traditional type I sourdough is dominated by *Fructolactobacillus sanfranciscensis*, *Lactiplantibacillus plantarum* subspecies *plantarum*, *Furfurilactobacillus rossiae*, *Levilactobacillus brevis*, *Companilactobacillus alimentarius*, along with other *Companilactobacillus* species (such as *L. paralimentarius*, *L. crustorum*, *L. mindensis*, and *L. nantensis*), *Leuconostoc* species, and *Weissella* species; due to the low incubation temperature and continuous backslopping (Zheng et al., 2020; Galli et al., 2019; Ganzle and Zheng, 2019; Di Cagno et al., 2014). A key LAB in type I sourdough has been found to be *F. sanfranciscensis*. *F. sanfranciscensis* has been well characterized as a key LAB species in sourdough due to its' production of EPS (Corsetti and Settanni et al., 2007). While the most common yeast species are *Saccharomyces cerevisiae*, *Candida milleri*, and *Issatchenkia orientalis* (Galli et al., 2019; Hammes et al., 2005).

1.6 Sensory Analysis Overview

The use of IWG in a variety of products has been under experimentation. A sparing application of IWG has pertained to beer and food (Marcus and Fox, 2022). On top of beer, whiskey has also been explored, as well as pancakes, cookies, banana cake, muffins, bread, extruded snacks and even ice cream. The results of bread have been the most acceptable when there is a blend with wheat flour. Bakery products where extensibility is desirable are likely candidates for development (flat breads, tortillas, crackers, and batter-based products) (Bharathi et al., 2022).

Sensory evaluation is important as it measures the sensory perception of a consumer towards a product (Delarue, 2022). There are two broad categories sensory evaluation fall under, analytical tests or affective tests, and within each category there are multiple types of tests. Analytical tests objectively evaluate the sensory characteristics of products, whereas affective tests measure consumer acceptability and/or preference. Each test requires varying types of panels, trained or untrained, with different amounts of panelists. Deciding the best test entirely depends on the goal and the over-arching question one wants answered from a sensory evaluation. Standardized terminology used across various audiences to assist communication is considered a lexicon and plays a large role in uniting countries with an exact sensory analysis to illustrate similar products (Tran et al., 2019).

Becker et al. (1991) first published the application of stone ground IWG flour with modified commercial recipes. Bread was made with 15% substitution of wheat flour with IWG, and the addition of 5% vital wheat gluten. The bread had a crust and crumb appearance similar to whole grain breads, with a distinct nutty-grain flavor. The muffins and cookies were produced with 100% IWG flour. The cookies had a sweet taste, with a texture that was soft and broke

easily without falling apart. The banana loaf cake was made with 50% each whole wheat IWG flour and whole wheat flour and 100% bananas. The overall flavor profile was a mild banana-nutty taste. The crumb appearance was described as good and moist, but not soggy. A sensory panel with 57 members of experience ranging from novel to expert judged the samples, using a 9-point scale (very poor-excellent) to judge appearance, texture, flavor, and overall characteristics. All products scored in the range between 5-9 (fair to excellent) for all attributes, with texture and flavor scoring lowest. No further product quality evaluation was presented (Becker et al., 1991).

In a study over the aroma profiles of whole grain IWG and whole wheat bread by Paravisini et al. (2019), they found the predominant aroma characteristics to be bran, roasted, earthy, green, oxidized, and mushroom in the crust of their bread samples. The difference between the two sets of samples was intensity as they had the same characteristics. For the wheat samples, roasted was the most intense aroma attribute and was higher compared to the IWG samples. For the IWG samples, bran and green attributes were significantly higher compared to the wheat, which could have been accentuated due to the decreased levels of Maillard compounds, and thus lower roasted notes. The notes of earthy and oxidized were similar between the wheat and IWG (Paravisini et al., 2019).

1.7 Overall Project Aim

The environmental benefits and good nutritional profile IWG possess make it an appealing alternative to annual grains. Efforts to find IWG's role in the food industry, along with improving the gluten structure and yield size have been emphasized. The aim of this project was to study 3 different areas: 1) evaluating the breeding efforts towards mixing quality of IWG, 2) evaluating the functionality (physical, chemical, and microbial characteristics) of IWG as a

substrate in sourdough fermentation, and 3) perform a sensory analysis on wheat bread with the addition of whole wheat or whole grain IWG sourdough.

1.8 References

- Arendt, E. K., Ryan, L. A. M., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*, 24(2), 165–174. <https://doi.org/10.1016/j.fm.2006.07.011>
- Arora, K., Ameer, H., Polo, A., Di Cagno, R., Rizzello, C. G., & Gobbetti, M. (2021). Thirty years of knowledge on sourdough fermentation: A systematic review. *Trends in Food Science & Technology*, 108, 71–83. <https://doi.org/10.1016/j.tifs.2020.12.008>
- Becker, R., Wagoner, P., Hanners, G. D., & Saunders, R. M. (1991). Compositional, nutritional and functional evaluation of Intermediate Wheatgrass (*Thinopyrum intermedium*). *Journal of Food Processing and Preservation*, 15(1), 63–77. <https://doi.org/10.1111/j.1745-4549.1991.tb00154.x>
- Bharathi, R., Muljadi, T., Tyl, C., & Annor, G. A. (2022). Progress on breeding and food processing efforts to improve chemical composition and functionality of intermediate wheatgrass (*Thinopyrum intermedium*) for the food industry. *Cereal Chemistry*, 99(2), 235–252. <https://doi.org/10.1002/cche.10482>
- Calvert, M. D., Madden, A. A., Nichols, L. M., Haddad, N. M., Lahne, J., Dunn, R. R., & McKenney, E. A. (2021). A review of sourdough starters: Ecology, practices, and sensory quality with applications for baking and recommendations for future research. *PeerJ*, 9, e11389. <https://doi.org/10.7717/peerj.11389>
- Corsetti, A. (2013). Technology of sourdough fermentation and sourdough applications. In M. Gobbetti & M. Gänzle (Eds.), *Handbook on Sourdough Biotechnology* (pp. 85–103). Springer US. https://doi.org/10.1007/978-1-4614-5425-0_4
- Corsetti, A., & Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Research International*, 40(5), 539–558. <https://doi.org/10.1016/j.foodres.2006.11.001>
- Dai, Y., Bharathi, R., Jungers, J., Annor, G. A., & Tyl, C. (2021). Effect of Bran Pre-Treatment with Endoxylanase on the Characteristics of Intermediate Wheatgrass (*Thinopyrum intermedium*) Bread. *Foods (Basel, Switzerland)*, 10(7), 1464. <https://doi.org/10.3390/foods10071464>
- DeHaan, L. R., Anderson, J. A., Bajgain, P., Basche, A., Cattani, D. J., Crain, J., Crews, T. E., David, C., Duchene, O., Gutknecht, J., Hayes, R. C., Hu, F., Jungers, J. M., Knudsen, S., Kong, W., Larson, S., Lundquist, P.-O., Luo, G., Miller, A. J., ... Westerbergh, A. (2023). Discussion: Prioritize perennial grain development for sustainable food production and environmental benefits. *Science of The Total Environment*, 895, 164975. <https://doi.org/10.1016/j.scitotenv.2023.164975>
- Delarue, J. (2022). 2—Overview of sensory methods for the evaluation of nonfood products. In A.-M. Pensé-Lhéritier, I. Bacle, & J. Delarue (Eds.), *Nonfood Sensory Practices* (pp. 17–37). Woodhead Publishing. <https://doi.org/10.1016/B978-0-12-821939-3.00020-8>

- Di Cagno, R., Pontonio, E., Buchin, S., De Angelis, M., Lattanzi, A., Valerio, F., Gobbetti, M., & Calasso, M. (2014). Diversity of the Lactic Acid Bacterium and Yeast Microbiota in the Switch from Firm- to Liquid-Sourdough Fermentation. *Applied and Environmental Microbiology*, 80(10), 3161–3172. <https://doi.org/10.1128/AEM.00309-14>
- Dobraszczyk, B. J., & Morgenstern, M. P. (2003). Rheology and the breadmaking process. *Journal of Cereal Science*, 38(3), 229–245. [https://doi.org/10.1016/S0733-5210\(03\)00059-6](https://doi.org/10.1016/S0733-5210(03)00059-6)
- Ercolini, D., Pontonio, E., De Filippis, F., Minervini, F., La Storia, A., Gobbetti, M., & Di Cagno, R. (2013). Microbial Ecology Dynamics during Rye and Wheat Sourdough Preparation. *Applied and Environmental Microbiology*, 79(24), 7827–7836. <https://doi.org/10.1128/AEM.02955-13>
- Erenstein, O., Jaleta, M., Mottaleb, K. A., Sonder, K., Donovan, J., & Braun, H.-J. (2022). Global Trends in Wheat Production, Consumption and Trade. In M. P. Reynolds & H.-J. Braun (Eds.), *Wheat Improvement: Food Security in a Changing Climate* (pp. 47–66). Springer International Publishing. https://doi.org/10.1007/978-3-030-90673-3_4
- Galle, S. (2013). Sourdough: A tool to improve bread structure. In M. Gobbetti & M. Gänzle (Eds.), *Handbook on Sourdough Biotechnology* (pp. 217–228). Springer US. https://doi.org/10.1007/978-1-4614-5425-0_8
- Galli, V., Venturi, M., Pini, N., Guerrini, S., Granchi, L., & Vincenzini, M. (2019). Liquid and firm sourdough fermentation: Microbial robustness and interactions during consecutive backsloppings. *LWT*, 105, 9–15. <https://doi.org/10.1016/j.lwt.2019.02.004>
- Gänzle, M. G., & Zheng, J. (2019). Lifestyles of sourdough lactobacilli – Do they matter for microbial ecology and bread quality? *International Journal of Food Microbiology*, 302, 15–23. <https://doi.org/10.1016/j.ijfoodmicro.2018.08.019>
- Hammes, W. P., Brandt, M. J., Francis, K. L., Rosenheim, J., Seitter, M. F. H., & Vogelmann, S. A. (2005). Microbial ecology of cereal fermentations. *Trends in Food Science & Technology*, 16(1), 4–11. <https://doi.org/10.1016/j.tifs.2004.02.010>
- Jungers, J. M., DeHaan, L. H., Mulla, D. J., Sheaffer, C. C., & Wyse, D. L. (2019). Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. *Agriculture, Ecosystems & Environment*, 272, 63–73. <https://doi.org/10.1016/j.agee.2018.11.007>
- Marcus, A., & Fox, G. (2022). Malting and Wort Production Potential of the Novel Grain Kernza (*Thinopyrum intermedium*). *Journal of the American Society of Brewing Chemists*, 0(0), 1–11. <https://doi.org/10.1080/03610470.2022.2026662>
- Marti, A., Qiu, X., Schoenfuss, T. C., & Seetharaman, K. (2015). Characteristics of Perennial Wheatgrass (*Thinopyrum intermedium*) and Refined Wheat Flour Blends: Impact on Rheological Properties. *Cereal Chemistry*, 92(5), 434–440. <https://doi.org/10.1094/CCEM-01-15-0017-R>

- Paravisini, L., Sneddon, K. A., & Peterson, D. G. (2019). Comparison of the aroma profiles of intermediate wheatgrass and wheat bread crusts. *Molecules*, 24(13), 2484. <https://doi.org/10.3390/molecules24132484>
- Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*, 35(9), 833–844. <https://doi.org/10.1038/nbt.3935>
- Rahardjo, C. P., Gajadeera, C. S., Simsek, S., Annor, G., Schoenfuss, T. C., Marti, A., & Ismail, B. P. (2018). Chemical characterization, functionality, and baking quality of intermediate wheatgrass (*Thinopyrum intermedium*). *Journal of Cereal Science*, 83, 266–274. <https://doi.org/10.1016/j.jcs.2018.09.002>
- Shewry, P. (2023). Wheat grain proteins: Past, present, and future. *Cereal Chemistry*, 100(1), 9–22. <https://doi.org/10.1002/cche.10585>
- Sun, X., Koksel, F., Scanlon, M. G., & Nickerson, M. T. (2022). Effects of water, salt, and mixing on the rheological properties of bread dough at large and small deformations: A review. *Cereal Chemistry*, 99(4), 709–723. <https://doi.org/10.1002/cche.10561>
- Taylor, K., Samaddar, S., Schmidt, R., Lundy, M., & Scow, K. (2023). Soil carbon storage and compositional responses of soil microbial communities under perennial grain IWG vs. Annual wheat. *Soil Biology and Biochemistry*, 184, 109111. <https://doi.org/10.1016/j.soilbio.2023.109111>
- Tieking, M., & Gänzle, M. G. (2005). Exopolysaccharides from cereal-associated lactobacilli. *Trends in Food Science & Technology*, 16(1), 79–84. <https://doi.org/10.1016/j.tifs.2004.02.015>
- Tran, T., James, M. N., Chambers, D., Koppel, K., & Chambers, E. (2019). Lexicon development for the sensory description of rye bread. *Journal of Sensory Studies*, 34(1), e12474. <https://doi.org/10.1111/joss.12474>
- Tyl, C., Bharathi, R., Schoenfuss, T., & Annor, G. A. (2019). Tempering Improves Flour Properties of Refined Intermediate Wheatgrass (*Thinopyrum intermedium*). *Foods* (Basel, Switzerland), 8(8), E337. <https://doi.org/10.3390/foods8080337>
- Van Der Borght, A., Goesaert, H., Veraverbeke, W. S., & Delcour, J. A. (2005). Fractionation of wheat and wheat flour into starch and gluten: Overview of the main processes and the factors involved. *Journal of Cereal Science*, 41(3), 221–237. <https://doi.org/10.1016/j.jcs.2004.09.008>
- Yazar, G. (2023). Wheat Flour Quality Assessment by Fundamental Non-Linear Rheological Methods: A Critical Review. *Foods*, 12(18), 3353. <https://doi.org/10.3390/foods12183353>
- Zhang, X., Ohm, J.-B., Haring, S., DeHaan, L. R., & Anderson, J. A. (2015). Towards the understanding of end-use quality in intermediate wheatgrass (*Thinopyrum intermedium*):

High-molecular-weight glutenin subunits, protein polymerization, and mixing characteristics. *Journal of Cereal Science*, 66, 81–88.

<https://doi.org/10.1016/j.jcs.2015.10.008>

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'Toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 70(4), 2782–2858.

<https://doi.org/10.1099/ijsem.0.004107>

Chapter 2 - Functional Evaluation of Intermediate Wheatgrass Cultivars Bred for Mixing Quality as Compared to Parent Lines

2.1 Introduction

Food security has become a pressing topic in today's world. The search for alternative grains has been a long road with the attention being turned to perennial crops. Not only do they expand on options for food use, but many can also offset ecological problems associated with annual crops (such as soil quality and fertilizer needs) (Taylor et al., 2023, Bharathi et al., 2022, Jungers et al., 2019). Intermediate wheatgrass (IWG) has been developed by The Land Institute within the last 20 years and is the first commercially available perennial crop (Bharathi et al., 2022). While IWG has a positive impact on carbon sequestration and prevention of soil erosion, there are still some challenges in end use quality (Bharathi et al., 2022; Jungers et al., 2019). Mixing quality has been identified as deficient, therefore, directing recent breeding efforts.

IWG has a weak gluten structure due to its lack of high-molecular-weight glutenin subunits (HMW-GS). HMW-GS are responsible for the majority of the gluten formation, and they play a key role in improving dough strength through the facilitation of protein-protein interactions (Bharathi et al., 2022). The amount of glutenin macropolymers correlates to the breadmaking quality (Shewry, 2022). In a study by Zhang et al. (2014), the breeders looked at genome cloning and protein biochemistry and discovered five HMW-GS genes. These genes resembled the same structure of HMW-GS as found in wheat, such as alike N-terminal regions, recurring domains, and C-terminal regions. One of the genes produced a protein product with an additional cysteine residue, which can engage in disulfide bond formations, therefore contributing to protein polymerization. This confirmed that IWG contained typical HMW-GS, same as found in common wheat and suggests with breeding efforts IWG could become suitable

for breadmaking (Zhang et al., 2014). Rahardjo et al. (2018), found a lack of mixing stability, staying less than two minutes and attributed it to the deficiency of HMW-GS. The farinograph measured a whole grain commercial hard red winter wheat's stability time to be around ten minutes (Rahardjo et al., 2018).

The Land Institute has been a front driver in the breeding efforts of IWG. They have been particularly focused on the functionality, while maintaining the high protein content. Efforts were put into improving the mixing quality and in forming a better gluten structure. A direct relationship between dough rheology and bread quality has been distinguished, and studying the dough rheological properties can predict a dough's processing performance throughout the breadmaking process (Sun et al., 2022). The objective of this project was to evaluate the effectiveness of breeding efforts focused on mixing quality among 4 different IWG cultivars: 701/702 (which will be referred to as 701), MQ, C5, and C9. MQ is the most advanced line bred and it was hypothesized that it would have better quality indicators.

2.2 Materials and methods

2.2.1 Ingredients

Four cultivars from four different blocks of IWG were obtained from crop breeding collaborators, The Land Institute. Samples MQ were obtained from breeding for mixing quality selection; C5 samples are starting materials prior to mixing quality selection; C9 samples are the most advanced types after breeding for seed size and yield, and 701/702 samples represent varieties currently being released to farmers. All samples were dehulled and cleaned prior to milling.

2.2.2 Milling

IWG kernels were milled with an Alpine Augsburg Pin Mill (Model #160z), yielding refined flour and bran. IWG flour and bran were sifted through 750, 500, 425, 355, 240, and 132 μm screens to evaluate particle size. Fractions above a 425 screen of the IWG flour and bran were reground the same way. To combine the bran and refined flour, the fractions were blended for five minutes in a 20-quart bowl (Hobart, Troy, Ohio) or hand-shaken until visually homogeneous.

One-stage moisture was measured following AACC Method 44-15.02 (AACC International, 1999) with slight modifications. Each cultivar and block were run in triplicates. The pan and lid were weighed together, then the lid was taken off and the pan weight was zeroed. 2 grams of sample was weighed in the pan and the dishes were placed in an air oven uncovered. Once the oven reached 130°C, the samples were heated for 60 minutes. Afterwards the lids were placed on the pans and placed in a desiccator using tongs. The samples were removed with tongs and reweighed after 45 minutes, once they reached room temperature. The moisture was calculated based on sample weight loss.

2.2.3 Mixograph

Using a 10-gram mixograph bowl, dough mixing characteristics were evaluated following AACC Method 54-40.02 (AACC International, 1999). Water absorption of 63% was used for all samples, as determined in preliminary trials. Parameters were selected for best description of mixing characteristics and differentiation between samples. They include midline peak time, midline peak value, midline peak integral, midline right slope plus 2 minutes, and midline slope peak plus 3 minutes. The parameters were evaluated using the MIXSMART

computer software (Version 2.0.609, (National Manufacturing Division, TMCO) Lincoln, Nebraska).

2.2.4 Dough Preparation and Baking

The optimized straight dough bread-baking AACC Method 10-10.03 was used with slight modifications (AACC International, 1999). Doughs were prepared using 100% flour basis (f.b.) of each whole grain milled flour, 63% distilled water, 1.7% f.b. instant active dry yeast, 3% f.b. all-purpose vegetable shortening, 6% f.b. sugar, and 1.5% f.b. salt. A pin mixer (National Manufacturing Co., Lincoln, Nebraska) was utilized and ran on empty for 5 minutes to warm up. To simulate the other runs the bowl was then washed prior to use. All dry ingredients were weighed together, except shortening and yeast were kept separate in their own containers. The dry ingredients were added to a 100-g pin mixer bowl, making sure to not pour the yeast over the sugar. Using a small spatula, a small well was created in the middle of the pins, and then the water was added. Everything was mixed to optimization previously determined from the mixograph results. The first fermentation time of 52 minutes began when mixing started as fermentation is initiated even during mixing. Each cultivar and block were run in triplicates.

The dough was placed in an oil sprayed 1.5-quart stainless steel mixing bowl and in an environmental chamber (Percival Scientific Inc., Perry, Iowa) to ferment at 30°C and a relative humidity (RH) of 85% for the remainder of the 52 minutes. After the first fermentation, using a sheeter (National Manufacturing Co., Lincoln, Nebraska) set to 3/16-inch roll and 3-inch roll width, the dough was punched once. A second fermentation for 25 minutes (30°C, 85 RH) occurred before the dough was punched again with the same settings, and then underwent a final fermentation period of 13 minutes. The dough was sheeted with readjusted sheeter settings to 5/16-inch roll and then run through a moulder (National Manufacturing Co., Lincoln, Nebraska).

Afterwards, the samples were placed into release-agent sprayed pans (top inside: 7.8 cm x 14.5 cm; bottom outside: 6.3 cm x 12.7 cm; height: 5.5 cm) and went back into the chamber for a proofing step of 35 minutes (30°C, 85 RH). In a preheated Reel oven (Reed Oven Co., Kansas City, Missouri), the samples were baked at 215°C for 20 minutes. The bread was then depanned and left to cool for one hour.

2.2.5 Physical Measurements of Breads

The cooled breads were weighed using an electronic balance. Loaf volume was measured using laser topography (Volscan, Stable Micro Systems, Godalming, Surrey, United Kingdom) using AACC Method 10-16.01 (AACC International, 2015). Specific loaf volume was calculated by dividing loaf volume by the loaf weight (cm³/g). The samples were then put into bags and stored at room temperature before further measurements.

2.2.6 Crumb Grain Analysis

The breads were hand sliced in the middle of the loaf to 2.5cm thickness using a wooden board with slits. The central slice was scanned with a C-Cell (Calibre Control International Ltd., Appleton, Warrington, United Kingdom) using AACC Method 10-18.01 (AACC International, 2017). Each slice was analyzed for slice brightness, number of cells, cell diameter, and wall thickness.

2.2.7 Statistical Analysis

Analysis of variance was run, using proc mixed with random blocks, using SAS (Version 9.4, SAS Institute). The effect of blocking was not significant and thus the treatments were considered as a single block for further analysis. P-values over 0.05 were used to establish significant differences and pairwise comparisons were made using Tukey's HSD.

2.3 Results and Discussion

2.3.1 Variation in Mixing Properties

Representative mixograph profiles for each cultivar are seen in Figure 2.1. MQ is statistically different than 701, C5, and C9 for peak time, peak value, and peak integral as seen in Table 1. Peak time relates to the minimum mobility or mix time and was used in the baking trials for each individual sample. The higher peak time is associated with better mixing quality as acknowledged by Liu et al. (2022) and Labuschagne et al. (2016). Peak value relates to the height of the curve at a percent of the full scale. The higher peak values are associated with higher protein quality and agrees with Mao et al. (2013) who found that higher HMW-GS resulted in higher peak values, along with longer mix times, higher resistance to breakdowns, and tendency towards a stronger dough. Peak integral time relates to the work used to mix to the peak. Peak integral higher value or work is associated with higher protein interactions, therefore a better gluten network and stronger gluten network confirmed by Isaak et al. (2019). This is highly relevant for industry and knowing how much work or energy the mixers use on a dough. The right slope and slope peak shows a doughs' stability and mixing tolerance. Right slope values were taken 2 minutes after peak, representing tolerance to 2 minutes of overmixing. Slope peak values were taken 3 minutes after peak, representing tolerance to 3 minutes of overmixing. The lower the right slope and slope peak, the higher the mixing tolerance and better dough stability and is in accordance with Liu et al. (2022).

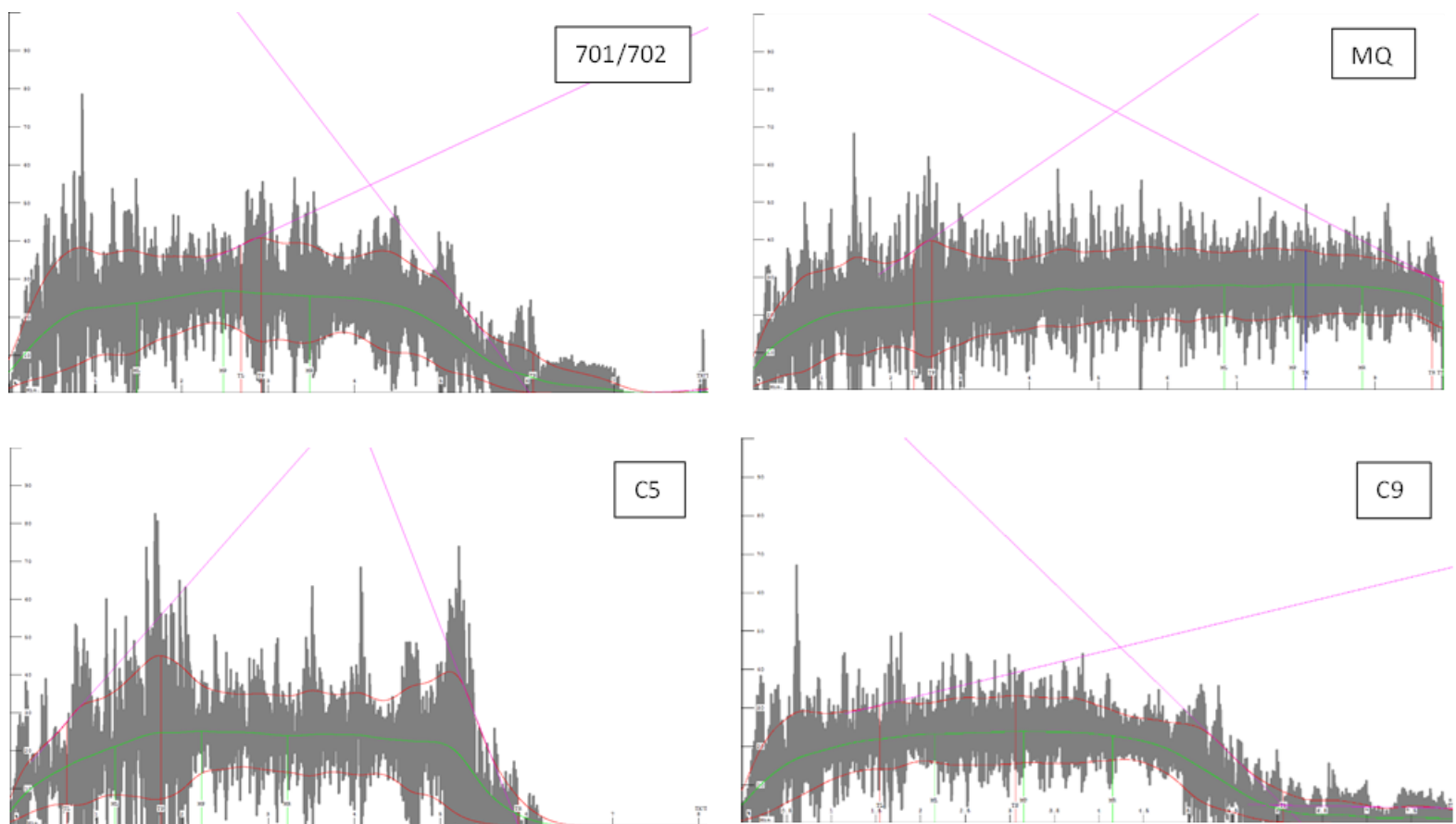


Figure 2.1 Mixograph profiles of IWG cultivars.

Cultivar MQ was bred to improve the mixing quality and hypothesized to perform better, which is seen for each parameter in Table 2.1. For the peak time, peak value, and peak integral MQ were significantly different to the other three cultivars, which were statistically similar. The mix time for MQ was the longest among the cultivars, while C5 had the shortest mix time. C9 had the smallest peak value, and MQ had the highest peak value. C5 also had the lowest peak integral, while MQ was the highest again. Mix time observations agreed with Marti et al. (2015) except for MQ which has been bred for mix time since then. The breeding efforts have improved the MQ cultivar to have a better mixing quality and better stability compared to the other cultivars, which were not bred for mixing quality. This could possibly be to changes in the HWM-GS structure and were able to be improved for MQ. There is a difference and dramatic

drop off in mixing tolerance between the right slope and slope peak that occurs within just a minute. MQ is the most stable, followed by C5. In contrast, the doughs with C9 and 701 deteriorate at a rate up to 3 times faster than that of MQ

Table 2.1 Mixograph properties of the 4 IWG cultivars.

	Midline Peak Time (min)	Midline Peak Value (%)	Midline Peak Integral (%TQ*min)	Midline Right Slope + 2 min (%/min)	Midline Slope Peak + 3 min (%/min)
701/702	2.37 ± 0.14 ^b	26.97 ± 0.97 ^b	51.50 ± 2.15 ^b	-1.83 ± 0.87	-11.41 ± 1.52
C5	2.24 ± 0.27 ^b	26.80 ± 0.72 ^b	45.51 ± 6.22 ^b	-0.68 ± 0.27	-10.10 ± 6.93
C9	3.07 ± 0.35 ^b	26.19 ± 1.07 ^b	65.52 ± 6.84 ^b	-2.33 ± -2.30	-8.37 ± 2.72
MQ	6.24 ± 0.82 ^a	29.75 ± 0.85 ^a	151.07 ± 18.27 ^a	-0.53 ± 0.34	-3.82 ± 2.18

Values followed by different letters in the same column are significantly different (p<0.05).

2.3.2 Variation in Final Product Properties

The better mixing properties of MQ are also reflected in its baking performance as seen in Table 2.2. The proof height was significantly higher, thus having a higher specific volume as well. The proof height of 701, C5, and C9 are all statistically similar, however only 701 and C5's specific volume are statistically similar. Despite C9 having the worst dough stability it had a higher specific volume than 701 and C5. While the midline peak time was used as mixing time for the baking trials, further processing during dough make-up will continue to change protein interactions, which is confirmed by Iwaki et al. (2021). Marti et al. (2015) found the pasting

temperature of IWG to be higher than wheat, which would explain the lower peak viscosity and final viscosity. The higher temperature would require IWG more time to reach the same viscosity, however the bread would be overbaked. The higher fiber content of IWG would also increase its water holding capacity, resulting in less swelling of the starch granules due to reduced water availability (Durmus et al., 2023). It is possible that these processes decreased dough viscosity for C9 more than the others, adjusting it to an optimum for expansion during proofing and early baking. The decrease in final viscosity also lowered the setback indicating a low retrogradation tendency for IWG, which is correlated with bread staling during storage (Bharathi et al., 2022; Marti et al., 2015).

Table 2.2 Baking properties and internal characteristics of the 4 IWG cultivars.

	Proof Height (mm)	Specific Volume (cm ³ /g)	Brightness	Number of Cells	Cell Diameter (mm)	Wall Thickness (mm)
701/702	46.34 ± 0.39 ^b	1.50 ± 0.07 ^c	47.13 ± 0.59 ^b	1723.59 ± 75.06 ^b	1.84 ± 0.07 ^{ab}	0.39 ± 0.01
C5	49.00 ± 1.44 ^b	1.45 ± 0.05 ^c	45.21 ± 1.65 ^b	1690.58 ± 69.43 ^b	1.96 ± 0.08 ^a	0.40 ± 0.01
C9	48.58 ± 1.45 ^b	1.66 ± 0.03 ^b	47.41 ± 1.91 ^b	1840.75 ± 88.90 ^b	1.77 ± 0.14 ^{ab}	0.39 ± 0.01
MQ	55.92 ± 3.03 ^a	1.82 ± 0.07 ^a	51.33 ± 0.87 ^a	2322.09 ± 96.42 ^a	1.75 ± 0.08 ^b	0.39 ± 0.01

Values followed by different letters in the same column are significantly different (p < 0.05).

Figure 2.2 depicts the final breads made with a representative from each block. Visual examination reveals a higher specific volume of MQ in comparison to the other cultivars. MQ is more rounded, while 701, C5, and C9 are more oblong and narrower. The lighter color of MQ is also apparent, as discussed below.



Figure 2.2 Cross-section of finished IWG bread from each cultivar. Left to right is block 1, 2, 3, and 4 of each cultivar.

Figure 2.3 is taken from the C-Cell imaging and shows the cell walls highlighted in black and the cells in blue. The darker the blue color, the smaller the cell size. MQ is seen to have more dark blue colors than light blue compared to the other three cultivars, which allows a visual representation of the uniform cell structure. This structure could be due to the breeding efforts increasing HMW-GS, leading to a better and tighter gluten network. Brightness indicates the light reflectance of a product slice and is influenced by bran content and crumb structure. A

lower value indicates a darker crumb, and MQ had the lightest crumb and is statistically significant. 701, C5, and C9 are statistically similar in brightness and the number of cells. A high number of cells is associated with a uniform crumb structure and is a desirable quality attribute for breads (Sun et al., 2023; Albasir et al., 2022). MQ is statistically significant with the highest number of cells. For cell diameter, the higher the value the coarser and more open cell structure the bread has. MQ has the lowest cell diameter indicating it has the tightest grain and statistically only differs from C5, which has the coarsest and most open cell structure. There was no difference for wall thickness.

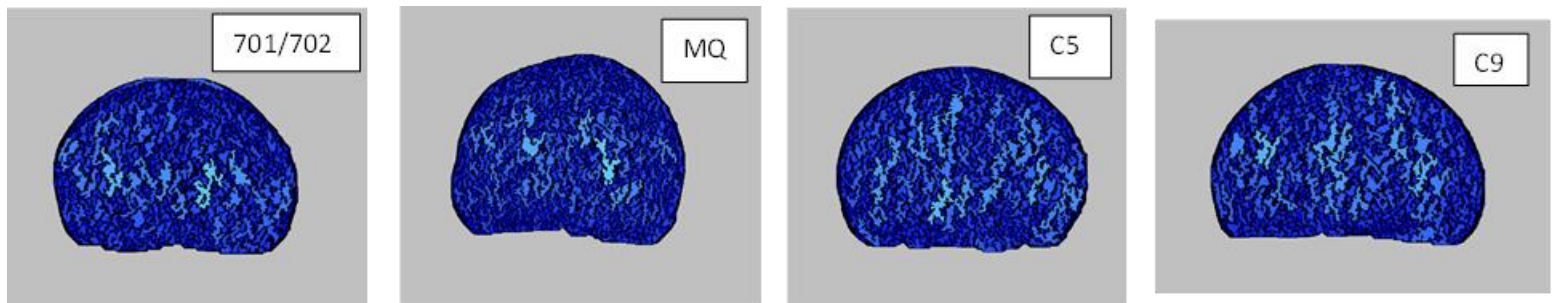


Figure 2.3 Cell images from C-Cell indicate how cells and cell wall are segmented.

2.4 Conclusion

Cultivar MQ was specifically bred for mixing quality and these efforts were found to be successful. The dough was better quality with an increased mixing tolerance. The mixing quality could be seen in the final product with an increased bread volume and higher number of cells. The use of IWG in food is limited mostly because of poor dough characteristics compared to wheat. IWG has many environmental and nutritional benefits. Therefore, targeted breeding and end-use quality studies are important to increase functionality and find new avenues for utilization.

2.5 References

- AACC International. Approved Methods of Analysis, 11th Ed. Method 10-10.03. Optimized Straight-Dough Bread-Making Method. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-10-10.03>
- AACC International. Approved Methods of Analysis, 11th Ed. Method 10-16.01. Volumetric and Dimensional Profile of Baked Products by Laser Topography-Volscan Profiler Method. Approved December 2015. AACC International, St. Paul, MN, U.S.A. <https://doi:10.1094/AACCIntMethod-10-16.01>
- AACC International. Approved Methods of Analysis, 11th Ed. Method 10-18.01. Measurement of Crumb Structure of Baked Products by C-Cell. Approved November 2017. AACC International, St. Paul, MN, U.S.A. <https://doi:10.1094/AACCIntMethod-10-18.01>
- AACC International. Approved Methods of Analysis, 11th Ed. Method 44-15.02. Moisture -Air-Oven Methods. Reapproved November 3, 1999. AACC International, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-44-15.02>
- AACC International. Approved Methods of Analysis, 11th Ed. Method 54-40.02. Mixograph Method. Reapproved November 3, 1999. AACC International, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-54-40.02>
- AACC International. Approved Methods of Analysis, 11th Ed. Method 74-09.01. Measurement of Bread Firmness by Universal Testing Machine. Reapproved November 3, 1999. AACC International, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-74-09.01>
- Albasir, M. O. S., Alyassin, M., & Campbell, G. M. (2022). Development of bread dough by sheeting: Effects of sheeting regime, bran level and bran particle size. *Foods*, 11(15), 2300. <https://doi.org/10.3390/foods11152300>
- Bharathi, R., Muljadi, T., Tyl, C., & Annor, G. A. (2022). Progress on breeding and food processing efforts to improve chemical composition and functionality of intermediate wheatgrass (*Thinopyrum intermedium*) for the food industry. *Cereal Chemistry*, 99(2), 235–252. <https://doi.org/10.1002/cche.10482>
- Durmus, Y., Anil, M., & Simsek, S. (2023). Discrimination of glutopex test and mixograph parameters for evaluation of wheat flour supplemented with hazelnut skin, cross-linked starch, and oxidized starch. *Foods*, 12(2), 328. <https://doi.org/10.3390/foods12020328>
- Jungers, J. M., DeHaan, L. H., Mulla, D. J., Sheaffer, C. C., & Wyse, D. L. (2019). Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. *Agriculture, Ecosystems & Environment*, 272, 63–73. <https://doi.org/10.1016/j.agee.2018.11.007>

- Labuschagne, M. T., Lindeque, R. C., & van Biljon, A. (2016). Dough mixing characteristics measured by Mixsmart software as possible predictors of bread making quality in three production regions of South Africa. *Journal of Cereal Science*, 70, 192–198. <https://doi.org/10.1016/j.jcs.2016.06.008>
- Liu, S., Xu, L., Wu, Y., Simsek, S., & Rose, D. J. (2022). End-use quality of historical and modern winter wheats adapted to the great plains of the United States. *Foods*, 11(19), 2975. <https://doi.org/10.3390/foods11192975>
- Isaak, C., Sapirstein, H., Wu, Y., & Graf, R. (2019). Effects of water absorption and salt on discrimination of wheat gluten strength assessed by dough mixing and protein composition. *Journal of Cereal Science*, 89, 102752. <https://doi.org/10.1016/j.jcs.2019.04.007>
- Iwaki, S., Hayakawa, K., Fu, B.-X., & Otake, C. (2021). Changes in hydrophobic interactions among gluten proteins during dough formation. *Processes*, 9(7), 1244. <https://doi.org/10.3390/pr9071244>
- Mao X, Li Y, Zhao S, Zhang J, Lei Q, et al. (2013) The Interactive Effects of Transgenically Overexpressed 1Ax1 with Various HMW-GS Combinations on Dough Quality by Introgression of Exogenous Subunits into an Elite Chinese Wheat Variety. *PloS ONE* 8(10): e78451. Doi:10.1371/ journal.pone.0078451
- Marti, A., Qiu, X., Schoenfuss, T. C., & Seetharaman, K. (2015). Characteristics of Perennial Wheatgrass (*Thinopyrum intermedium*) and Refined Wheat Flour Blends: Impact on Rheological Properties. *Cereal Chemistry*, 92(5), 434–440. <https://doi.org/10.1094/CCHEM-01-15-0017-R>
- Rahardjo, C. P., Gajadeera, C. S., Simsek, S., Annor, G., Schoenfuss, T. C., Marti, A., & Ismail, B. P. (2018). Chemical characterization, functionality, and baking quality of intermediate wheatgrass (*Thinopyrum intermedium*). *Journal of Cereal Science*, 83, 266–274. <https://doi.org/10.1016/j.jcs.2018.09.002>
- Shewry, P. (2023). Wheat grain proteins: Past, present, and future. *Cereal Chemistry*, 100(1), 9–22. <https://doi.org/10.1002/cche.10585>
- Sun, X., Koksel, F., Scanlon, M. G., & Nickerson, M. T. (2022). Effects of water, salt, and mixing on the rheological properties of bread dough at large and small deformations: A review. *Cereal Chemistry*, 99(4), 709–723. <https://doi.org/10.1002/cche.10561>
- Taylor, K., Samaddar, S., Schmidt, R., Lundy, M., & Scow, K. (2023). Soil carbon storage and compositional responses of soil microbial communities under perennial grain IWG vs. Annual wheat. *Soil Biology and Biochemistry*, 184, 109111. <https://doi.org/10.1016/j.soilbio.2023.109111>
- Zhang, X., Ohm, J.-B., Haring, S., DeHaan, L. R., & Anderson, J. A. (2015). Towards the understanding of end-use quality in intermediate wheatgrass (*Thinopyrum intermedium*): High-molecular-weight glutenin subunits, protein polymerization, and mixing

characteristics. *Journal of Cereal Science*, 66, 81–88.
<https://doi.org/10.1016/j.jcs.2015.10.008>

Chapter 3 - Functionality of Intermediate Wheatgrass in Spontaneous Sourdough Fermentation and Bread Quality

3.1 Introduction

Intermediate wheatgrass (IWG) has been experimented in bread products and found to have a poor performance due to the lack of high molecular weight-glutenin subunits (HMW-GS), causing a weak gluten structure. The search to find a functional end-use for IWG has been ongoing, with breeding efforts being led by The Land Institute. The positive environmental impact and nutritional composition make IWG an attractive new food ingredient. Recently, sourdough fermentation has increased interest due to its capability to substitute additives in breads, alongside its potential health benefits (Gil-Cardoso et al., 2021; Gobbetti et al., 2019; Poutanen et al., 2009). The incorporation of IWG in sourdough fermentation could potentially offer enhanced environmental and consumer health benefits.

Although there are limitations in directly replacing wheat for IWG, its potential as a substrate for lactic acid bacteria (LAB) has not yet been thoroughly explored. The high protein content, high ash (2.6%), and adequate starch (46.7%, Marti et al., 2015) supports the possibility of IWG's application in sourdough for food products. Differences in substrate composition and fermentation conditions result in unique LAB/yeast consortium, influencing the flavor and functional characteristics of the bread (De Vuyst et al., 2009). Health-promoting characteristics were revealed to be linked to specific LAB strains (Corsetti and Settanni et al., 2007). While flavor is important, the impact of sourdough addition on bread's functional aspects such as

volume, texture, and shelf-life are also being investigated (Abarquero et al., 2022; Gobbetti et al., 2019; Corsetti and Settanni, 2007). Fermentation of a novel substrate, such as IWG, is expected to lead to variations in fermentation rates and metabolites, potentially offering technological advantages over wheat.

The goal was to explore another potential end use for IWG, including as an ingredient. The overall aim of this work is to evaluate the functionality of IWG as a substrate in sourdough fermentation as compared to whole wheat. The main objectives to achieve this were: develop a stable sourdough starter, characterize the starter, and determine the functionality in a wheat-based bread. It is hypothesized that due to the higher ash content the fermentation rate may be accelerated; spontaneous fermentation will lead to different microbial communities for IWG and wheat, thus different metabolite production; and compared to wheat sourdoughs IWG sourdoughs may present distinct technological outcomes when added to wheat-based bread.

3.2 Materials and Methods

3.2.1 Ingredients

Hard red winter wheat kernels (variety Kansas Western Star) were provided by the Kansas State University Department of Agronomy. Two cultivars of IWG were provided by The Land Institute in Salina, KS, C6 and 701/702. The IWG grains were slender and smaller than the wheat kernels. A picture can be seen in Chapter 1 Section 1.1.

For the baking trial bread flour (Kansas Sun) was donated by Grain Craft. Instant active dry yeast (Lesaffre saf), all-purpose vegetable shortening, sugar, salt, and deionized (DI) water were used.

3.2.2 Milling

Milling steps were as described in Chapter 2 Section 2.2.2. Briefly, IWG and HRW kernels were milled with an Alpine Augsburg Pin Mill (Model #160z), yielding refined flour and bran. HRW and IWG flour and bran were sifted through 750, 500, 425, 355, 240, and 132 μm screens to evaluate particle size. After comparing to whole wheat particle sizes, fractions above a 425 screen of the IWG flour and bran was reground the same way. To combine the bran and refined flour, each flour was blended for five minutes at first speed in a 60-quart bowl on a Hobart floor mixer (Hobart, Troy, Ohio). The HRW was stored in a plastic container, while the two IWG cultivars were stored in separate paper bags. Whole wheat flour was labeled WW. Whole grain IWG flours were labeled K6 and K7, referring to cultivars C6 and 701/702, respectively.

3.2.3 Raw Material Characterization

The flour samples were characterized for various factors. Dietary fiber was analyzed using AOAC 991.43 method for total, soluble, and insoluble dietary fiber in foods. The α - and β -amylase activity were measured using enzyme activity kits (Ceralpha and Betamyl-3, Megazyme Ireland; AACC Method 22-02.01 and RACI, respectively). Mineral analysis was done using inductively coupled plasma (ICP) spectrometry following Nitric-Perchloric digestion (ICP Spectrometer, Model 5800 ICP OES Agilent Technologies). Nitrogen was analyzed using a LECO TruSpec CN combustion analyzer, and protein was computed using Nx5.7 for wheat and Nx6.2 for IWG. Particle size analysis was conducted using an Alpine Jet Air Sieve (model E200 LS, Hosokawa Alpine Ag & Co, Augsburg, Germany) with sieve opening sizes of 850, 425, 212, 150, 75, 45, and 25 μm . Size distribution of the flour was measured with a Morphologi G3

(Malvern Panalytical, Grovewood, UK) as previously described by Suprabha Raj et al., 2023 but with an adjustment for 10,000 particles.

3.2.4 Sourdough Establishment and Characterization

Sourdough starters began as a 1:1 mixture of DI water and either WW, K6, or K7 flour. Once the mixture was made, 10 grams of the starter was blended in 100 grams of DI water and used to measure pH and total titratable acidity (TTA) using an automatic titrator. Titration was carried out using NaOH 0.1N to an endpoint of pH 8.5. Starters were then incubated in 2-liter polycarbonate containers in an environmental chamber (Percival Scientific Inc., Perry, Iowa) at 28°C and a relative humidity (RH) of 85%. The starters underwent spontaneous fermentation for 24 hours and were then backslopped every 24 hours using a ratio of 1:1:1 (sourdough: DI water: respective whole grain flour) for 11 days. pH and TTA were measured daily prior to backslopping. At the end of the 11 days, the sourdough was sealed in polyethylene bags and kept frozen at -18°C, until use in the baking trial. Fermentations were conducted in triplicates.

Yeast and mold enumeration and LAB enumerations were analyzed on the initial and final days. To prepare the samples 10 grams of sourdough were mixed with 90 mL of 0.1% peptone solution in a stomacher bag and hand massaged for 5 minutes to homogenize. A serial dilution was made from 10⁻¹ to 10⁻⁴. For the yeast and mold enumerations, each dilution was spread on a Petrifilm count plate and incubated at 25°C for 3-5 days. For the LAB enumerations, each dilution was spread on MRS agars and incubated at 35°C ± 2°C for 48-72 hours.

The concentrations of organic acids, saccharides, and alcohols in final day sourdough samples were analyzed using high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan), as described previously (Liu et al., 2024; Liu et al., 2023). A five-point standard curve was generated for each compound with an R-squared value above 0.99. To prepare the

samples, 2 grams of sourdough was dispersed in 20 grams of DI water, vortexed, and centrifuged at 4,000 rpm for 10 minutes. 2 mL of the supernatant were removed and filtered through a 0.2 μ m filter into the HPLC vial. The fermentation quotient (FQ) was calculated from these results and is defined as the molar ratio between lactic and acetic acids.

3.2.4.1 Acidification Kinetics and LAB Evolution

To further understand the acidification of the different substrates, another set of starters was initiated following the same method as described above. Based on the data from the first fermentation trial, indicating no statistically significant change in acidity after 7 days, the time frame was adjusted to 7 days of fermentation. To establish the sourdough a 1:1 mixture of DI water and either WW, K6, or K7 flour was prepared. Starters were incubated in 2-quart polycarbonate containers in an environmental chamber (Percival Scientific Inc., Perry, Iowa) at 28°C and a RH of 85%. Sourdoughs were backslopped every 24 hours using a ratio of 1:1:1 (sourdough: DI water: whole grain flour) for 7 days. Yeast and mold enumerations were measured on the initial and final days. For days 0-4 pH and TTA were measured every 8 hours to gain a better understanding of the acidification observed during the first sourdough establishment. Days 5-7 were then measured every 24 hours before backslopping. Fermentations were conducted in triplicates. On the final day, the color of the sourdough starters was measured using a colorimeter (Konica Minolta) before being sealed in polyethylene bags and frozen at 18°C, which would be used later for the sensory evaluation in chapter 4 of this thesis.

To calculate color differences, Altan et al. (2008) was used. ΔL^* calculates the difference in lightness and darkness. The more positive the lighter sample, the more negative the darker. Δa^* calculates the difference in red and green hues. The more positive the redder, and the more negative the greener. Δb^* calculates the difference in yellow and blue hues. The more positive

the yellower, and the more negative the bluer. ΔE^*ab calculates the total difference. The higher the value the more different the samples are.

A final trial of sourdough starters was established to further understand the LAB and Organic acids evolution during a 7-day fermentation period. Sourdoughs were prepared as a 1:1 mixture of DI water and either WW, K6, or K7 flour and incubated in glass 600 mL beakers in an environmental chamber (Percival Scientific Inc., Perry, Iowa) at 28°C and a relative humidity (RH) of 85%. Sourdoughs were backslopped every 24 hours using a ratio of 1:1:1 (sourdough: DI water: whole grain flour) for 7 days. Yeast and mold enumerations, LAB enumerations, pH, TTA, and HPLC analyses were measured on day 0, 1, 3, 5, and 7. Each method followed the same procedures as previously mentioned, with a slight modification to the sample preparation for HPLC method was used (samples were centrifuged at 5,000 rpm for 15 minutes to obtain a clearer supernatant). The final day FQ was also calculated. Fermentations were conducted in duplicates.

3.2.5 Baking Trial

To determine proper absorption, moisture content for the bread flour was analyzed on a near-infrared (NIR) (Blue Sun Scientific, Jessup, Maryland). The average of two tests were taken to determine a moisture content of 14.8% and was used for the baking trial.

Sourdoughs from the 11-day backsloppings were used in baking trial, at 20% (flour basis) inclusion in the dough. Frozen sourdough was allowed 2 hours to thaw before utilizing. Optimized straight dough bread-baking AACC Method 10-10.03 was used with slight modifications (AACC International, 1999). Doughs were mixed in Hobart mixers fitted with McDuffy bowls and 2-prong attachments. Jacketed bowls were attached to a water bath at 5°C. The mixers ran empty for 5 minutes to warm up the motor. All dry ingredients were weighed,

except shortening and yeast kept separate in their own containers. The dry ingredients were added to the mixer bowl. Using a spatula, a well was created in the middle around the pin, and then the water and 20% (f.b.) of sourdough was added. The dough was mixed to optimum development based on farinograph evaluation of the base flour and visual observation (one minute on first speed and four and a half minutes on second speed). The first fermentation time of 52 minutes began when mixing started as fermentation is initiated even during mixing. Each dough was divided into six, 180-gram doughs. Doughs were placed in an oil sprayed 1.5 qt mixing bowl and in an environmental chamber to ferment (Percival Scientific Inc., Perry, Iowa) at 30°C and a relative humidity (RH) of 85 for the remainder of the 52 minutes. After the first fermentation, using a sheeter (National Manufacturing Co., Lincoln, Nebraska) set to 3/16-inch roll and 3-inch roll width the doughs were punched once. Then underwent a second fermentation for 25 minutes (30°C, 85 RH) and punched again with the same settings, then underwent a final fermentation period of 13 minutes. The dough was sheeted with readjusted sheeter settings to 5/16-inch roll and then run through a moulder (National Manufacturing Co., Lincoln, Nebraska). Afterwards, samples were placed into release-agent sprayed pans (top inside: 7.8 cm x 14.5 cm; bottom outside: 6.3 cm x 12.7 cm; height: 5.5 cm) and placed back into the chamber for a proofing step of 35 minutes (30°C, 85 RH). The samples were baked at 215°C for 20 minutes in a gas-fired Reel oven (Reed Oven Co., Kansas City, Missouri). The bread was then depanned and left to cool for one hour.

3.2.5.1 Physical Measurements of Breads

The cooled breads were weighed using an electronic balance. Loaf volume was measured using laser topography (Volscan, Stable Micro Systems, Godalming, Surrey, United Kingdom) using AACC Method 10-16.01 (AACC International, 2015). Specific loaf volume was calculated

by dividing loaf volume by the loaf weight (cm³/g). At 1-day post-bake samples were analyzed for pH and TTA. Four slices of bread of 25 mm thickness were measured for firmness at 1-day and 5-days post-bake (AACC method 74-09.01, AACC International, 1999). The bread firmness was measured using a TA.XT2 Texture Analyzer (Texture Technologies or Stable Micro Systems, Scarsdale, New York) with a 25 mm acrylic probe. Texture Exponent 32 version 5.07.0 was used to analyze the firmness. 5 loaves from each treatment were sealed in polyethylene bags and stored at room temperature for evaluation of day to first visual mold.

3.2.6 Statistical Analysis

All analysis was conducted in triplicates, except were otherwise noted (LAB evolution trial). JMP Pro 17.2.0 was used to run ANOVA and Tukey's HSD (significance established at $p < 0.05$).

3.3 Results and Discussion

3.3.1 Raw Material

The WW, K6, and K7 flours were characterized by the shape and size of the flour, protein, fiber, and other nutritional factors. According to 21CFR137 to be considered whole wheat flour at least 50% has to pass through a 850 μm sieve, which can be seen in figure 3.1. The particle size distribution shows the WW flour to have a higher percentage of particles between 75 μm to 300 μm . At the 425 μm to 850 μm K6 and K7 flours have a higher percentage compared to WW flour. Although after regrinding, the K6 and K7 flours are similar to the WW flour. The K6 and K7 flours were similar in particle size analysis and visually. They were more cohesive than the WW flour and had a yellow tint to them.

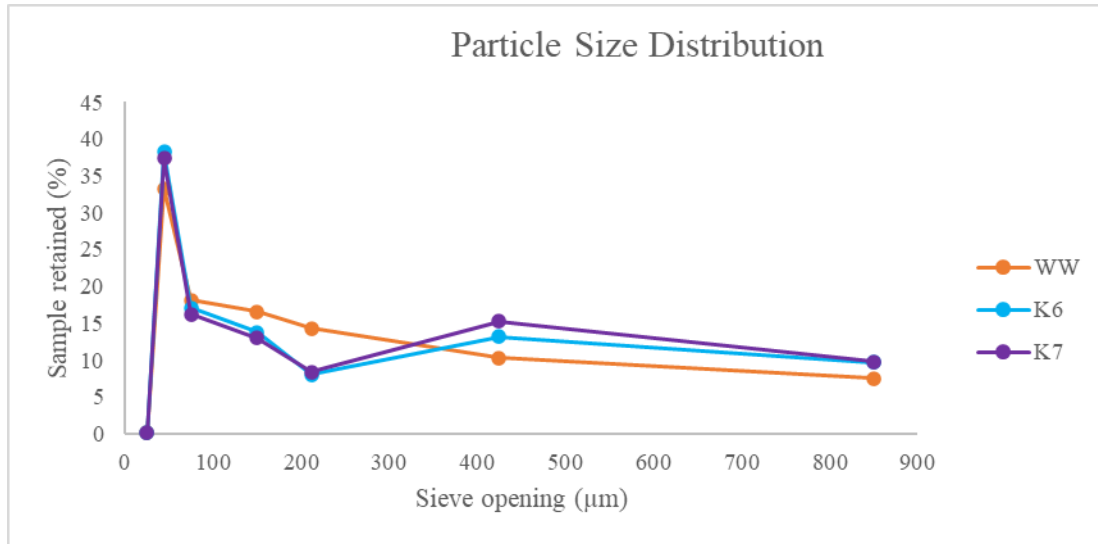


Figure 3.1 Particle size distribution curve of WW, K6, and K7. Sieve opening sizes of 850 μm, 425 μm, 212 μm, 150 μm, 75 μm, 45 μm, and 25 μm were used.

Particle size and shape could influence fermentation, which is why particle morphology was assessed (table 3.1a, b). The d_{10} , d_{50} , and d_{90} show again that K6 and K7 flours have bigger particle sizes compared to WW flour and that K7 is the largest. The Morphologi captures a 2D image of the 3D flour particle and converts it to a circle of equivalent area to the 2D image. The diameter of this circle is the circle equivalent (CE) diameter. The larger the CE diameter the larger the diameter of the circle, thus the larger the particle size. High sensitivity (HS) circularity measures how similar a shape is to a perfect circle. The closer to 1, the more circular the shape is. Elongation measures how elongated the particle is, the closer to 1, the more elongated the shape is. Convexity measures the surface roughness of a particle. The closer to 1, the smoother the shape, while a spiky or irregular shape is closer to 0. All these factors could relate to substrate activity. The bigger the particle, the more elongated, and the more irregular a particle the more surface area there is for enzymatic activity. WW, K6, and K7 flour particles all have a

similar, more circular shape and can be seen in figure 3.2. Overall, fermentation is unlikely to be affected by particle size and shape, since all three substrates displayed similar morphology.

Table 3.1 (a) Volume based d10, d50, and d90 analysis and (b) WW, K6, and K7 flour particle size and shape analysis.

(a)

Volume-Based (μm)			
Sample	<i>d10</i>	<i>d50</i>	<i>d90</i>
WW	20.6	85.4	218.1
K6	13.8	106.7	230.1
K7	15.8	266.6	300.9

(b)

Sample	CE Diameter Mean (μm)	HS Circularity Mean	Aspect Ratio Mean	Elongation Mean	Solidity Mean	Convexity Mean
WW	8.68	0.81	0.72	0.28	0.97	0.98
K6	8.21	0.82	0.73	0.27	0.97	0.98
K7	9.22	0.81	0.73	0.28	0.96	0.98

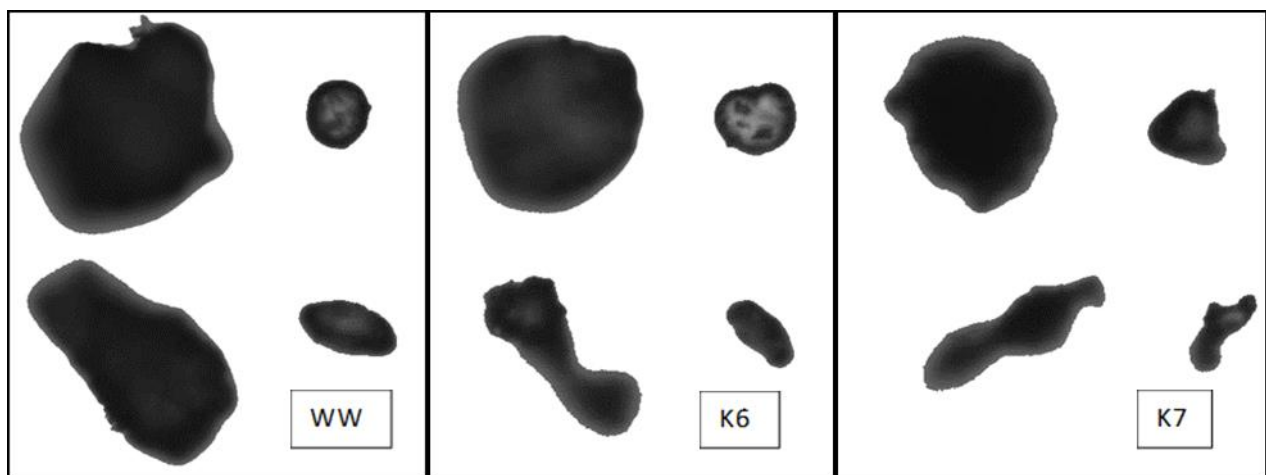


Figure 3.2 Select Morphologi photos of WW, K6, and K7 flour particles representing the various size and shapes.

In agreement with previous literature, protein content, dietary fiber, and minerals are higher for IWG compared to wheat. Table 3.2 shows the analytical results of the characterization. Total and insoluble dietary fiber were statistically significant with K6 and K7 differing from WW. While α -amylase was not different statistically, β -amylase was statistically different, where K6 and WW were different than K7. Looking at the mineral results, all the minerals except copper and zinc were statistically different, where K6 and K7 differed from WW. Sulfate and manganese had a variation between the K cultivars as well. All the minerals are higher for both K6 and K7, than WW. The protein content was higher for both K6 and K7 samples compared to WW, and they are all statistically different.

Table 3.2 Raw material characterization: dietary fiber, α - and β -amylase, protein content, and mineral analysis.

	WW	K6	K7
TDF % $\pm$ SD	11.4 $\pm$ 0.3 ^b	18.0 $\pm$ 2.5 ^a	16.1 $\pm$ 0.9 ^a
IDF % $\pm$ SD	9.8 $\pm$ 0.8 ^b	15.8 $\pm$ 2.1 ^a	13.5 $\pm$ 0.3 ^a
SDF % $\pm$ SD	1.7 $\pm$ 1.0	2.1 $\pm$ 0.5	2.6 $\pm$ 0.8
α -amylase (units/g) $\pm$ SD	2.77 $\pm$ 1.76	2.84 $\pm$ 0.69	0.76 $\pm$ 0.72
β -amylase (units/g) $\pm$ SD	34.88 $\pm$ 2.36 ^a	39.35 $\pm$ 0.19 ^a	26.20 $\pm$ 0.57 ^b
Protein % $\pm$ SD	13.28 $\pm$ 0.08 ^c	18.63 $\pm$ 0.27 ^b	20.84 $\pm$ 0.04 ^a
P ppm $\pm$ SD	34.8 $\pm$ 0.0 ^b	53.2 $\pm$ 0.0 ^a	51.6 $\pm$ 0.0 ^a
K ppm $\pm$ SD	37.0 $\pm$ 0.0 ^b	66.0 $\pm$ 0.0 ^a	65.0 $\pm$ 0.0 ^a
Ca ppm $\pm$ SD	5.7 $\pm$ 0.0 ^b	12.7 $\pm$ 0.0 ^a	13.2 $\pm$ 0.0 ^a
Mg ppm $\pm$ SD	14.5 $\pm$ 0.0 ^b	17.5 $\pm$ 0.0 ^a	16.7 $\pm$ 0.0 ^a
SO4-S ppm $\pm$ SD	14.3 $\pm$ 0.0 ^c	22.1 $\pm$ 0.0 ^b	24.9 $\pm$ 0.0 ^a
Cu ppm $\pm$ SD	4.0 $\pm$ 0.3	3.9 $\pm$ 0.5	4.2 $\pm$ 0.8
Fe ppm $\pm$ SD	40.9 $\pm$ 1.8 ^b	75.4 $\pm$ 2.5 ^a	69.4 $\pm$ 4.0 ^a
Mn ppm $\pm$ SD	56.3 $\pm$ 1.6 ^c	68.2 $\pm$ 0.4 ^b	74.2 $\pm$ 1.3 ^a
Zn ppm $\pm$ SD	25.0 $\pm$ 0.5	37.0 $\pm$ 8.6	33.1 $\pm$ 4.3

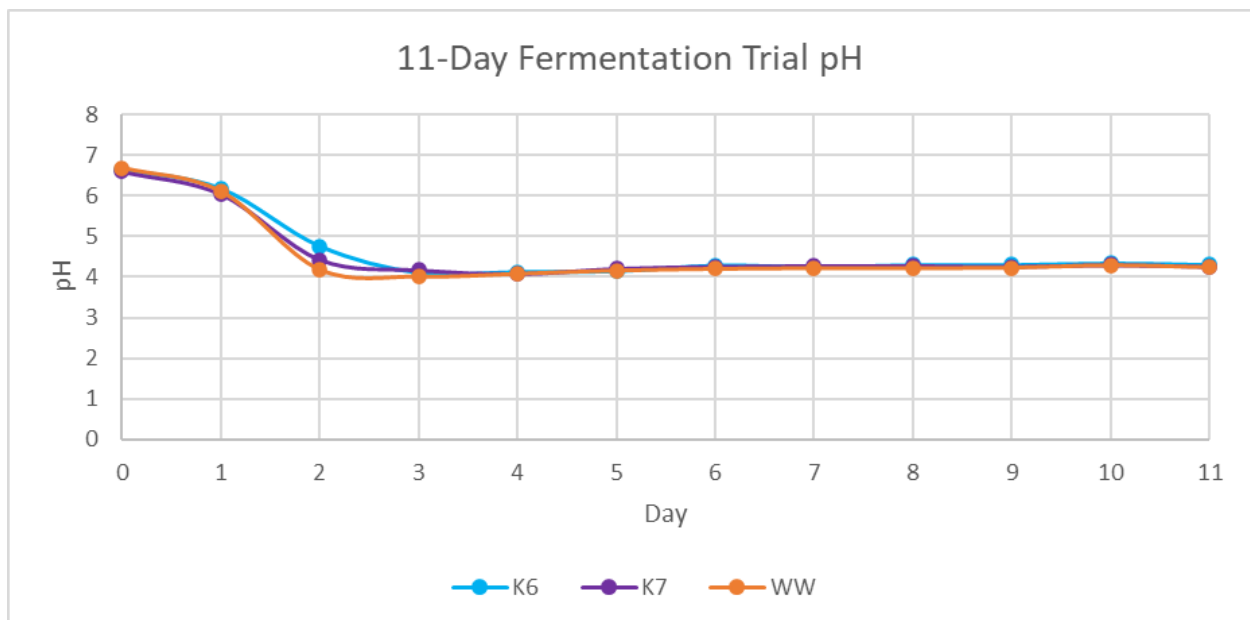
Values followed by different letters in the same row are significantly different (p<0.05).

3.3.2 Sourdough

3.3.2.1 Sourdough Establishment—11-day Fermentation Trial

The acidification of the sourdough can be seen in figure 3.3a and b. The trend of the pH was similar among all three samples. They all began around 6.5 and by day 2 dropped down to a pH of 4 and stayed consistent through day 11. The TTA is not as consistent. All three samples begin a steep increase between day 1 to 2. For WW, Day 2 to 3 is when it reaches its peak at 18 mL NaOH 0.1N. Then begins to level out at 15 mL NaOH 0.1N. K6 and K7 continue acidifying past day 2 until day 4, peaking at 25 mL NaOH 0.1N, and then begins to level out and at 20 mL NaOH 0.1N. The sourdough industry typically measures TTA, as it measures the total organic acids produced during sourdough fermentation (Corsetti, 2013). Reduction in time necessary to achieve maximum acid production is of interest, as it may reduce process time and increase capacity of sourdough plants.

(a)



(b)

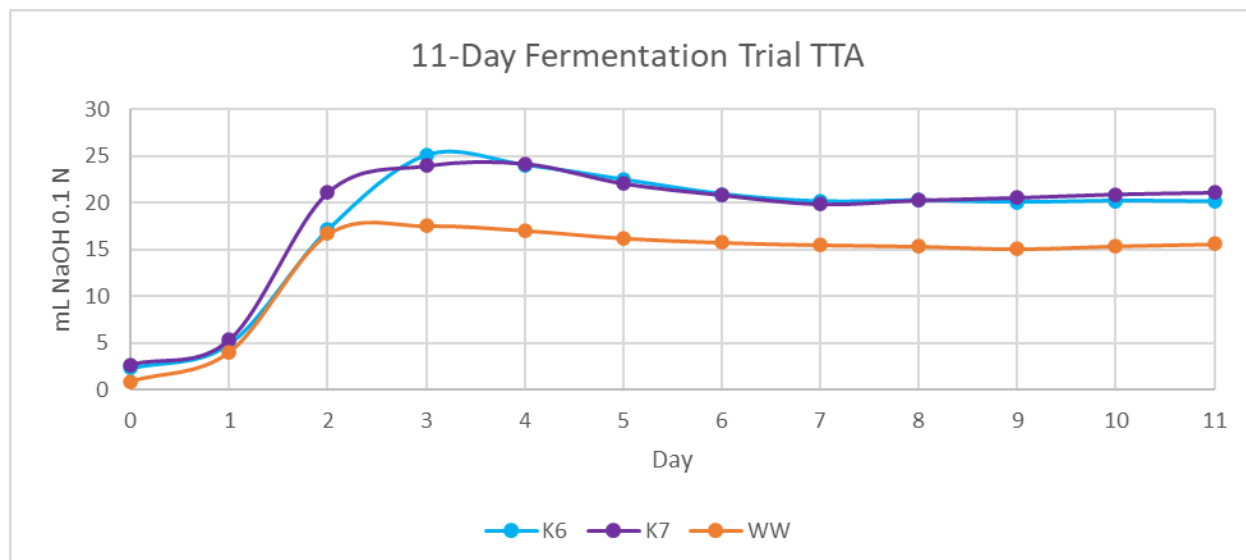


Figure 3.3 (a) 11-day fermentation trial pH progression and (b) 11-day fermentation trial TTA progression.

In figure 3.4 the HPLC results are shown. Succinic acid and butyric acid were just at or below detection limits for most samples, as is expected for microflora dominated by LAB bacteria. These metabolites are therefore excluded from the discussion. Lactic acid is most prevalent for WW, K6, and K7, but is significantly higher for K6 and K7 compared to WW. The assumption can be made that there is LAB present in the sourdoughs. Ethanol is the second most prevalent, particularly for K6 and K7. K7 has a high amount of citric acid produced. For glucose, malic acid, and formic acid K6 and K7 are both higher than WW. However, WW is higher in acetic acid than both K6 and K7, and is reflected in the fermentation quotient, or molar ratio of lactic/acetic acid (table 3.3). Both K6 and K7 have higher lactic acid production, therefore the FQ is larger than the WW FQ. FQ of K6 is double the FQ of WW. Lactic acid is the major end product of homo-fermentative LAB, while hetero-fermentative LAB also produce acetic acid

(Lim et al., 2017). Bread shelf-life and sensory attributes are strongly influenced by FQ, particularly acetic acid (Lim et al., 2017; Corsetti, 2013).

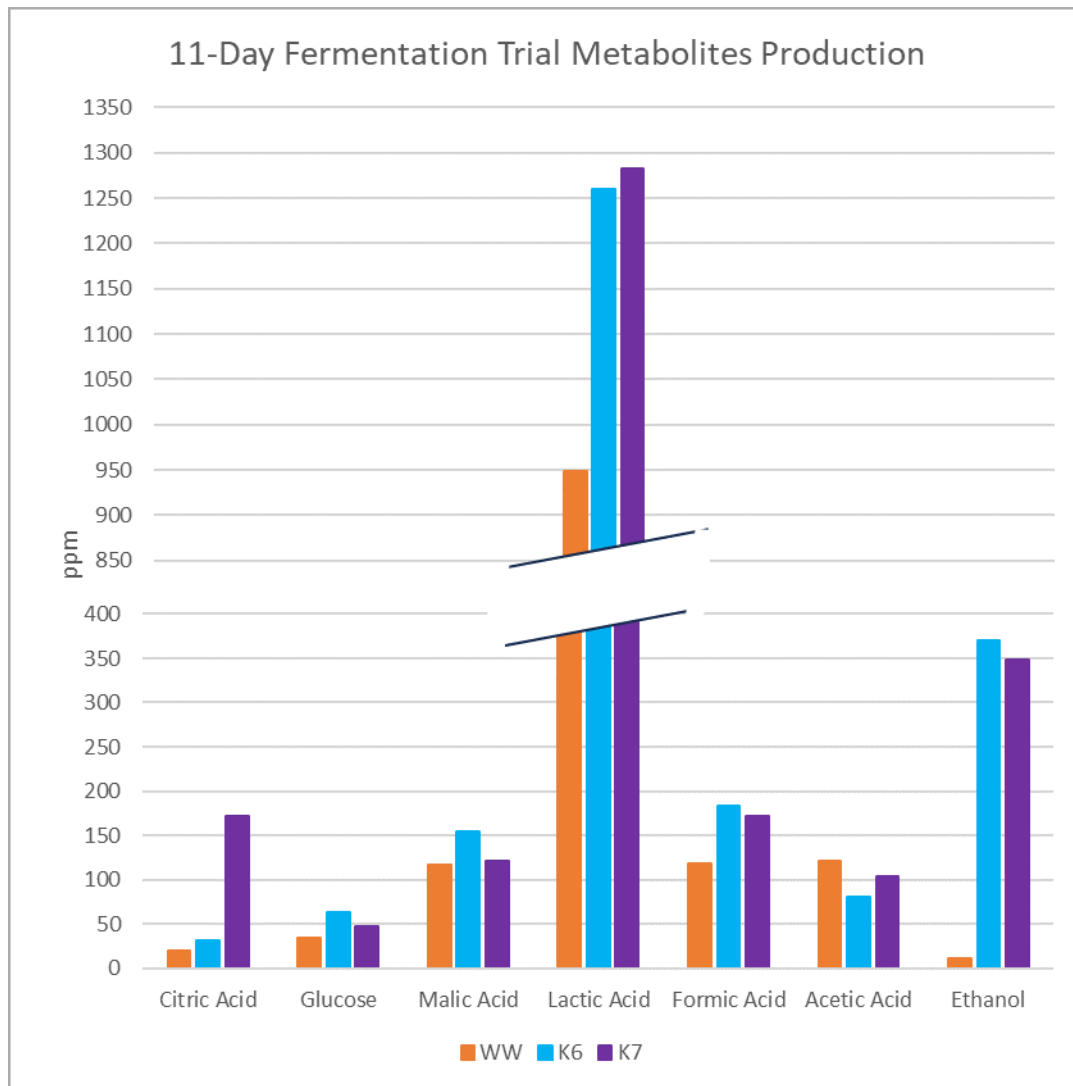


Figure 3.4 11-day fermentation trial final day metabolites production.

Table 3.3 11-day fermentation trial final day fermentation quotient.

Sample	FQ
WW	5.2
K6	10.5
K7	8.3

3.3.2.2 Acidification Kinetics Fermentation Trial

After the successful establishment of sourdough utilizing K6 and K7, there was a gap in knowledge of the acidification progression within the first 4 days. No statistical differences were found in the acidification in the first fermentation trial post 7 days; thus the secondary trial time was shortened. A consensus from various previous literature determined a stable, mature sourdough is established in about 5-7 days through spontaneous fermentation and continuous feedings (Galli et al., 2019; Ercolini et al., 2013). This also helped support the decision to shorten the fermentation time period.

Figure 3.5 shows the progression of pH over 168 hours. The pH trend of this fermentation trial agrees with the previous trial. Focusing on the gap area of hours 24-72 (day 1-3), it is evident that the WW drops in pH faster than K6 between hours 24-48. The largest drop in pH for all three samples was between hours 32-40. At hour 40 the WW is fairly consistent the remainder of the time. K7 follows closely at hour 48, but K6 only reached pH 4 at 56 hours. All three sourdoughs have a slight increase from hours 72-88, which is most likely due to the backslopping effect and microorganisms still competing for dominance. $V_{\max}$ is the maximum acidification rate and was found to be at a time maximum of 40 hours for each sample. K6 and WW are significantly different, while K7 is similar to both. The bigger the $V_{\max}$, the steeper the slope. At the 40-hour mark, there is a significant drop in pH for WW and is reflected in the $V_{\max}$. K6 has the smallest $V_{\max}$, and is reflected in the graph at 40 hours, but all three samples are

similar again by 56 hours. ΔpH is the difference between the final and initial pH and can be seen in table 3.4, along with the V_{max} . The ΔpH is not significantly different as the final and initial pH for all three samples are similar.

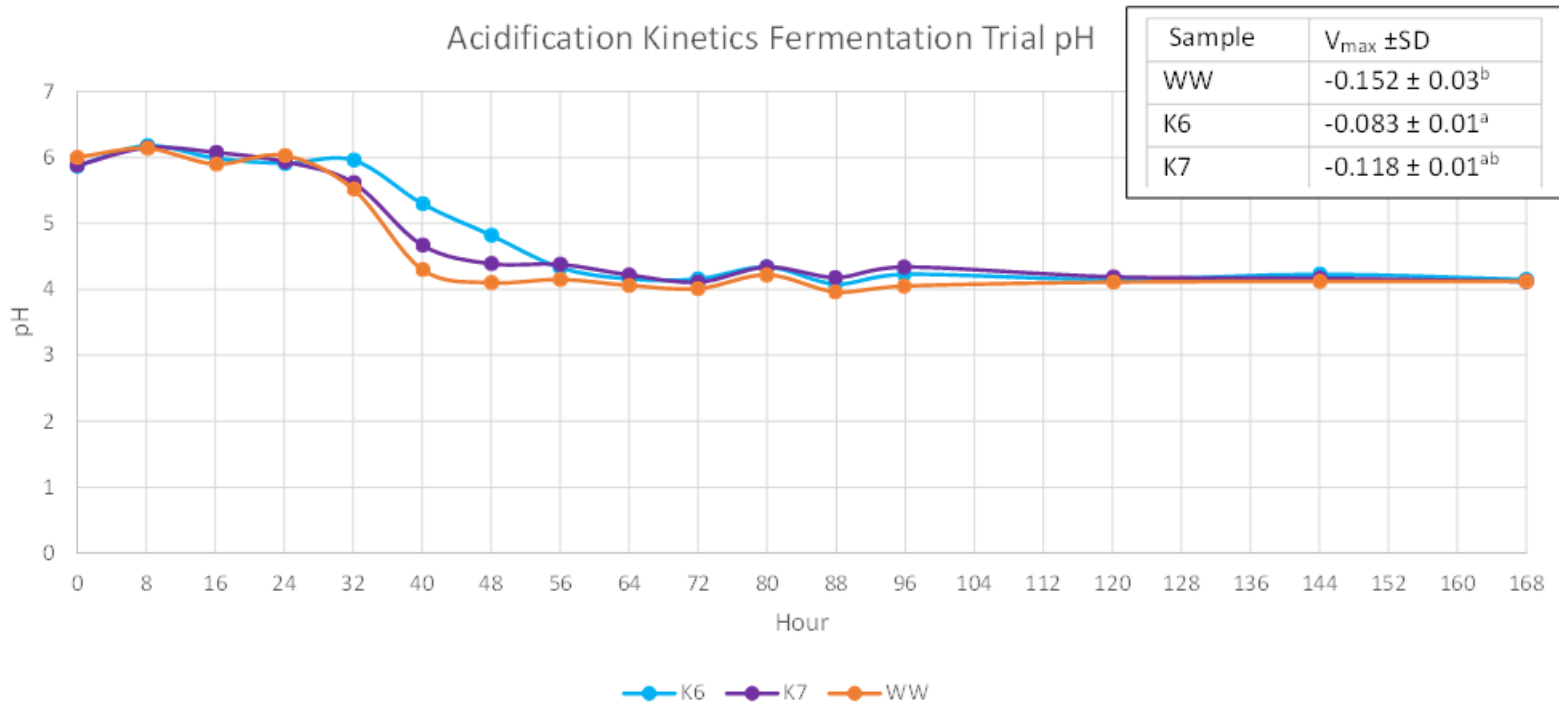


Figure 3.5 Acidification kinetics over 7 days, measured every 8 hours for the first 96 hours and then measured every 24 hours for the remainder. A table with V_{max} at a T_{max} of 40 hours is included, where different letters signify significant statistical difference at $p < 0.05$.

Table 3.4 Acidification Kinetics Fermentation Trial ΔpH (final minus initial pH) and V_{max} .

	ΔpH (D7-D0)	V_{max}
WW	-1.871	-0.152^b
K6	-1.723	-0.083^a
K7	-1.756	-0.118^{ab}
P-value	0.18	0.008

Different letters in column indicate significant difference ($p < 0.05$) and significant p-values bolded for impact.

The TTA progression of the acidification kinetics fermentation trial is similar to the 11-day fermentation trial and can be seen in figure 3.6. Similar to the acidification kinetics fermentation trial pH graph there is a significant increase in TTA from hours 32-48. K6 is reduced in comparison to WW and K7, with the first big increase from hours 40-56. K7 and WW drop down between hours 48-56, most likely due to the backslopping effect and recent feeding at 48 hours. However, acid production quickly resumes, and all three samples concentrate acid from hours 56-72. All three samples reach their peak TTA of 20 mL NaOH and 26 mL NaOH for WW and K6/K7, respectively, at 72 hours. There is one last drop in TTA, similar to pH, between hours 72-96 again most likely due to the backslopping effect and dominant microorganisms establishing themselves before plateauing at 96 hours.

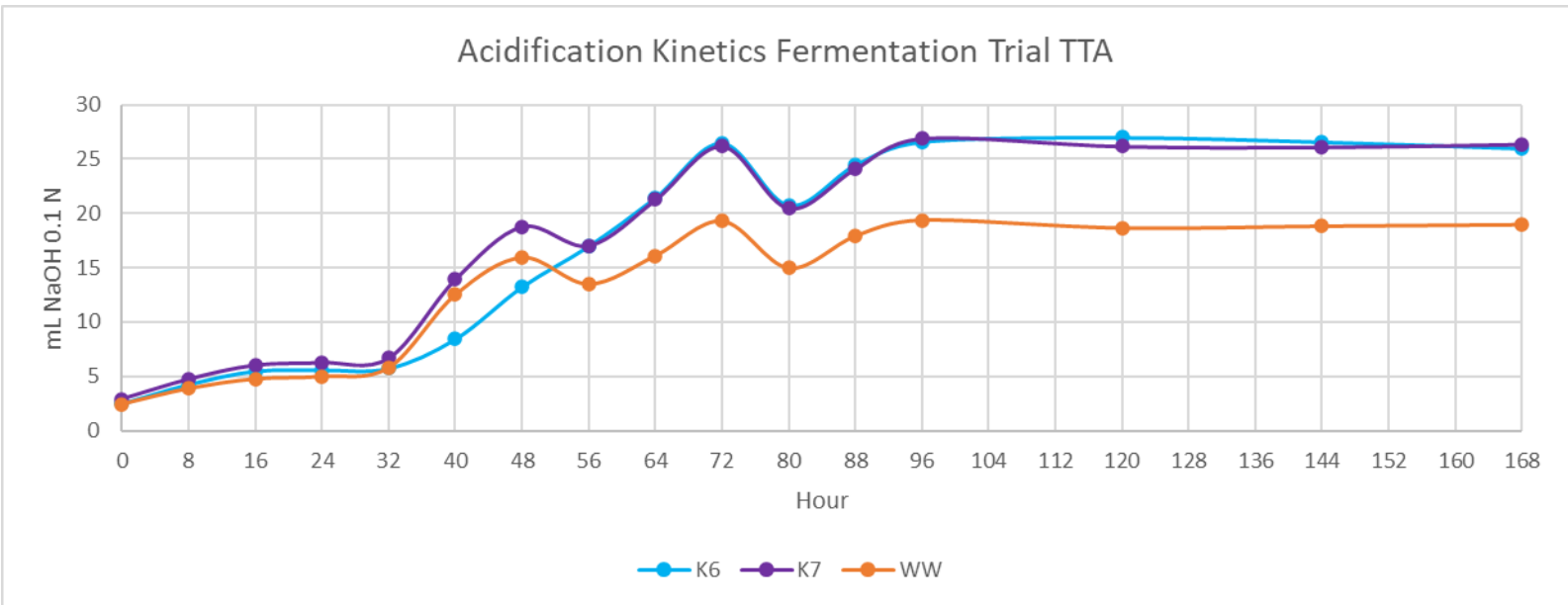


Figure 3.6 Acidification kinetics fermentation trial TTA progression over 7 days, measured every 8 hours for the first 96 hours and then measured every 24 hours for the remainder.

Figure 3.7 depicts the sourdoughs on the final day of fermentation. There is a visible difference in color between K6 and K7 to the WW sourdough. The ΔL^* , Δa^* , Δb^* , and ΔE^*_{ab} in

table 3.5 shows the difference of K6 and K7 to WW are similar. The biggest difference between K6 and K7 is the ΔL^* and Δb^* . This reflects as they have significantly darker color with strong yellow hues. The Δa^* signifies the redder hues present. This could be explained from the observations in the flour. While all the flours were stored in a light protecting container, the K6 and K7 flour over time had visible bran flakes that turned red from oxidation whereas the WW did not. As previously mentioned, the flour also had a more visible yellow tint compared to the WW flour. They are also significantly darker than the WW and the total difference is high. The texture of the K6 and K7 also appears to be lumpier and firmer compared to the WW, which was more liquid-like. This is due to the high fiber content of the K6 and K7 samples, as they can retain more water than the WW.

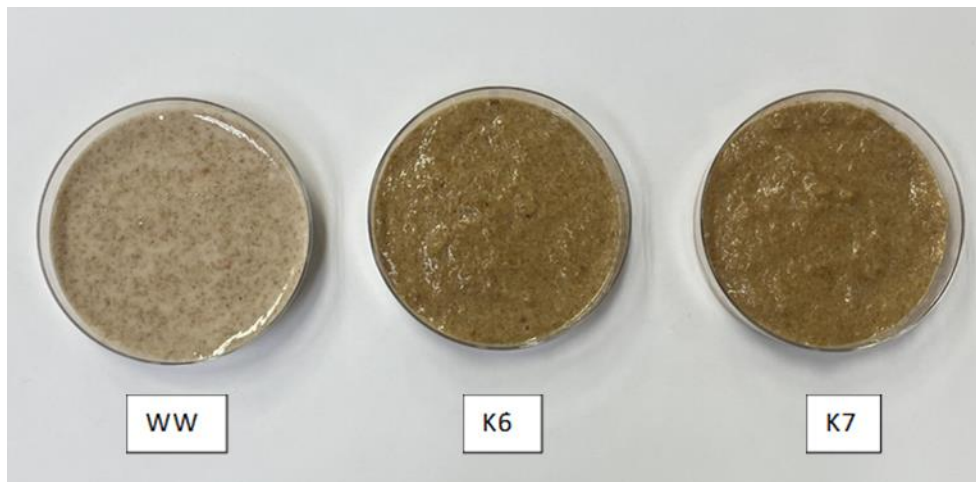


Figure 3.7 Photo of WW, K6, and K7 sourdoughs on final day.

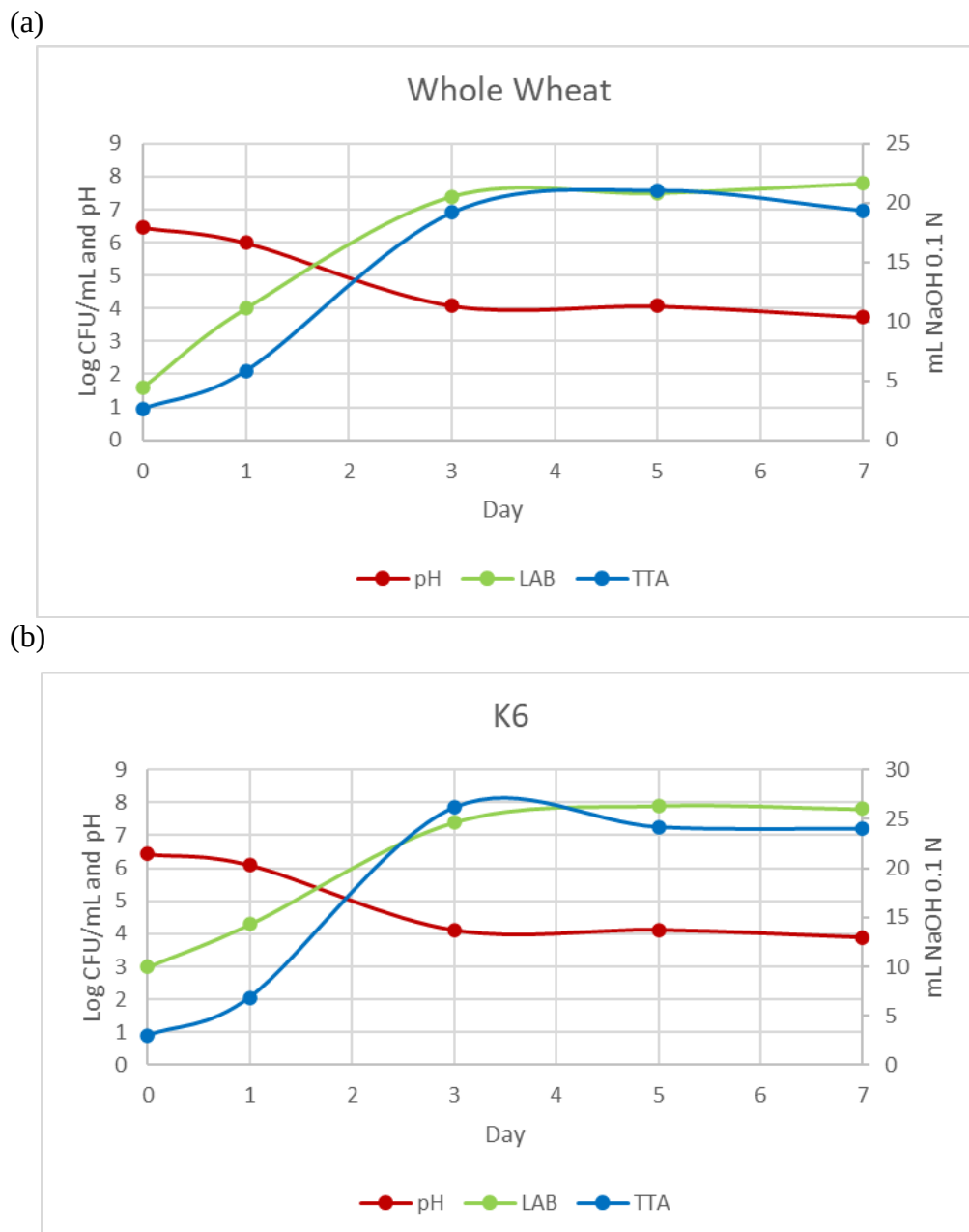
Table 3.5 Color analysis of K6 vs. WW and K7 vs WW.

	K6 vs. WW	K7 vs. WW
ΔL^*	-10.2	-11.2
Δa^*	2.4	2.7
Δb^*	6.5	5.6
ΔE^*_{ab}	12.4	12.8

3.3.2.3 LAB and Organic Acid Evolution Fermentation Trial

A final fermentation trial was conducted to explore the LAB, yeast and mold, and organic acid evolution. Microbial diversity is typically observed to simplify and remain consistent over time after about 5 days (Ercolini et al., 2013). This is very apparent in figure 3.8a, b, and c, where pH, TTA, and LAB enumerations are shown. From day 5 to day 7 the lines begin to flatten out and stay consistent agreeing with Ercolini et al. (2013). The overall trend of these graphs is as pH decreases the LAB population and TTA increases with the biggest change between day 1 and day 3. The pH and TTA trends follow the same patterns as the previous two fermentation trials. There is little to no difference in pH for all three samples, but K6 and K7 have a higher TTA than WW by day 3. Compared to wheat, K6 and K7 reach a higher LAB enumeration and as previously mentioned, K6 and K7 have a significantly higher production of lactic acid than WW. These results suggest differences in LAB communities, as further discussed below. According to Galli et al. (2019), the dominating LAB of a mature and stable Type 1 sourdough ranges in concentration from 6 to 9 CFU/g, agreeing with the LAB enumeration found in this study. The decrease in pH and acid production is a crucial mechanism to enhance mineral bioavailability for LAB (Poutanen et al., 2009). K6 and K7 have higher initial LAB enumerations, however acidification is similar to WW at day 3. It can be hypothesized that this acidification increases mineral availability in K6 and K7, supporting higher acidification in the

following days. According to Hammes et al. (2005), manganese is an important growth factor for LAB. The manganese content of the substrates is 68.2, 74.2 and 56.3 ppm for K6, K7 and WW, respectively. Calcium and magnesium are also found to enhance LAB metabolism (Matevosyan et al., 2019). K6 and K7 have approximately 2x the calcium content of WW and 20% higher magnesium content (Table 3.2).



(c)

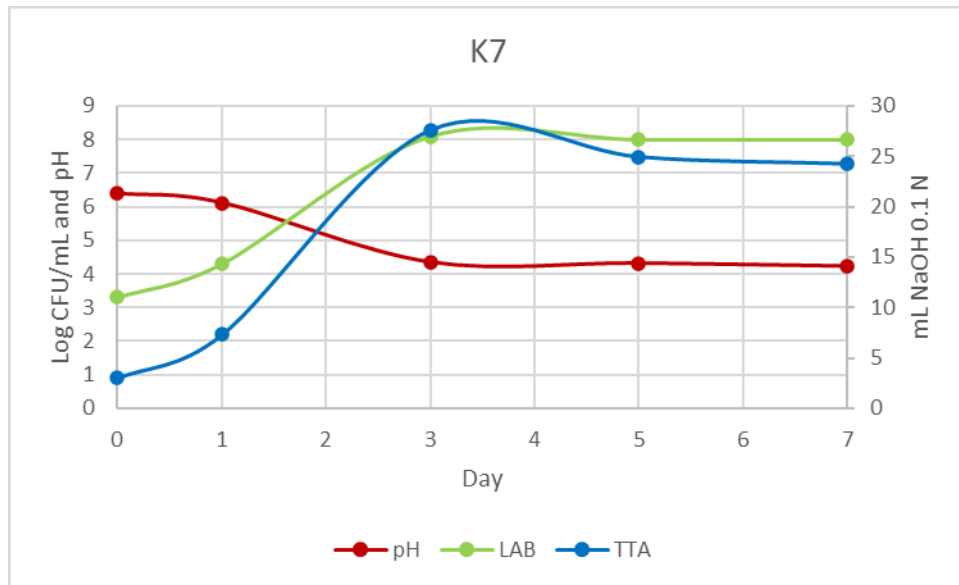


Figure 3.8 (a) LAB evolution fermentation trial WW pH, TTA, and LAB evolution and (b) LAB evolution fermentation trial K6 pH, TTA, and LAB evolution and (c) LAB evolution fermentation trial K7 pH, TTA, and LAB evolution.

The yeast and mold evolution can be seen in figure 3.9. WW increased from day 0 to day 1, but then stayed fairly consistent the remainder of the time. On day 1, K7 and K6 were higher than WW, particularly K6. On day 3, K7 dropped down slightly below WW, but significantly increased afterwards until day 7 by over 2.0 log CFU/mL. K6 dropped down close to WW on day 5, but quickly rose to 4.5 log CFU/mL by day 7. The fluctuation of yeast and mold enumerations for K6 and K7 shows the variability between substrates and suggests the possibility of differing microbial communities compared to WW.

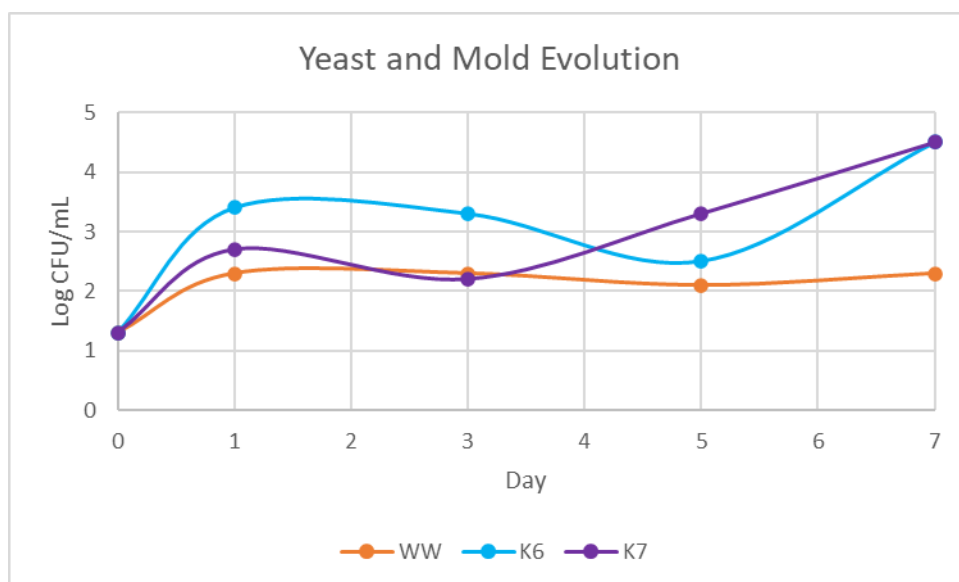


Figure 3.9 LAB evolution fermentation trial yeast and mold evolution for WW, K6, and K7.

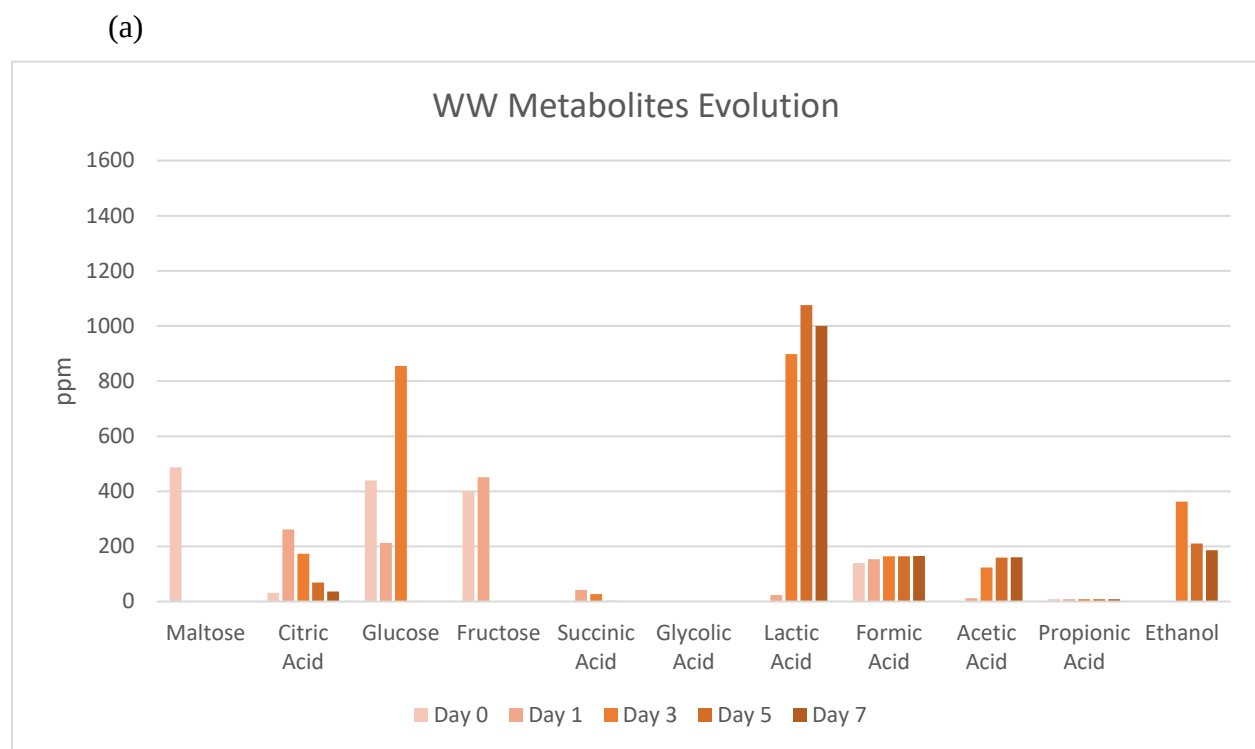
The evolution of metabolite accumulation can be seen in figure 3.10a, b, and c. Succinic acid, glycolic acid, and propionic acid had low levels and were similar among all three samples. Maltose allows LAB species the ability to produce large amounts of lactic and acetic acid and plays an important role in the souring activity of bread, which helps gas production and dough leavening (De Vuyst and Neysens, 2005). This is most likely why maltose was only detected on the initial day and WW was over twice as high as K6 and K7. Citric acid had a similar trend among the three samples with a spike on day 1 and then a decrease, although K7 had another spike on day 7. K6 and K7 had a similar trend for glucose with a large amount on the initial and a dramatic drop by day 1, which never recovered. WW had a large spike in glucose on day 3, with nothing to follow. Fructose was present for WW on day 0 and day 1, then had very low levels. On the other hand, K6 had an increase on day 1-5, whereas K7 was significantly present day 1-5 as well, but the largest production was on day 3. The trend for lactic acid was similar among the three samples with an increase in production from day 3-7, and K6 and K7 had a

higher production than WW, which is discussed in more detail below. Formic acid for WW is fairly consistent, while K6 and K7 increase in production over time. Acetic acid increases from day 0 to day 1, then stays fairly consistent. WW does have a higher acetic production than K6 and K7. Further details are discussed below as well. Finally, ethanol is present day 1 for K6 and K7, then disappears. K6 only has ethanol production on day 7, while K7 has ethanol day 5 and 7. WW only has a spike on day 3, then decreases on day 5 and 7.

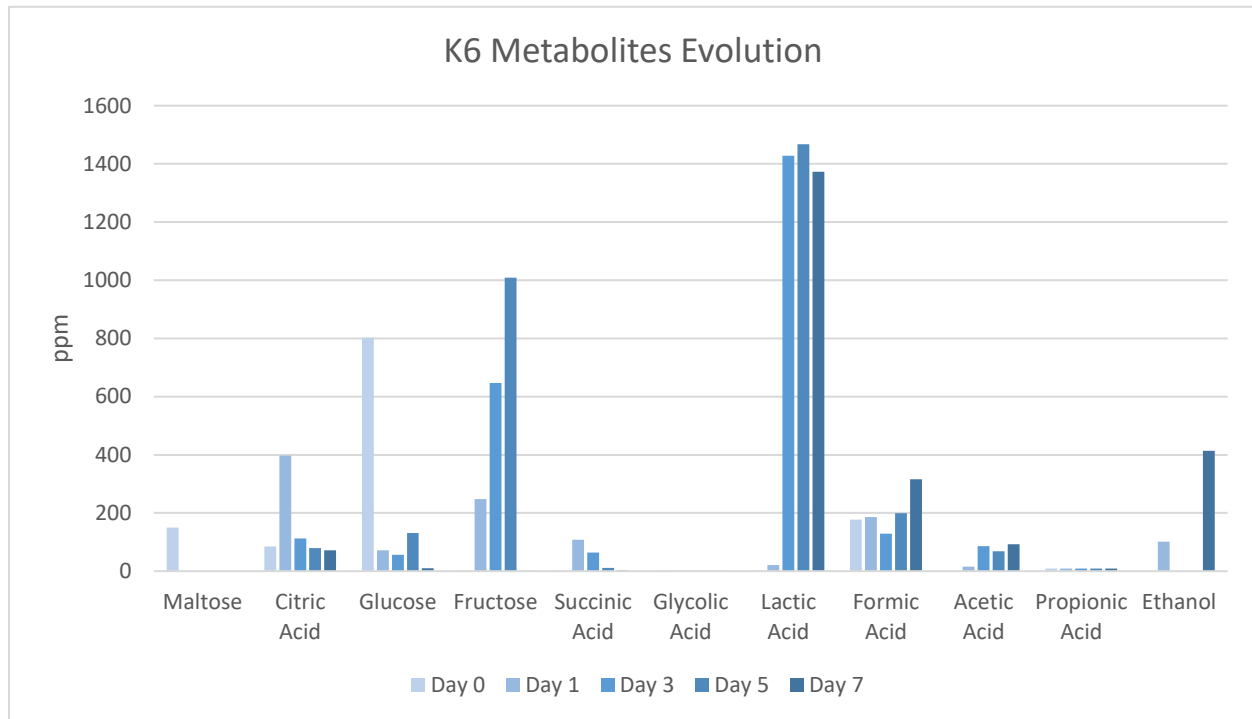
Limosilactobacillus fermentum and *Fructilactobacillus sanfranciscensis* break down maltose into a mix of lactic acid, acetic acid, and some ethanol (Harth et al., 2016). *F. sanfranciscensis* prefers maltose as an energy source (Liu et al., 2023; Stolz et al., 1996). WW has a higher amount of maltose on day 0 with a production of lactic acid, acetic acid, and high ethanol on day 3. There is typically an accumulation of glucose associated with *F. sanfranciscensis* (De Vuyst and Neysens, 2005), which can be seen with a spike on day 3 for the WW sample. This may suggest the presence of *L. fermentum* and/or *F. sanfranciscensis* in the WW sourdough. The higher ethanol content for WW could likely be derived from bacterial metabolism as well, as the yeast and mold enumeration was lower than K6 at day 3. A higher pentose content in rye flour has been associated with presence of *Levilactobacillus brevis*, resulting in higher lactic acid production as compared to wheat sourdoughs (Lau et al., 2021). Given the higher fiber content and higher lactic acid accumulation of K6 and K7 compared to wheat, it can be hypothesized that this microorganism is present in the K6/K7 sourdoughs. Fructose levels were found to be higher for K6 and K7 between days 1-5, compared to the WW sample suggesting a difference in the overall microbial community.

Yeast is mainly responsible for producing ethanol (Wang and Wang, 2024), which could explain the twofold higher K6 ethanol content and threefold higher K7 ethanol content,

compared to wheat on day 7. Although some hetero-fermentative LAB can also produce ethanol. Since, yeast and LAB have a symbiotic relationship (Wang and Wang, 2024), as the microorganisms are establishing the dominant LAB, the yeast enumerations fluctuated in the first 5-7 days, thus impacting the ethanol content to be inconsistent until day 7. It is also possible that the open vessel fermentation allowed for release of any ethanol in the headspace, thus contributing to inconsistent results.



(b)



(c)

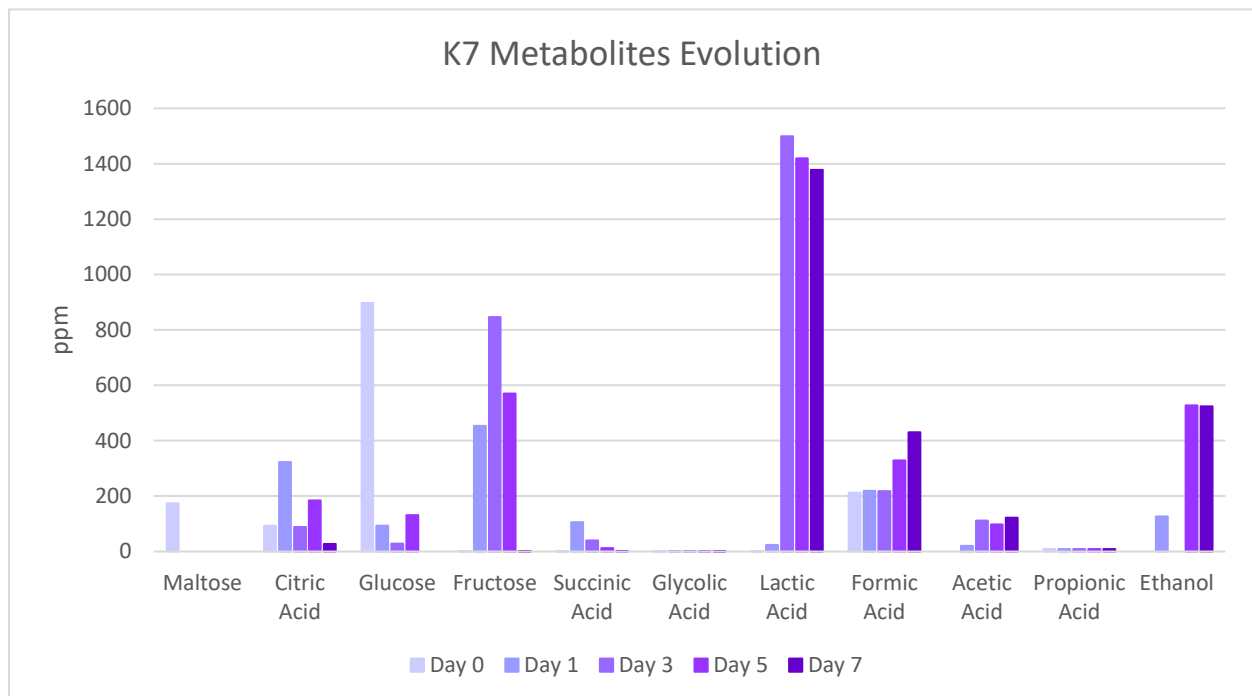
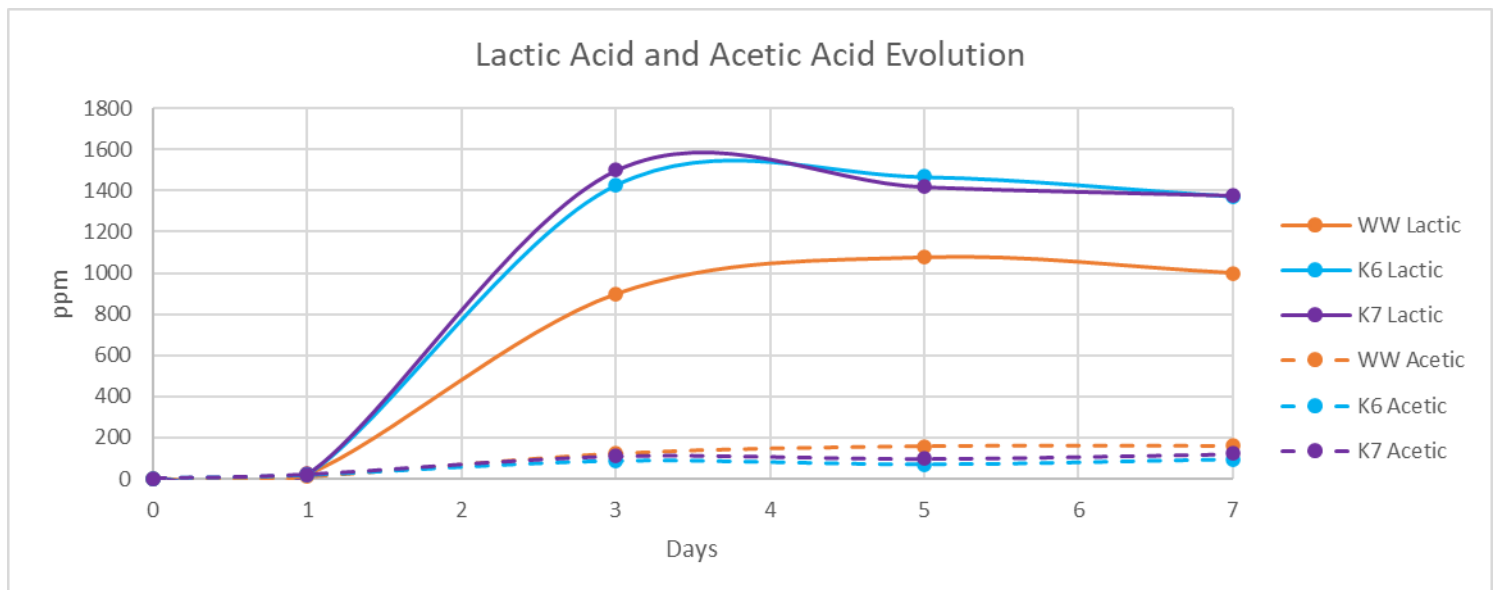


Figure 3.10 (a) LAB evolution fermentation trial organic acid, ethanol, and glucose evolution for WW and (b) LAB evolution fermentation trial organic acid, ethanol, and glucose evolution for K6 and (c) LAB evolution fermentation trial organic acid, ethanol, and glucose evolution for K7.

The production of acetic acid was smaller compared to the lactic acid production over 7 days (figure 3.11). However, WW was slightly higher than K6 and K7 for day 5 and 7. The lactic acid production increased dramatically between day 1 to day 3 for all three samples. K6 and K7 peaked between days 3 and 5 and were almost twofold higher than WW. On day 5 WW lactic acid production peaked, closing the gap between K6 and K7. The difference on day 7 was 400 ppm between WW and K6/K7. FQ was calculated for the final day of fermentation (Figure 3.11). K7 has a slightly lower FQ than K6, signifying a higher production of acetic acid than K6. As previously mentioned, FQ strongly influences bread shelf-life and sensory attributes (Lim et al., 2017; Corsetti, 2013). The FQ from the 11-day fermentation trial is similar to this fermentation trial, with increased lactic acid accumulation as compared to WW.



Sample	FQ
WW	4.2
K6	9.9
K7	7.5

Figure 3.11 LAB evolution fermentation trial lactic acid vs acetic acid evolution for WW, K6, and K7, along with a table of the fermentation quotient (FQ) from Day 7.

3.3.3 Baking Trial

Di Cagno et al., (2014) mentions sourdough has been revealed to positively impact baked goods' sensory, nutritional, texture, and shelf-life. In this study, a 20% inclusion of sourdough was added to the bread formula and was found to have no negative effects to the final product quality in table 3.6. The specific volume, 1-day post-bake firmness, and 5-days post-bake firmness were not statistically significant. Bread samples made with K6, K7, and WW sourdoughs all had an average of 6 days to first visual mold. There were no mold inhibitors or preservatives used in the formula which would change the results. Some sourdoughs produce exopolysaccharides which may contribute to softness and extended shelf-life (Galle, 2013). However, there was no evidence of this. The pH and TTA of the final bread were statistically significant, showing the K6 and K7 breads to be more acidic than the WW.

Table 3.6 Final product analysis: pH, TTA, 1-day post-bake firmness, 5-days post-bake firmness, and specific volume.

	Bread pH $\pm$ SD	Bread TTA mL $\pm$ SD	Day 1 Firmness grams $\pm$ SD	Day 5 Firmness grams $\pm$ SD	Specific Volume cm ³ /g $\pm$ SD
WW	5.212 $\pm$ 0.06 ^a	4.174 $\pm$ 0.06 ^b	267.2 $\pm$ 24.81	616.32 $\pm$ 96.89	4.04 $\pm$ 0.19
K6	5.115 $\pm$ 0.01 ^{ab}	4.814 $\pm$ 0.01 ^a	261.53 $\pm$ 35.97	561.48 $\pm$ 74.07	4.04 $\pm$ 0.21
K7	4.978 $\pm$ 0.11 ^b	5.058 $\pm$ 0.13 ^a	253.29 $\pm$ 54.59	562.56 $\pm$ 54.20	4.15 $\pm$ 0.19
P-value	0.03	0.0007	0.83	0.37	0.77

Different letters in column indicate significant difference ($p < 0.05$) and significant p-values bolded for impact.

3.5 Conclusion

End uses for IWG are still being explored, as there is a lack of HMW-GS and poor performance in bread. The exploration of K6 and K7 in a spontaneous fermentation process was successful. Compared to a WW, K6 and K7 had a higher acid production. This could be

beneficial in the industry. The acidification kinetics, LAB, and yeast and mold evolution were also explored. LAB and yeast and mold enumerations were found to be higher for K6 and K7 compared to WW. Lactic acid production was significantly higher for K6 and K7, but acetic acid was slightly higher for WW. This signifies a difference in microbial communities where K6 and K7 may have more homo-fermentative LAB, compared to a more hetero-fermentative LAB dominant WW. K6 and K7 were also utilized in a wheat-based bread trial and found to have more acidic final products. While, 1-day and 5-days post-bake firmness, specific volume, and first day to visual mold analysis were not significant there were no negative effects, even with a higher inclusion of sourdough. K6 and K7 were found to perform well in a sourdough process and wheat-based bread trial. This could be a potential application for IWG as a novel substrate in sourdough fermentation.

3.6 References

- AACC International. Approved Methods of Analysis, 11th Ed. Method 10-10.03. Optimized Straight-Dough Bread-Making Method. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-10-10.03>
- AACC International. Approved Methods of Analysis, 11th Ed. Method 10-16.01. Volumetric and Dimensional Profile of Baked Products by Laser Topography-Volscan Profiler Method. Approved December 2015. AACC International, St. Paul, MN, U.S.A. <https://doi.org/10.1094/AACCIntMethod-10-16.01>
- AACC International. Approved Methods of Analysis, 11th Ed. Method 74-09.01. Measurement of Bread Firmness by Universal Testing Machine. Reapproved November 3, 1999. AACC International, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-74-09.01>
- Abarquero, D., Renes, E., Fresno, J. M., & Tornadijo, M. E. (2022). Study of exopolysaccharides from lactic acid bacteria and their industrial applications: A review. *International Journal of Food Science & Technology*, 57(1), 16–26. <https://doi.org/10.1111/ijfs.15227>
- Altan, A., McCarthy, K. L., & Maskan, M. (2008). Twin-screw extrusion of barley–grape pomace blends: Extrudate characteristics and determination of optimum processing conditions. *Journal of Food Engineering*, 89(1), 24–32. <https://doi.org/10.1016/j.jfoodeng.2008.03.025>
- Corsetti, A. (2013). Technology of sourdough fermentation and sourdough applications. In M. Gobbetti & M. Gänzle (Eds.), *Handbook on Sourdough Biotechnology* (pp. 85–103). Springer US. https://doi.org/10.1007/978-1-4614-5425-0_4
- Corsetti, A., & Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Research International*, 40(5), 539–558. <https://doi.org/10.1016/j.foodres.2006.11.001>
- De Vuyst, L., & Neysens, P. (2005). The sourdough microflora: Biodiversity and metabolic interactions. *Trends in Food Science & Technology*, 16(1), 43–56. <https://doi.org/10.1016/j.tifs.2004.02.012>
- De Vuyst, L., Vrancken, G., Ravyts, F., Rimaux, T., & Weckx, S. (2009). Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiology*, 26(7), 666–675. <https://doi.org/10.1016/j.fm.2009.07.012>
- Di Cagno, R., Pontonio, E., Buchin, S., De Angelis, M., Lattanzi, A., Valerio, F., Gobbetti, M., & Calasso, M. (2014). Diversity of the Lactic Acid Bacterium and Yeast Microbiota in the Switch from Firm- to Liquid-Sourdough Fermentation. *Applied and Environmental Microbiology*, 80(10), 3161–3172. <https://doi.org/10.1128/AEM.00309-14>
- Ercolini, D., Pontonio, E., De Filippis, F., Minervini, F., La Stora, A., Gobbetti, M., & Di Cagno, R. (2013). Microbial Ecology Dynamics during Rye and Wheat Sourdough

- Preparation. *Applied and Environmental Microbiology*, 79(24), 7827–7836.
<https://doi.org/10.1128/AEM.02955-13>
- Galle, S. (2013). Sourdough: A tool to improve bread structure. In M. Gobbetti & M. Gänzle (Eds.), *Handbook on Sourdough Biotechnology* (pp. 217–228). Springer US.
https://doi.org/10.1007/978-1-4614-5425-0_8
- Galli, V., Venturi, M., Pini, N., Guerrini, S., Granchi, L., & Vincenzini, M. (2019). Liquid and firm sourdough fermentation: Microbial robustness and interactions during consecutive backsloppings. *LWT*, 105, 9–15. <https://doi.org/10.1016/j.lwt.2019.02.004>
- Gil-Cardoso, K., Saldana, G., Luengo, E., Pastor, J., Virto, R., Alcaide-Hidalgo, J.M., del Bas, J.M., Arola, L., Caimari, A. 2021. Consumption of sourdough bread improves postprandial glucose response and produces sourdough-specific effects on biochemical and inflammatory parameters and mineral absorption. *Journal of Agricultural and Food Chemistry*, 69, 3044-3059.
- Gobbetti, M., De Angelis, M., Di Cagno, R., Calasso, M., Archetti, G., Rizzello, C.G. 2019. Novel insights on the functional/nutritional features of the sourdough fermentation. *International Journal of Food Microbiology*, 302, 103-113.
- Hammes, W. P., Brandt, M. J., Francis, K. L., Rosenheim, J., Seitter, M. F. H., & Vogelmann, S. A. (2005). Microbial ecology of cereal fermentations. *Trends in Food Science & Technology*, 16(1), 4–11. <https://doi.org/10.1016/j.tifs.2004.02.010>
- Harth, H., Van Kerrebroeck, S., & De Vuyst, L. (2016). Community dynamics and metabolite target analysis of spontaneous, backslopped barley sourdough fermentations under laboratory and bakery conditions. *International Journal of Food Microbiology*, 228, 22–32. <https://doi.org/10.1016/j.ijfoodmicro.2016.04.011>
- Lau, S. W., Chong, A. Q., Chin, N. L., Talib, R. A., & Basha, R. K. (2021). Sourdough microbiome comparison and benefits. *Microorganisms*, 9(7), 1355.
<https://doi.org/10.3390/microorganisms9071355>
- Lim, S. B., Tingirikari, J. M. R., Seo, J. S., Li, L., Shim, S., Seo, J.-H., & Han, N. S. (2017). Isolation of lactic acid bacteria starters from Jeung-pyun for sourdough fermentation. *Food Science and Biotechnology*, 27(1), 73–78.
<https://doi.org/10.1007/s10068-017-0274-0>
- Liu, M., Li, S., Weiss, T., Li, Y., Wang, D., Zheng, Y., 2023. Solid-state fermentation of grain sorghum to produce Chinese liquor: Effect of grain properties and fermenting culture. *Journal of Cereal Science* 114, 103776. <https://doi.org/10.1016/j.jcs.2023.103776>
- Liu, M., Mahata, C., Wang, Z., Kumar, S., Zheng, Y., 2024. Comparative exploration of biological treatment of hydrothermal liquefaction wastewater from sewage sludge: Effects of culture, fermentation conditions, and ammonia stripping. *Journal of Environmental Management* 349, 119527.
<https://doi.org/10.1016/j.jenvman.2023.119527>

- Liu, Y., Danial, M., Dong, H., Zhang, M., Jia, R., & Zhang, G. (2023). Breeding of *Fructilactobacillus sanfranciscensis* with excellent ethanol tolerance based on adaptive evolution technology. *Food Bioengineering*, 2(1), 15–24. <https://doi.org/10.1002/fbe2.12039>
- Marti, A., Qiu, X., Schoenfuss, T. C., & Seetharaman, K. (2015). Characteristics of Perennial Wheatgrass (*Thinopyrum intermedium*) and Refined Wheat Flour Blends: Impact on Rheological Properties. *Cereal Chemistry*, 92(5), 434–440. <https://doi.org/10.1094/CCHEM-01-15-0017-R>
- Matevosyan, L., Bazukyan, I., & Trchounian, A. (2019). Comparative analysis of the effect of Ca and Mg ions on antibacterial activity of lactic acid bacteria isolates and their associations depending on cultivation conditions. *AMB Express*, 9, 32. <https://doi.org/10.1186/s13568-019-0758-9>
- Poutanen, K., Flander, L., Katina, K. 2009. Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*, 26, 693-699.
- Stolz, P., Hammes, W., & Vogel, R. (1996). Maltose-phosphorylase and hexokinase activity in lactobacilli from traditionally prepared sourdoughs. *Advances in Food Sciences*. <https://www.semanticscholar.org/paper/Maltose-phosphorylase-and-hexokinase-activity-in-Stolz-Hammes/1825e06518a41d08a52467278dcf849a1e5db163>
- Suprabha Raj, A., Boyacioglu, M. H., Dogan, H., & Siliveru, K. (2023). Investigating the contribution of blending on the dough rheology of roller-milled hard red wheat. *Foods*, 12(10), 2078. <https://doi.org/10.3390/foods12102078>
- Wang, Z., & Wang, L. (2024). Impact of sourdough fermentation on nutrient transformations in cereal-based foods: Mechanisms, practical applications, and health implications. *Grain & Oil Science and Technology*. <https://doi.org/10.1016/j.gaost.2024.03.001>

Chapter 4 - Degree of Difference Sensory Testing of Breads Made with Intermediate Wheatgrass Versus Whole Wheat Spontaneously Fermented Sourdoughs

4.1 Introduction

Intermediate wheatgrass (IWG) has been experimented with in a variety of products up to date. However, IWG is still only sparingly used in beer and food (Marcus and Fox, 2022). The most acceptable result for bread has been blended with wheat flour.

Sensory analysis uses all the panelist's senses to investigate a product's characteristics or attributes (Ruiz-Capillas and Herrero, 2021). The significance of sensory evaluation lies in its ability to gauge how consumers perceive a product (Delarue, 2022). There are many types of sensory tests, but the goal then becomes choosing the best evaluation to answer the principal question. This will dictate how an evaluation is set up.

One type of evaluation is the degree of difference (DOD) test, which measures how similar or different one product is to another. It takes the production variation into consideration and was found to be better suited in determining if a heterogeneous product is truly different to another than a triangle test would (Aust et al., 1985). For DOD an action criteria is chosen prior to the sensory analysis. An action criteria of 2 is typical for breads, signifying at the action criteria and below there is a low risk of consumers being able to notice a difference in the product. Whereas above the action criteria it is assumed that there is a risk that consumers would notice a difference in the product, and as the score increases, the intensity of the difference also increases.

In sensory evaluation, consumers can also be asked what they like or dislike about a product they are evaluating. A Just About Right (JAR) scales is one of the more favored methods to pinpoint areas in which enhancements can be made (Delarue, 2022). This type of scale can be used to identify if product attribute levels are met. They are usually paired with an overall liking evaluation, and are a simple, effective way to supply quantitative data (Delarue, 2022). Although with both, DOD and JAR, consumers perceptions will differ due to various factors. For instance, a key factor that impacts consumers' outlooks and expectations towards food is culture (Jeong and Lee, 2021).

The IWG in a sourdough process was found to be successful with the potential for technological advantages (Chapter 3). The use of IWG sourdough in a wheat-based bread was also found to perform as well as a whole wheat sourdough, with no negative effects to firmness or specific volume. The following steps were to determine how similar or different these sourdoughs would be. The overall aim of this project was to evaluate whether consumers would detect the difference between a wheat-based bread with the addition of whole wheat sourdough or whole grain IWG sourdough. A lexicon was adapted from Tran et al. (2018) and Lotong et al. (2000), which were based on attributes of wheat sourdough bread and rye bread. It was hypothesized the breads would have subtle differences, but overall, the IWG sourdough added bread would be similar to the whole wheat added sourdough bread that consumers would not notice.

4.2 Materials and Methods

4.2.1 Sourdough Fermentation

IWG (K6) and whole wheat (WW) flours were milled as previously described (Chapter 3 Section 3.2.2). The flours were used to establish a stable sourdough through spontaneous

fermentation. Briefly, a 1:1 mixture of DI water and either WW or IWG flour (cultivar C6) was prepared and incubated in an environmental chamber (Percival Scientific Inc., Perry, Iowa) at 28°C and a relative humidity (RH) of 85%. Sourdoughs were backslopped every 24 hours using a ratio of 1:1:1 (sourdough: DI water: whole grain flour) for 7 days. Fermentations were conducted in triplicates. 10 grams of sourdough was blended in 100 grams of DI water to measure pH and total titratable acidity (TTA) using an automatic titrator. Titration was carried out using NaOH 0.1N to an endpoint of pH 8.5. Final day sourdough metabolites were analyzed using high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan), as described previously (Liu et al., 2024; Liu et al., 2023). A five-point standard curve was generated for each compound with an R-squared value above 0.99. To prepare the samples, 2 grams of sourdough was dispersed in 20 grams of DI water, vortexed, and centrifuged at 5,000 rpm for 15 minutes. 2 mL of the supernatant were removed and filtered through a 0.2 µm filter into the HPLC vial. The color of the sourdough starters was measured on day 7 using a colorimeter (Konica Minolta). After 7 days of fermentation, samples were sealed in polyethylene bags and frozen at 18°C until use. Prepared sourdoughs are named WW for samples made with whole wheat flour and K for samples made with IWG.

4.2.2 Baking

To prepare the bread samples, bread flour (Kansas Sun) was donated by Grain Craft. Instant active dry yeast (Lesaffre saf), all-purpose vegetable shortening, sugar, salt and tap water.

Sourdough was thawed at ambient temperature for 2 hours prior to use, and pH and TTA were measured immediately before dough preparation. A no-time baking method was used, meaning no bulk fermentation prior to shaping and panning. A 20-quart mixer bowl with a dough hook attachment was utilized (Centerline by Hobart, Troy, Ohio). All dry ingredients were

weighed, except shortening and yeast were kept separate in their own containers. The dry ingredients were added to the mixer bowl. Water was used at 3°C for a desired dough temperature of 23°C. Absorption level was determined from previous studies (62% flour basis). The dough was mixed for 1 minute on 1st speed. The sourdough was added at 20% flour basis and mixed for 1 minute on 1st speed. Each dough was mixed to development on 2nd speed (15 minutes for WW sourdough added dough and 12 minutes for IWG sourdough added dough). The doughs were allowed to rest for a 5-minute floor time before dividing into 5 dough pieces of 565 grams each. The doughs were then rounded and allowed to rest another 5 minutes for an intermediate proof time. Afterwards the doughs were sheeted and moulded using an Oshikiri moulder (model MG, Oshikiri Machinery Ltd., Fujisawa, Japan) (top roller: 110 mm; bottom roller: 50 mm; infeed belt: 32.5 mm; guide rails in: 32 mm; guide rails out: 24 mm; pressure board in: 42.5 mm; pressure board out: 40.5 mm) and put seam side down into release-agent sprayed pans (top inside: 12.8cm x 25.5cm; bottom outside: 11.0 cm x 23.7 cm; height: 7.6 cm). The dough was placed into a proofing chamber at 40.6°C and 85% RH for 60 minutes, until the dough reached 2 cm above the top rim of the pan) The samples were baked at 205°C until internal temperature reached 93.3°C in a gas-fired Reel oven (Reed Oven Co., Kansas City, Missouri). The WW sourdough added dough baked for 22 minutes, while the IWG sourdough added dough baked for 20 minutes. The bread was then depanned and left to cool for one hour before analysis.

4.2.3 Physical Measurement of Breads

The cooled breads were weighed using an electronic balance. Loaf volume was measured using laser topography (Volscan, Stable Micro Systems, Godalming, Surrey, United Kingdom) using AACC Method 10-16.01 (AACC International, 2015). Specific loaf volume was calculated

by dividing loaf volume by the loaf weight (cm³/g). The crust color of the breads was measured using a colorimeter (Konica Minolta). To calculate color differences, Altan et al. (2008) was used. ΔL^* calculates the difference in lightness and darkness. The more positive the lighter sample, the more negative the darker. Δa^* calculates the difference in red and green hues. The more positive the redder, and the more negative the greener. Δb^* calculates the difference in yellow and blue hues. The more positive the yellower, and the more negative the bluer. ΔE^*_{ab} calculates the total difference. The higher the value the more different the samples are.

The samples were then sliced using a reciprocating blade slicer (model 797, Oliver, Grand Rapids, Michigan) put into bags and stored at room temperature for further measurements the next day. For crumb grain analysis, 3 slices were scanned with a C-Cell (Calibre Control International Ltd., Appleton, Warrington, United Kingdom) using AACC Method 10-18.01 (AACC International, 2017). Each slice was analyzed for slice brightness, number of cells, area of cells, and cell diameter. The bread firmness at 1-day post-bake was measured using a TA.XT2 Texture Analyzer (Texture Technologies or Stable Micro Systems, Scarsdale, New York) with a 25 mm acrylic probe following AACC Method 74-09.01 (AACC International, 1999). Texture Exponent 32 version 5.07.0 was used to analyze the firmness. pH and TTA were measured as previously mentioned, but with 10 grams of crumb in 100 grams of DI water.

4.2.4 Sensory Evaluation by the Degree of Difference (DOD) Test

Sensory evaluation of the overall flavor and 4 guiding attributes (appearance, aroma, flavor, and texture) between breads with K and WW sourdoughs was accomplished using a degree of difference test. A total of 50 panelists from Kansas State University (KSU) participated in the study for a total of 2 sessions over 2 days with approximately 20 panelists per session. Between the 2 sessions, panelists were allowed to participate again if available. Before

participating, panelists were screened for allergens and to be regular consumers of bread made with whole grain flour. Panelists were given a practice ballot beforehand to become familiar with how to use the scale with store bought samples. Despite the training on ballot use, the panelists are considered untrained (naive).

In each session K and WW samples were evaluated. Before panelists arrived, bread slices were cut in half vertically and kept in bags, then placed in a tote to prevent samples from drying out. When panelists arrived, a set of samples of a reference (WW) and 2 test samples (K or WW used as a blind control) were presented to panelists on a 7-inch Chinnet crystal plastic plate with half a slice of each. The ordering of presenting test samples was randomized across panelists. To compare each test sample against the reference, panelists were instructed to taste the reference initially, followed by the test sample. The DOD scale ranged from 0 to 7, with descriptors below the anchors explaining the difference in size: 0 correlating to ‘not at all different’ and 7 correlating to ‘extremely different’ (figure 4.1). A Just About Right scale ranged from 1 to 5, with descriptors below the number explaining the difference in size: 1 correlated to ‘does not meet expectations’, 2 correlated to ‘slightly meets expectations’, 3 correlated to ‘meets expectations’, 4 correlated to ‘slightly exceeds expectations’, and 5 correlated to ‘significantly exceeds expectations’ (figure 4.2). Followed by two questions to ‘briefly describe anything they liked or disliked about the sample’. Total there were two sample comparisons with 12 questions for each test sample (complete ballot can be found in appendix B.1). To remove the possibility of flavor transfer, panelists were provided with water for in between the two comparisons. Study protocols were approved by the KSU Institutional Review Board (#12013).

1. How similar or different overall is the sample compared to the reference sample?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different						Extremely Different	

Figure 4.1 DOD scale ranging from 0 to 7.

6. How well does the sample meet your overall expectations?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

Figure 4.2 JAR scale ranging from 1 to 5.

4.2.5 Statistical Analysis

All analysis was conducted in duplicates. JMP Pro 17.2.0 was used to run ANOVA and T-test (significance established at $p < 0.05$), and to conduct text analysis and build word clouds (Text Explorer function).

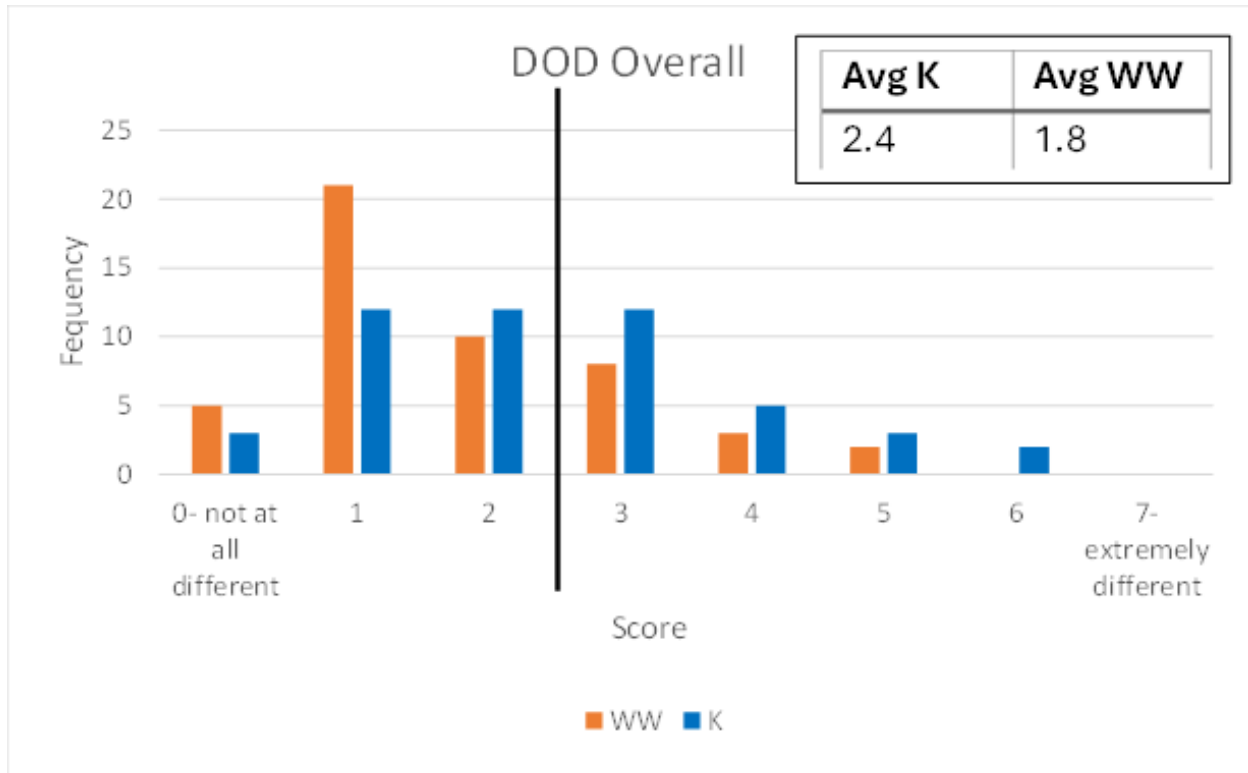
4.3 Results and Discussion

The results of the DOD questions can be seen in figure 4.3. The black line represents the action criteria chosen (2). Low scores signify that K and WW are similar. The averages can also be seen in the figure. Based on the average for the WW sample, an assumption can be made that the participants were looking for a difference since WW was a blind control. In this case, the difference between K and WW is decreased and are more similar to each other.

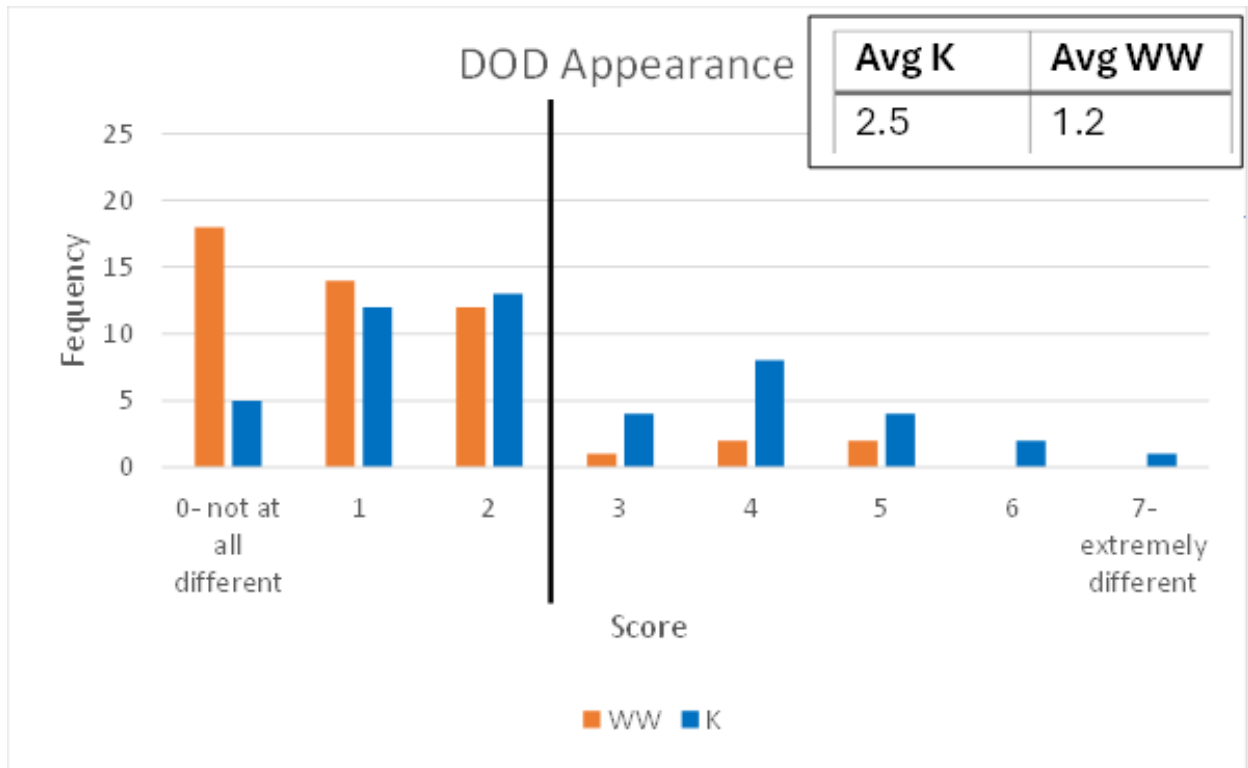
The overall score has a higher percentage of WW below the action criteria, indicating a lower perception of difference. The majority of the K scores are between scores 1-3 (region closer to “not at all different”) with a few responses between scores 4-6 (closer to “extremely different”). The biggest difference between WW and K is in the low difference region (1 score). For the appearance attribute, the majority of WW and K are below the action criteria, with a slight increase at a score of 4 for K. This was also the only attribute that scored a 7 (anchor for extremely different). For the aroma attribute, the majority of WW was below the action criteria, whereas K had a more level response between scores 0-3. The flavor attribute has a large percentage of both K and WW responses below the action criteria line. There was a slight increase for K at a score of 4 again. For the texture attribute K makes a U-shape between scores 1-3 and is similar to WW for scores 4-6. The majority of WW responses were below the action criteria, particularly between the scores of 0-1.

Across all five histograms K consistently had 1-2 responses at a score of 6 (close to “extremely different”), and a response at a score of 7 for appearance. Whereas, WW only had a few responses at a score of 6 for the flavor and texture attributes. The appearance and aroma attributes have the biggest differences in averages. While overall, flavor, and texture attributes are more similar between WW and K averages. Based on the trends from the graphs, it becomes clear there is a slight difference to consumers between WW and K. Formulation and processing can be optimized to enhance similarity between the samples. For instance, the TTA added to each bread can be matched to more closely align aroma and flavor. A lower amount of K sourdough can also impact appearance.

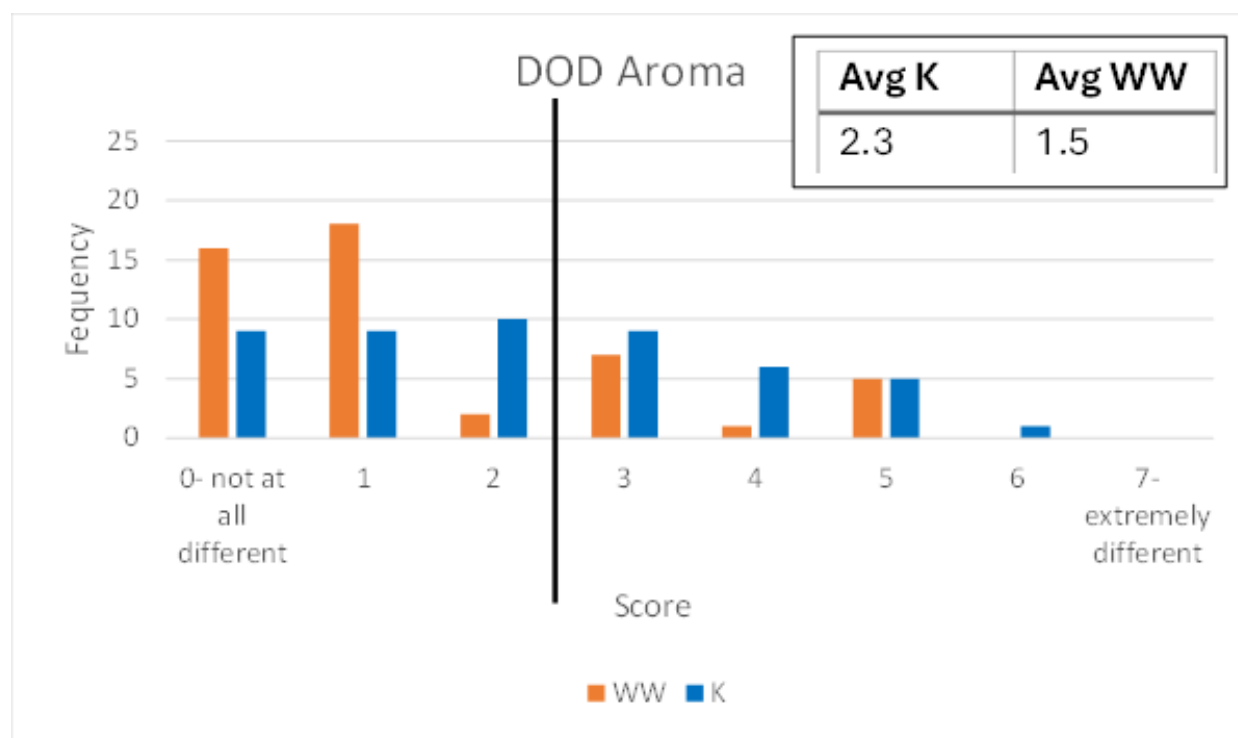
(a)



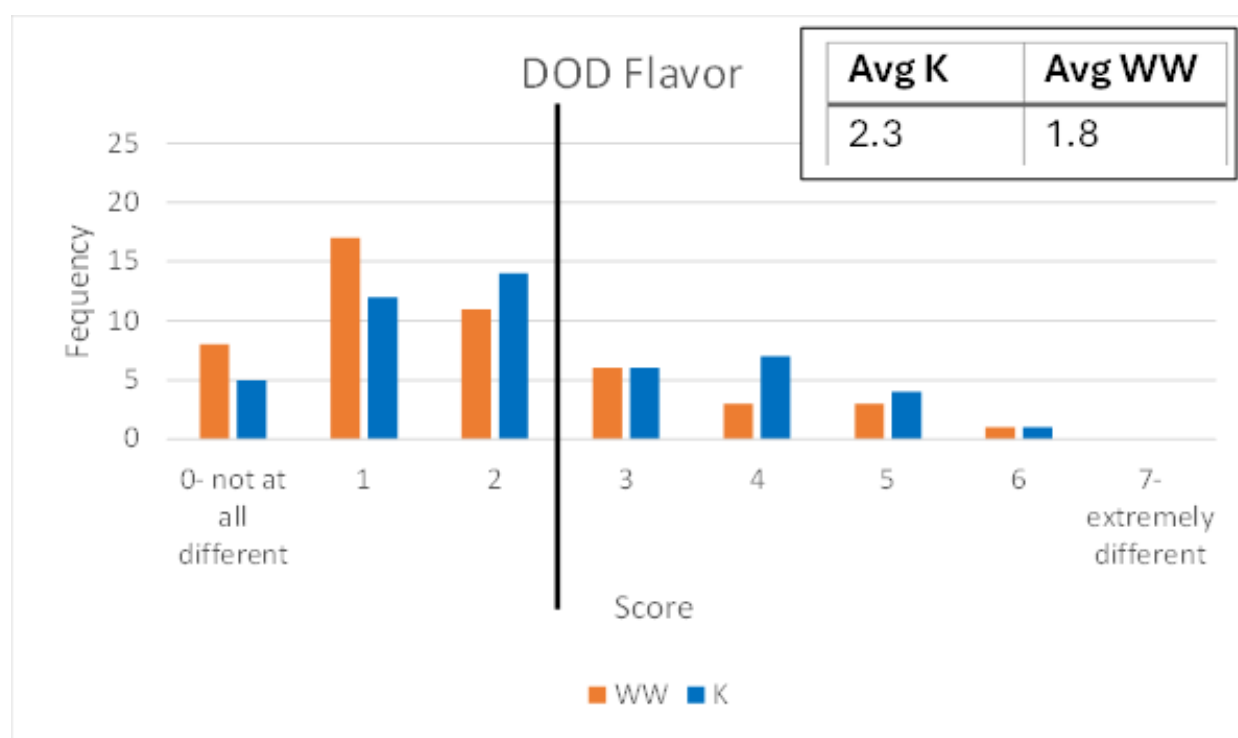
(b)



(c)



(d)



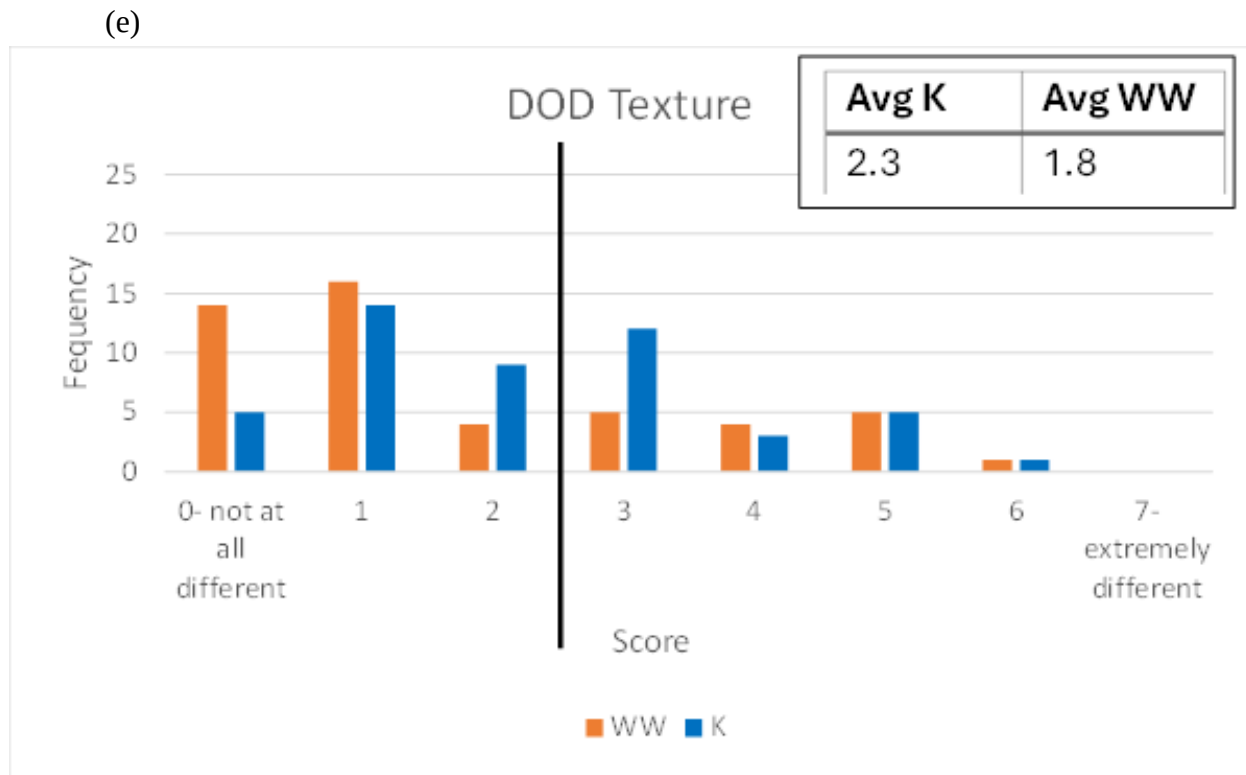


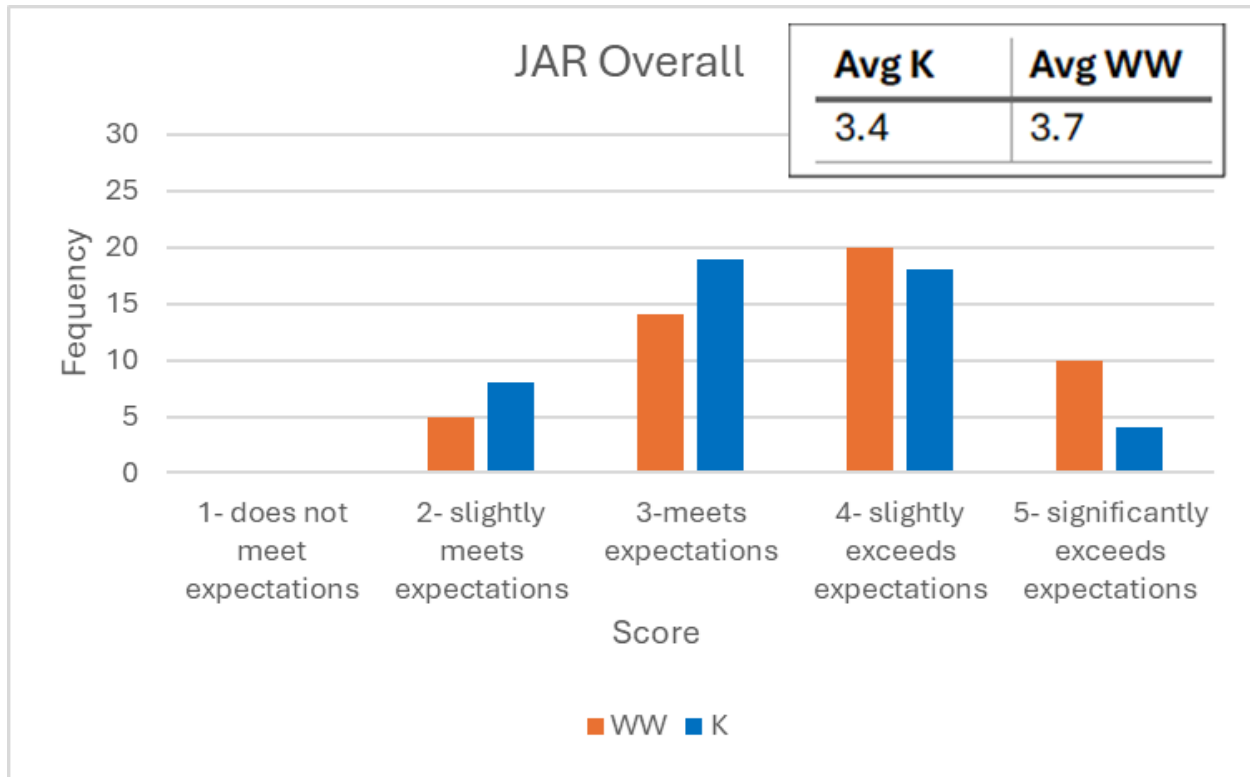
Figure 4.3 Degree of difference histograms (a) overall (b) appearance (c) aroma (d) flavor and (e) texture with each attributes average scores for K and WW. Black line represents action criteria chosen at 2. Signifying at the action criteria and below there is a low risk of consumers being able to notice a difference in the product. Above the action criteria it is assumed that there is a risk that consumers would notice a difference in the product, and as the score increases, the intensity of the difference also increases.

The objective of the Just About Right (JAR) questions was to determine the consumer's expectations and whether the product does or does not meet those expectations. The target of this scale is to have a score of 3 or higher ("meets expectations" to "significantly exceeds expectations"). A score of 1 and 2 ("does not meet expectations" and "slightly meets expectations," respectively) could signify that particular attribute is not acceptable. Averages of the overall and each attribute are above 3 for both WW and K (figure 4.4). All of the averages are similar with 0.3 being the largest difference.

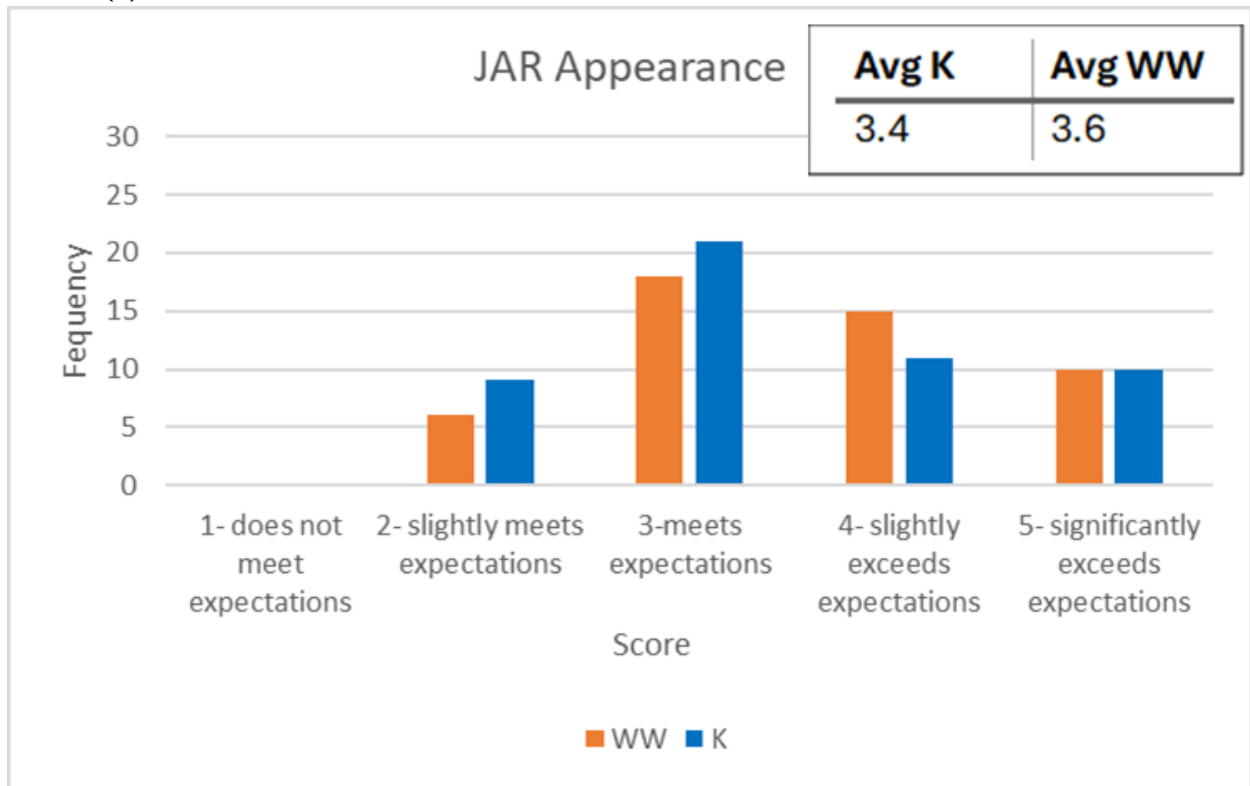
The overall graph, K has a higher frequency at a score of 3 than WW but is not as high at a score of 5 and slightly higher at a score at 2. The appearance attribute trend is similar to the overall trend, with a higher frequency at a score of 3 for K, but also a slightly higher frequency at a score of 2. The aroma attribute stands out the most with a pronounced increase above a frequency of 25 for both K and WW at a score of 3. WW does have a small presence at a score of 1. The flavor attribute's highest peak is also at a score of 3 for both WW and K. Both of the samples have a small presence at a score of 1 and are the only attribute to do so. The texture attribute also has a pronounced peak at a score of 3 where K is greater than WW. Although K does have a small presence at a score of 1.

Across all five histograms, the largest peak is typically at a score of 3. Indicating WW and K meet expectations for a higher percentage of the panelists. While there is a presence at a score of 2 for both samples, they do not exceed a higher frequency than scores at 4 or 5. Seldomly were there scores present at 1, indicating WW and K at least "slightly exceeded expectations" for a large percentage of the panelists.

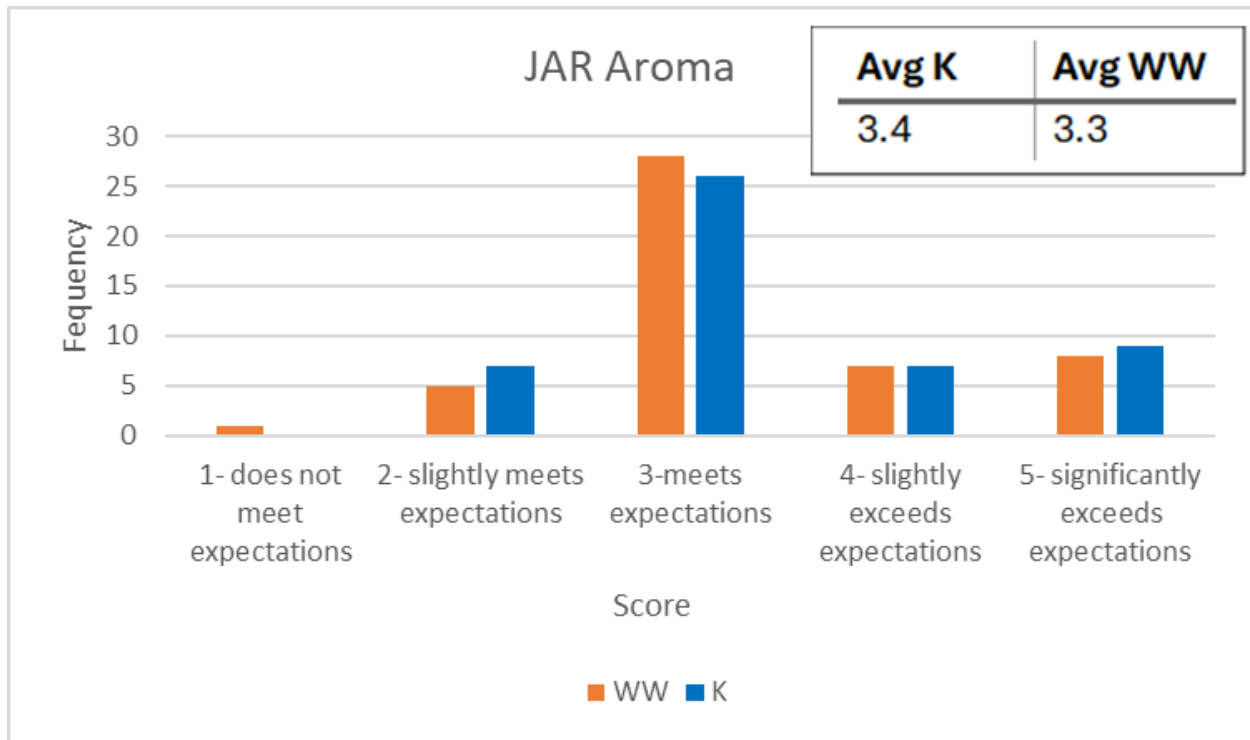
(a)



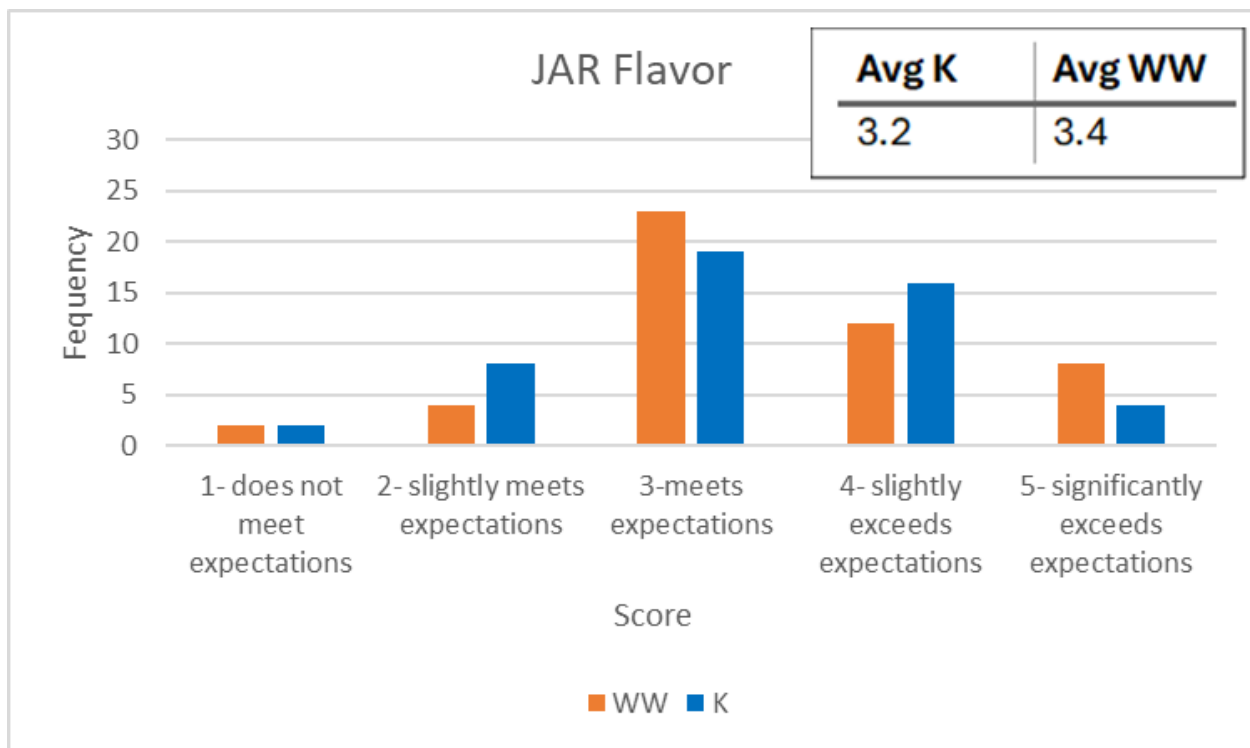
(b)



(c)



(d)



(e)

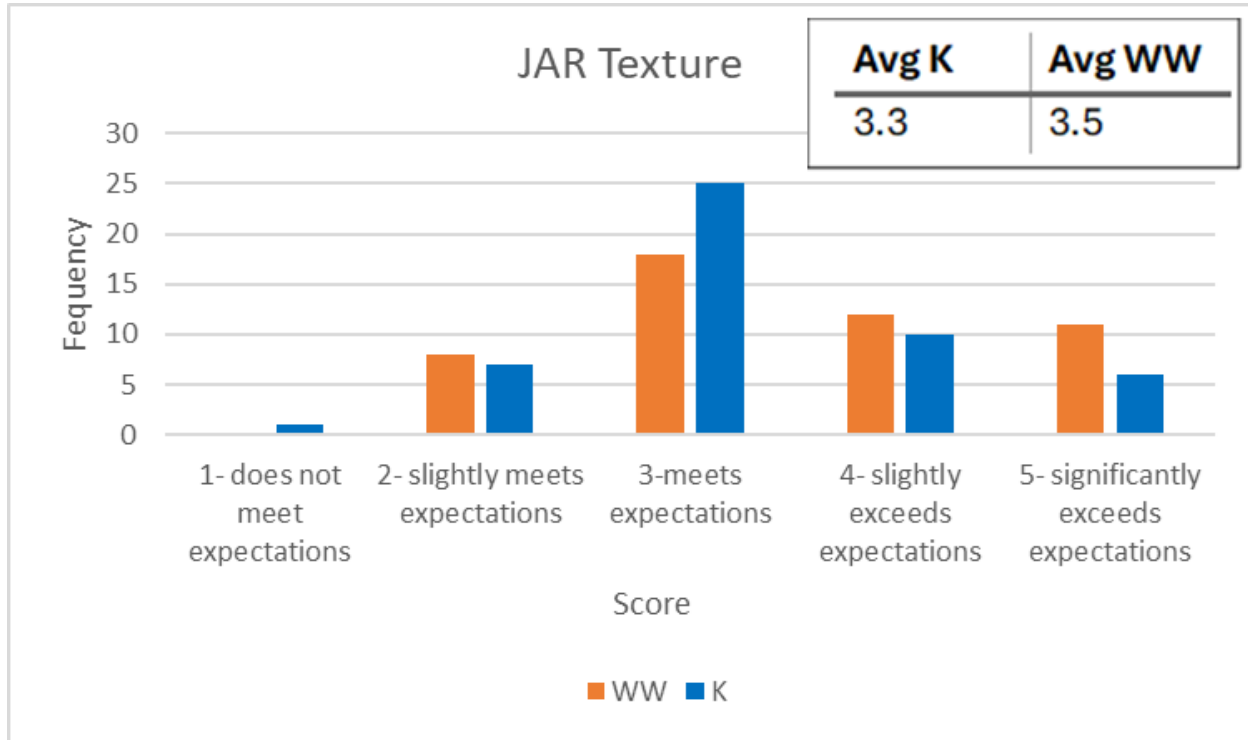
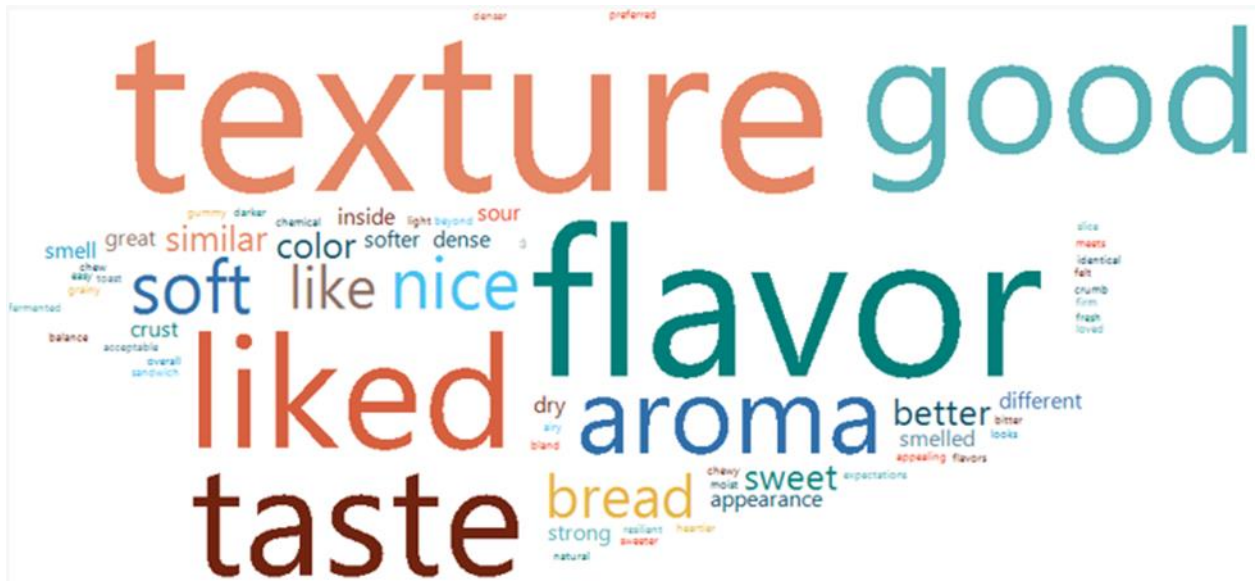


Figure 4.4 Just About Right histograms (a) overall (b) appearance (c) aroma (d) flavor and (e) texture with each attributes average scores for K and WW.

Comparing the DOD and JAR results, consumers did notice slight differences between WW and K, however K still met consumers expectations and even exceeded them. This is where process and formulation optimization could help K be more similar or better than WW. Despite the slight differences noticed for K, consumers still liked it and even preferred K sometimes. This could be where consumers' cultural differences impact their attitude and outlook towards food becomes the most evident, as previously mentioned by Jeong and Lee (2021). Figure 4.5a and b and figure 4.6a and b shows the word clouds of the likes and dislikes for both K and WW. The main attributes panelists enjoyed for K were the texture, flavor/taste, and aroma, whereas they disliked color, texture, bitter favor/taste. But a high percentage also said they disliked

nothing. The main attributes panelists enjoyed for WW were the texture, flavor, and aroma, whereas they disliked the crust, flavor, aroma and nothing at all as well.

(a)



(b)



Figure 4.5 (a) Word cloud of open-ended question for liking K sample and (b) Word cloud of open ended question for disliking K sample. The larger, bolder words signify a higher association of characteristics among various panelists.

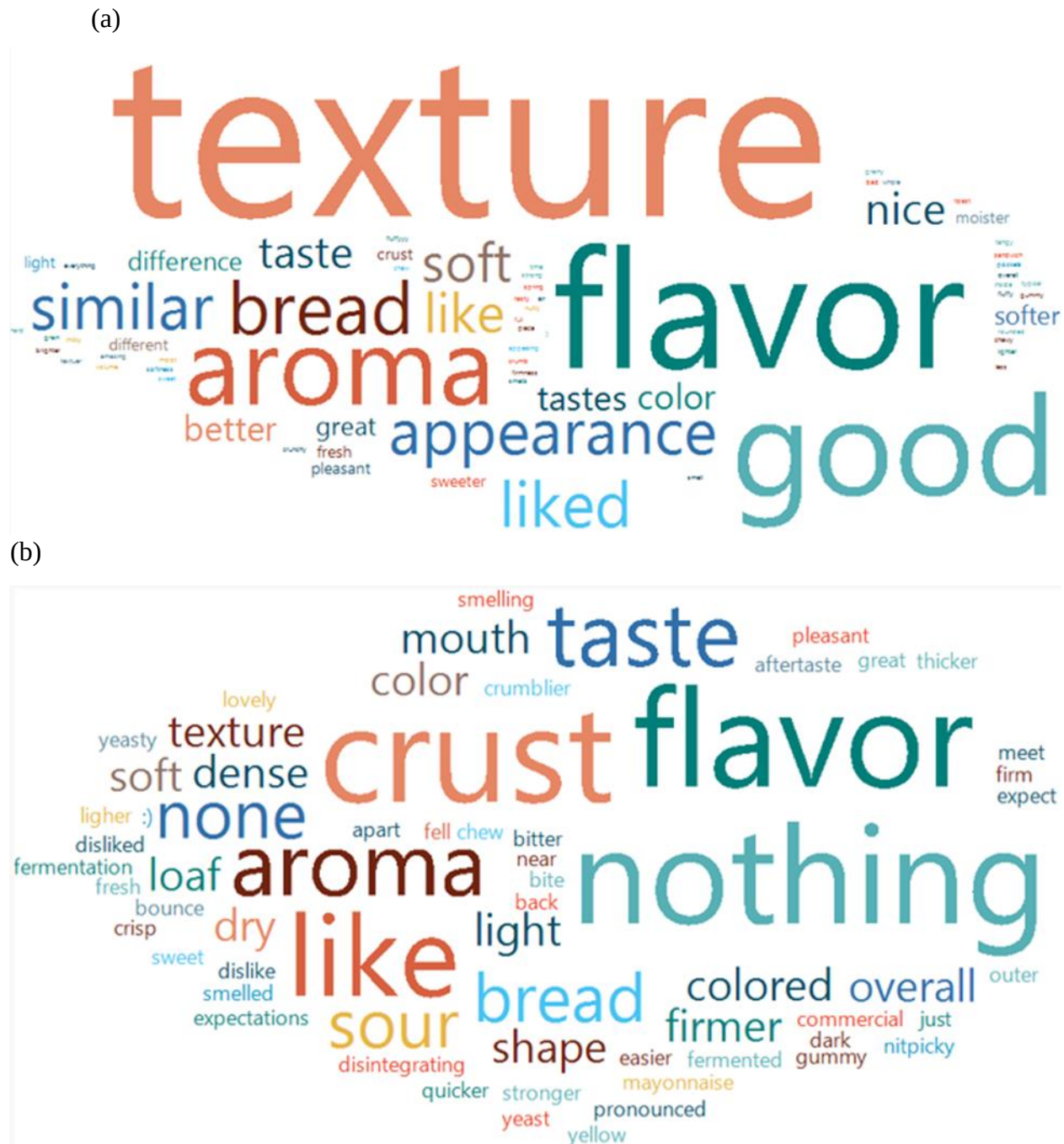


Figure 4.6 (a) Word cloud of open-ended question for liking WW sample and (b) Word cloud of open-ended question for disliking WW sample. The larger, bolder words signify a higher association of characteristics among various panelists.

Table 4.1 shows the analytical results for the pH and TTA of the sourdough before baking as well as the analytical tests of the final products. The pH and TTA of the final breads

were statistically significantly different, which would have an impact on the flavor and aroma attributes. Doughs with a pH of 4 exhibit heightened proteolysis, leading to the degradation of the gluten structure and diminishing the firmness and elasticity of the sourdough (Arendt et al., 2007). This would cause a softer, more extensible dough with increased gas retention. This is typically correlated with an improvement of bread volume and thus reduced retrogradation and agrees with Arendt et al. (2007). The acidified dough would influence firmness, which would impact the texture attribute however no statistical difference was found between WW and K. Crust color BCU, L*, and b* were found to be significant, which to a lesser degree could impact crumb color appearance. The brightness is significantly different and will impact the appearance attribute, which is reflected in the DOD of appearance and can be seen in figure 7.

Table 4.1 Analytical results on the sourdough and final product.

	WW	K	P-value
Sourdough pH $\pm$ SD	4.127 $\pm$ 0.07	4.272 $\pm$ 0.18	0.18
Sourdough TTA $\pm$ SD	20.689 $\pm$ 0.29	27.603 $\pm$ 0.13	<.0001
Bread pH $\pm$ SD	5.311 $\pm$ 0.08	5.106 $\pm$ 0.05	0.005
Bread TTA $\pm$ SD	4.391 $\pm$ 0.27	5.326 $\pm$ 0.13	0.0008
Specific Volume $\pm$ SD	5.05 $\pm$ 0.51	4.68 $\pm$ 0.60	0.17
BCU $\pm$ SD	2.19 $\pm$ 0.16	2.01 $\pm$ 0.08	0.006
L* $\pm$ SD	40.26 $\pm$ 2.04	37.96 $\pm$ 0.94	0.005
a* $\pm$ SD	16.87 $\pm$ 0.67	16.88 $\pm$ 0.36	0.96
b* $\pm$ SD	24.7 $\pm$ 1.46	23.23 $\pm$ 0.73	0.01
Firmness $\pm$ SD	154.29 $\pm$ 46.59	153.54 $\pm$ 17.47	0.96
Brightness $\pm$ SD	100.7 $\pm$ 2.62	110.5 $\pm$ 5.12	0.002
Number of Cells $\pm$ SD	6279 $\pm$ 245.86	6367 $\pm$ 332.30	0.61
Area of Cells $\pm$ SD	53.85 $\pm$ 0.82	54.27 $\pm$ 0.47	0.31
Cell Diameter $\pm$ SD	2.14 $\pm$ 0.20	2.01 $\pm$ 0.19	0.27

Bolded p-values indicate significant difference (p<0.05).

Figure 4.7 shows the images for brightness, and the K samples are slightly darker. The brightness significance is reflected in the appearance attributes DOD results. The results from the

colorimeter calculations show the crust of K is darker, slightly redder, and bluer compared to WW crust (table 4.2). There is a total difference between K and WW, signifying consumers would be able to perceive a difference in crust color and appearance. The sourdoughs before use in bread were evaluated for color in Chapter 3 Section 3.3.2.2. The data shows a significant difference between the K and WW samples. As the type of sourdough was the only difference in formulation, the impact would be expected to carry into the final product, but to a lower degree. The ΔL^* , Δa^* , and ΔE^*_{ab} values align with the final sourdough color difference but to a smaller degree. The only difference is the Δb^* value, which has more yellow hues in the sourdough, but bluer hues in the final product crust.

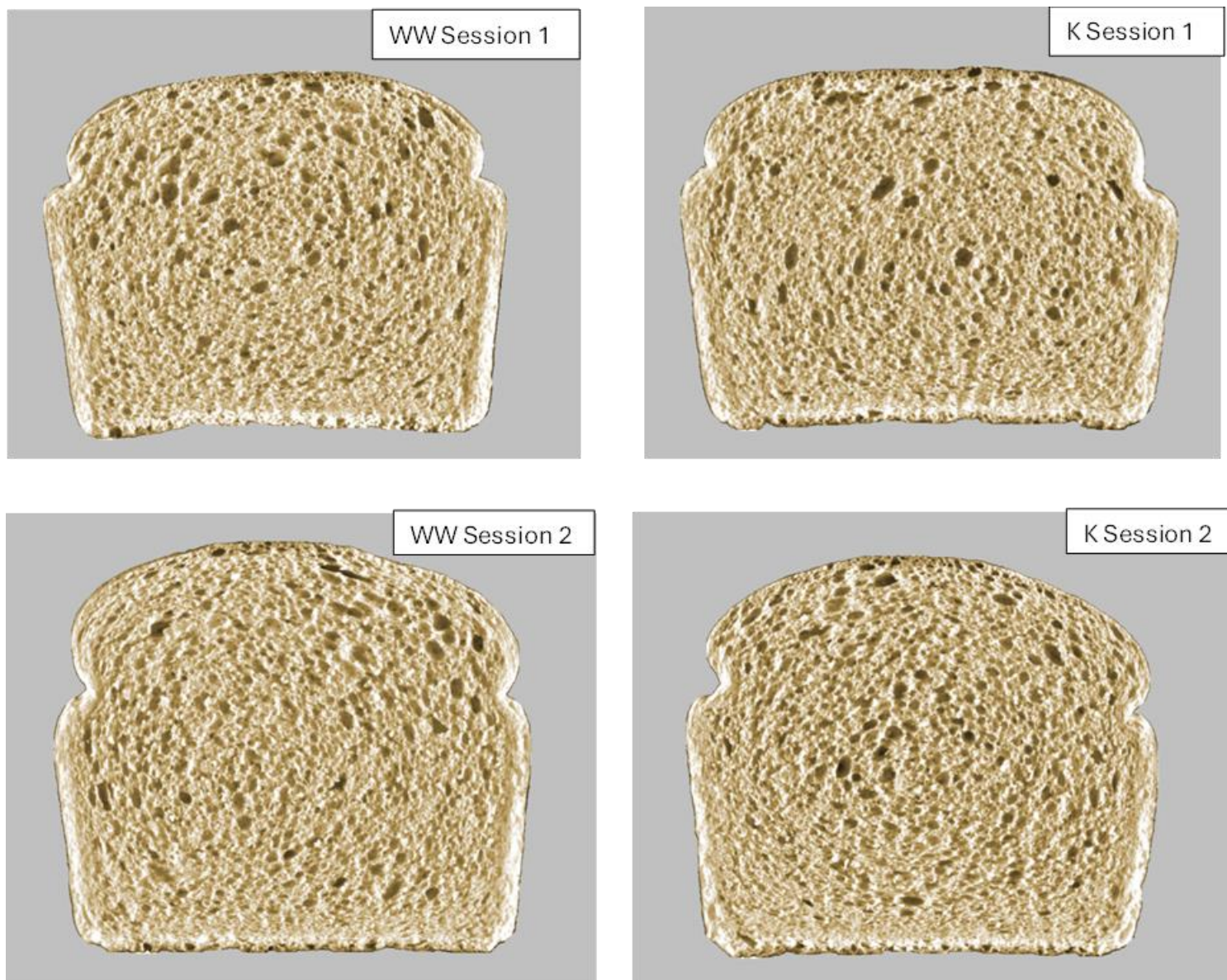


Figure 4.7 C-Cell images for brightness of the final breads from each session.

Table 4.2 ΔL^* , Δa^* , Δb^* , and ΔE^*_{ab} averages of crust color K vs. WW.

ΔL^*	-2.11
Δa^*	0.05
Δb^*	-1.31
ΔE^*_{ab}	2.90

Acetic acid, lactic acid, and citric acid have different flavors and aroma profiles. Fruity, sweet, and alcoholic aromas are impacted by the primary volatile compound groups of acids, alcohols, and aldehydes (De Luca et al., 2021). The varying degrees of organic acids are shown in figure 4.8 and are reflected in the flavor and aroma profiles. Citric acid is similar between K and WW. For malic acid, K is almost double the amount of WW. K is also higher than WW for formic acid. Lactic acid, acetic acid, and ethanol will have the biggest influence on flavor and aroma. Lactic acid is significantly higher for K than WW but is milder in aroma and flavor compared to acetic acid. Sharp vinegar and sour aromas are found to be responsible by acetic acid (Cera et al., 2024). Acetic acid is typically the biggest contributor to flavor and aroma in a sourdough and is desired in sourdough. The common belief is the higher quantity of acetic acid, the more enhanced flavor and aroma a sourdough will have (Arora et al., 2021; Lim et al., 2017). The enhancement may or may not translate into greater consumer acceptance. K is seen to be higher than WW in acetic acid and would impact the flavor and aroma attributes. K had a higher average than WW for aroma in the JAR results but were closer for the flavor attribute. Strong alcoholic and medicinal aromas are associated with ethanol content (Cera et al., 2024), which may not meet expectations for the majority of consumers. Ethanol is higher for WW than K and aroma appears relatively equally in disliking and liking term usage for WW (figure 6a and b).

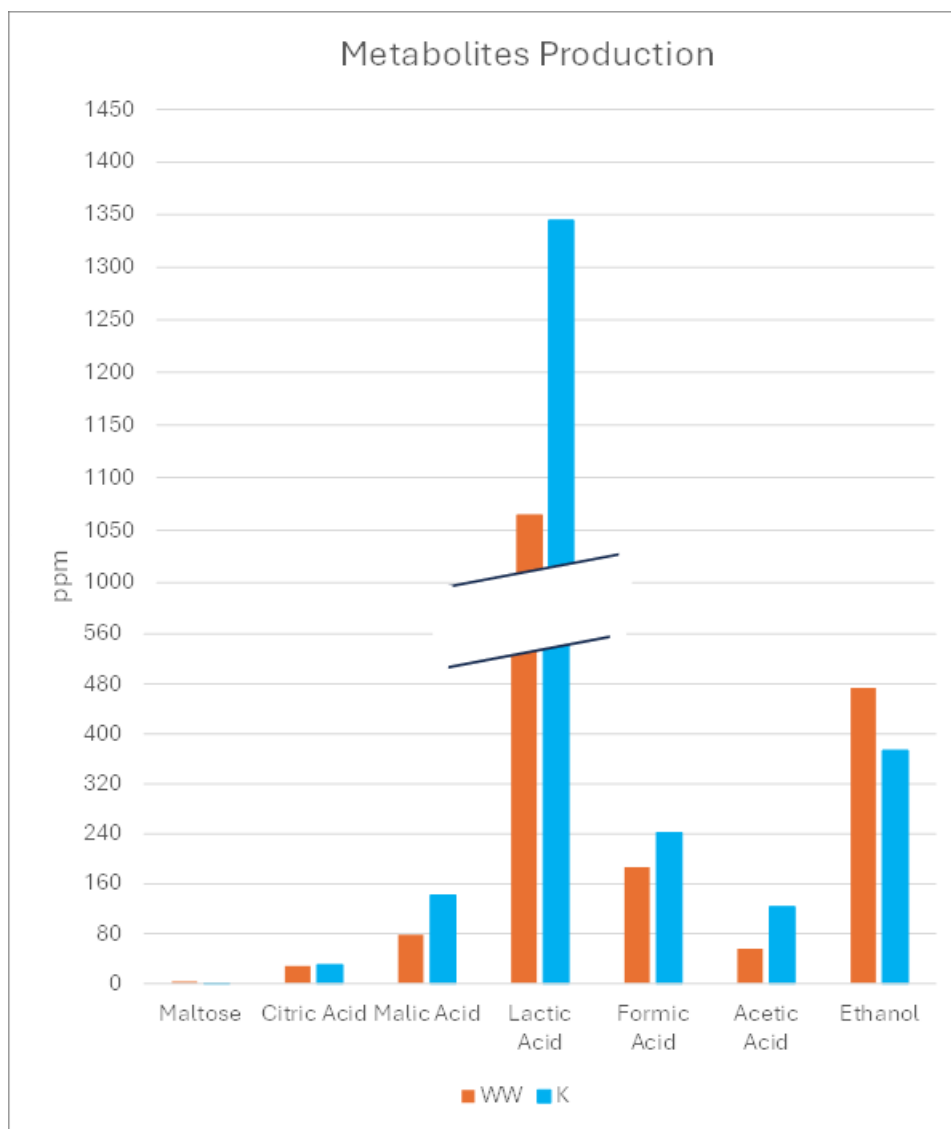


Figure 4.8 Metabolites production result of WW and K sourdough from final day of establishment.

4.4 Conclusion

There are differences between wheat bread made with whole wheat sourdough compared to whole grain IWG sourdough. In this study, the only difference in ingredients was the type of sourdough used at a 20% addition. The difference between the samples is slight and could be adjusted with formula and process optimization. However, despite the differences the K sample

still meet and even exceeded consumers expectations. There is potential for IWG to be used in a sourdough process and incorporated as an ingredient of wheat-based bread.

4.5 References

- AACC International. Approved Methods of Analysis, 11th Ed. Method 10-16.01. Volumetric and Dimensional Profile of Baked Products by Laser Topography-Volscan Profiler Method. Approved December 2015. AACC International, St. Paul, MN, U.S.A. [https://doi: 10.1094/AACCIntMethod-10-16.01](https://doi.org/10.1094/AACCIntMethod-10-16.01)
- AACC International. Approved Methods of Analysis, 11th Ed. Method 10-18.01. Measurement of Crumb Structure of Baked Products by C-Cell. Approved November 2017. AACC International, St. Paul, MN, U.S.A. [https://doi: 10.1094/AACCIntMethod-10-18.01](https://doi.org/10.1094/AACCIntMethod-10-18.01)
- AACC International. Approved Methods of Analysis, 11th Ed. Method 74-09.01. Measurement of Bread Firmness by Universal Testing Machine. Reapproved November 3, 1999. AACC International, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-74-09.01>
- Arendt, E. K., Ryan, L. A. M., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*, 24(2), 165–174. <https://doi.org/10.1016/j.fm.2006.07.011>
- Arora, K., Ameer, H., Polo, A., Di Cagno, R., Rizzello, C. G., & Gobbetti, M. (2021). Thirty years of knowledge on sourdough fermentation: A systematic review. *Trends in Food Science & Technology*, 108, 71–83. <https://doi.org/10.1016/j.tifs.2020.12.008>
- Aust, L. B., Jr., M. C. G., Beard, S. A., & Ii, R. W. W. (1985). Degree of difference test method in sensory evaluation of heterogeneous product types. *Journal of Food Science*, 50(2), 511–513. <https://doi.org/10.1111/j.1365-2621.1985.tb13439.x>
- Cera, S., Tuccillo, F., Knaapila, A., Sim, F., Manngård, J., Niklander, K., Verni, M., Rizzello, C. G., Katina, K., & Coda, R. (2024). Role of tailored sourdough fermentation in the flavor of wholegrain-oat bread. *Current Research in Food Science*, 8, 100697. <https://doi.org/10.1016/j.crfs.2024.100697>
- Delarue, J. (2022). 2—Overview of sensory methods for the evaluation of nonfood products. In A.-M. Pensé-Lhéritier, I. Bacle, & J. Delarue (Eds.), *Nonfood Sensory Practices* (pp. 17–37). Woodhead Publishing. <https://doi.org/10.1016/B978-0-12-821939-3.00020-8>
- De Luca, L., Aiello, A., Pizzolongo, F., Blaiotta, G., Aponte, M., & Romano, R. (2021). Volatile organic compounds in breads prepared with different sourdoughs. *Applied Sciences*, 11(3), 1330. <https://doi.org/10.3390/app11031330>
- Jeong, S., & Lee, J. (2021). Effects of cultural background on consumer perception and acceptability of foods and drinks: A review of latest cross-cultural studies. *Current Opinion in Food Science*, 42, 248–256. <https://doi.org/10.1016/j.cofs.2021.07.004>
- Lim, S. B., Tingirikari, J. M. R., Seo, J. S., Li, L., Shim, S., Seo, J.-H., & Han, N. S. (2017). Isolation of lactic acid bacteria starters from Jeung-pyun for sourdough

- fermentation. *Food Science and Biotechnology*, 27(1), 73–78.
<https://doi.org/10.1007/s10068-017-0274-0>
- Lotong, V., Iv, E. C., & Chambers, D. H. (2000). Determination of the sensory attributes of wheat sourdough bread ¹. *Journal of Sensory Studies*, 15(3), 309–326.
<https://doi.org/10.1111/j.1745-459X.2000.tb00273.x>
- Marcus, A., & Fox, G. (2022). Malting and Wort Production Potential of the Novel Grain Kernza (*Thinopyrum intermedium*). *Journal of the American Society of Brewing Chemists*, 0(0), 1–11. <https://doi.org/10.1080/03610470.2022.2026662>
- Ruiz-Capillas, C., & Herrero, A. M. (2021). Sensory analysis and consumer research in new product development. *Foods*, 10(3), 582. <https://doi.org/10.3390/foods10030582>
- Tran, T., James, M. N., Chambers, D., Koppel, K., & Chambers, E. (2019). Lexicon development for the sensory description of rye bread. *Journal of Sensory Studies*, 34(1), e12474. <https://doi.org/10.1111/joss.12474>

Chapter 5 - Overall Conclusions and Future Work

This study highlighted the environmental, nutritional, and technological benefits of IWG, and the potential for this perennial plant to be utilized as a food source. IWG has a lack in HMW-GS and throughout various research shown a poor performance in bread products alone. However, breeding efforts spearheaded by The Land Institute, have targeted the mixing quality. This was evaluated and found the breeding efforts to be successful. The cultivar that was specifically bred for mixing (MQ) was found to have an increased mix time, higher peak integral, and better stability. This translated into the final bread quality. The specific volume increased, along with a higher number of cells, which are both desirable traits in bread.

Besides breeding efforts, potential end uses are still being explored. IWG has a higher ash, protein, and starch content as compared to wheat, indicating suitability as a substrate in a sourdough process. Spontaneously fermented whole grain IWG (K6/K7) was found to acidify to higher TTA when compared to whole wheat (WW), despite the faster acidification (lowest $V_{\max}$) for WW. Due to the higher fiber content and an increased water holding capacity, IWG sourdough was firmer and was also significantly darker in color. The LAB evolution trail revealed IWG to reach a higher LAB enumeration faster, possibly due to the higher magnesium, manganese, and calcium contents. IWG was found to be significantly higher in lactic acid production, but WW typically had slightly higher acetic acid production. Through a baking trial with 20% flour basis added sourdough in a wheat-based bread, IWG bread was found to be more acidic, however the shelf-life (without mold inhibitors or preservatives) was found to be an average of 6 days for both IWG and WW breads. There were no negative effects in firmness 1-day or 5-days post-bake as well to the specific volume. Thus, results support sourdough to be a potential end use for IWG.

Taking that a step farther, the IWG sourdough or WW sourdough was added to wheat-based bread and tested in a sensory evaluation. Degree of difference (DOD) between overall characteristics and specific attributes were evaluated. A Just About Right (JAR) scale was used to measure if consumers' expectations were met (overall and within the same attributes of the DOD scale). An action criteria of 2 was chosen for the DOD, signifying low risk of consumers noticing a difference. IWG had a DOD average of 2.4. However, panelists also found differences in a WW blind control (average of 1.8). This is an indication that panelists were looking for a difference, thus actual difference can be assumed to be smaller. For the JAR scale a score of 3 ("meets expectations") or higher is desired, and the averages for both IWG and WW were higher than 3. Appearance and aroma had the biggest differences in DOD averages. While consumers noticed a slight difference between IWG (K) and WW, IWG still met expectations. With formula optimization the samples could align more, allowing for the potential use of IWG in a sourdough process and wheat-based bread.

Future work includes further characterization of the sourdoughs from chapter 3 through metagenomics. This would allow additional understanding of the microflora present in IWG and the effects seen. A Type 1 sourdough was explored, however there may be benefit in exploring IWG in a Type II sourdough system as well and comparing performance.

Appendix A - Chapter 2: Multivariate Analysis Tables

A.1 Mixograph SAS results with blocking.

Variable	Source	Degrees of Freedom (DF)	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
MPT	ID	3	42.155169	14.051723	Var(Residual) + Q(ID)	MS(Residual)	9	61.14	<.0001
	BLK	3	0.591719	0.197240	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	0.86	0.4970
	Residual	9	2.068456	0.229828	Var(Residual)	--	--	--	--
MPV	ID	3	30.058119	10.019373	Var(Residual) + Q(ID)	MS(Residual)	9	10.02	0.0032
	BLK	3	0.961869	0.320623	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	0.32	0.8105
	Residual	9	9.003006	1.000334	Var(Residual)	--	--	--	--
MPI	ID	3	29006	9668.823317	Var(Residual) + Q(ID)	MS(Residual)	9	92.05	<.0001
	BLK	3	337.258250	112.419417	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	1.07	0.4092
	Residual	9	945.324100	105.036011	Var(Residual)	--	--	--	--
MRS	ID	3	9.309650	3.103217	Var(Residual) + Q(ID)	MS(Residual)	9	1.82	0.2143
	BLK	3	3.259650	1.086550	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	0.64	0.6105
	Residual	9	15.378000	1.708667	Var(Residual)	--	--	--	--

A.2 Baking Trial SAS Results with blocking.

Variable	Source	Degrees of Freedom (DF)	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
PH	ID	3	205.686969	68.562323	Var(Residual) + Q(ID)	MS(Residual)	9	22.30	0.0002
	BLK	3	12.754169	4.251390	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	1.38	0.3096
	Residual	9	27.668706	3.074301	Var(Residual)	--	--	--	--
SV	ID	3	0.340350	0.113450	Var(Residual) + Q(ID)	MS(Residual)	9	45.58	<.0001
	BLK	3	0.012950	0.004317	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	1.73	0.2293
	Residual	9	0.022400	0.002489	Var(Residual)	--	--	--	--
B	ID	3	79.182500	26.394167	Var(Residual) + Q(ID)	MS(Residual)	9	21.50	0.0002
	BLK	3	11.301950	3.767317	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	3.07	0.0837
	Residual	9	11.048350	1.227594	Var(Residual)	--	--	--	--
CNUM	ID	3	1026052	342017	Var(Residual) + Q(ID)	MS(Residual)	9	40.76	<.0001
	BLK	3	7440.817250	2480.272417	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	0.30	0.8278
	Residual	9	75522	8391.314883	Var(Residual)	--	--	--	--
CD	ID	3	0.102769	0.034256	Var(Residual) + Q(ID)	MS(Residual)	9	3.26	0.0733
	BLK	3	0.015469	0.005156	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	0.49	0.6970
	Residual	9	0.094456	0.010495	Var(Residual)	--	--	--	--
WT	ID	3	0.000350	0.000117	Var(Residual) + Q(ID)	MS(Residual)	9	2.33	0.1423
	BLK	3	1.479114E-31	4.930381E-32	Var(Residual) + 4 Var(BLK)	MS(Residual)	9	0.00	1.0000
	Residual	9	0.000450	0.000050000	Var(Residual)	--	--	--	--

Appendix B - Chapter 4: Supplemental Information

B.1 Sensory Ballot

Ballot

Name: _____

You are about to participate in a difference from control test. This is an individual exercise about **your** opinion, so please only look at your own samples and ballot. You will be evaluating breads containing whole grain flours. These products do contain wheat, so if you are allergic, or have sensitivities then you should not participate. You will be tasting 2 samples today in pairs with a reference sample. There are no incorrect answers, as we are simply interested in your opinion. This will take about 15-20 minutes total.

- Please taste the sample marked reference.
- Now taste the other sample and write the sample code # _____
- Take a sip of water to rinse your mouth.

Answer the following questions based on the two samples just tasted.

1. How similar or different overall is the sample compared to the reference sample?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different				Extremely Different			

2. How similar or different is the sample compared to the reference in terms of appearance?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different				Extremely Different			

3. How similar or different is the sample compared to the reference in terms of aroma?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different				Extremely Different			

4. How similar or different is the sample compared to the reference in terms of flavor?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different				Extremely Different			

5. How similar or different is the sample compared to the reference in terms of texture?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different				Extremely Different			

6. How well does the sample meet your overall expectations?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

7. How well does the sample **meet your expectation** in terms of appearance?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

8. How well does the sample **meet your expectation** in terms of aroma?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

9. How well does the sample **meet your expectation** in terms of flavor?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

10. How well does the sample meet your expectation in terms of texture?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

11. Briefly describe anything you liked about the sample.

12. Briefly describe anything you disliked about the sample.

**You have now completed your first sample evaluation.
Please raise your hand to be served your second sample.**

You will now taste another sample and compare it to the reference sample.

- Please re-taste the sample marked reference.
- Then taste the other sample and write the sample code # _____

Answer the following questions based on the two samples just tasted.

13. How similar or different overall is the sample compared to the reference sample?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different						Extremely Different	

14. How similar or different is the sample compared to the reference in terms of appearance?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different						Extremely Different	

15. How similar or different is the sample compared to the reference in terms of aroma?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different						Extremely Different	

16. How similar or different is the sample compared to the reference in terms of flavor?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different						Extremely Different	

17. How similar or different is the sample compared to the reference in terms of texture?

☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7
Not at all Different						Extremely Different	

18. How well does the sample meet your overall expectations?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

19. How well does the sample **meet your expectation** in terms of appearance?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

20. How well does the sample **meet your expectation** in terms of aroma?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

21. How well does the sample **meet your expectation** in terms of flavor?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

22. How well does the sample meet your expectation in terms of texture?

- ☐ 1- does not meet expectations
- ☐ 2- slightly meets expectations
- ☐ 3- meets expectations
- ☐ 4- slightly exceeds expectations
- ☐ 5- significantly exceeds expectations

23. Briefly describe anything you liked about the sample.

24. Briefly describe anything you disliked about the sample.

You have now completed your final sample evaluation. Ensure your name and the code numbers are written down on your paper. Then raise your hand when you have finished.

****Please do not speak about the sensory in any aspect to anyone afterwards, so as not to create any bias for anyone that may participate in the future session. Thank you for understanding!****

B.2 IRB Approval Letter



TO: Elisa Karkle
Grain Science and Industry

Proposal Number: IRB-12013

FROM: Lisa Rubin, Chair
Committee on Research Involving Human Subjects

DATE: 01/22/2024

RE: Proposal Entitled, "Functionality of Kernza (Intermediate wheatgrass) in sourdough fermentation and bread quality."

The Committee on Research Involving Human Subjects / Institutional Review Board (IRB) for Kansas State University has reviewed the proposal identified above and has determined that it is EXEMPT from further IRB review. This exemption applies only to the proposal - as written - and currently on file with the IRB. Any change potentially affecting human subjects must be approved by the IRB prior to implementation and may disqualify the proposal from exemption.

Based upon information provided to the IRB, this activity is exempt under the criteria set forth in the Federal Policy for the Protection of Human Subjects, **45 CFR §104(d), category:Exempt Category 6 Subsection i.**

Certain research is exempt from the requirements of HHS/OHRP regulations. A determination that research is exempt does not imply that investigators have no ethical responsibilities to subjects in such research; it means only that the regulatory requirements related to IRB review, informed consent, and assurance of compliance do not apply to the research.

Any unanticipated problems involving risk to subjects or to others must be reported immediately to the Chair of the Committee on Research Involving Human Subjects, the University Research Compliance Office, and if the subjects are KSU students, to the Director of the Student Health Center.

Electronically signed by Lisa Rubin on 01/22/2024 5:37 PM ET

B.3 Text Explorer Complete Table

Text Explorer for Like Sample=K						Text Explorer for Dislike Sample=K					
Number of Terms	Number of Cases	Total Tokens	Tokens per Case	Number of Non-Empty Cases	Portion of Non-Empty Cases	Number of Terms	Number of Cases	Total Tokens	Tokens per Case	Number of Non-Empty Cases	Portion of Non-Empty Cases
89	47	503	10.7021	47	1.0000	78	47	355	7.55319	45	0.9574
Term and Phrase Lists						Term and Phrase Lists					
Term	Count		Phrase	Count	N	Term	Count		Phrase	Count	N
texture	19		flavor and aroma	3	3	reference	12		crumb structure was slightly	2	4
flavor	17		liked the taste	3	3	slightly	8		slightly tighter than reference	2	4
good	14		liked the taste better	2	4	color	6		structure was slightly tighter	2	4
liked	13		aroma is good	2	3	texture	5		compared to reference	2	3
taste	13		aroma was slightly	2	3	bitter	4		structure was slightly	2	3
sample	11		like the flavor	2	3	crumb	4		tighter than reference	2	3
aroma	9		liked the flavor	2	3	disliked	4		crumb structure	2	2
reference	7		liked the texture	2	3	dry	4		slightly dry	2	2
nice	6		similar to control	2	3	flavor	4		slightly tighter	2	2
soft	6		taste is good	2	3	like	4				
bread	5		texture was nice	2	3	little	4				
like	5		taste better	2	2	n	4				
slightly	5					nothing	4				
better	3					sample	4				
color	3					taste	4				
control	3					appearance	3				
similar	3					aroma	3				
sweet	3					compared	3				
tasted	3					dull	3				
appearance	2					none	3				
crust	2					quite	3				
dense	2					aftertaste	2				
different	2					bread	2				
dry	2					chewy	2				
great	2					crust	2				
inside	2					good	2				
smell	2					much	2				
smelled	2					slight	2				
softer	2					smell	2				
sour	2					soft	2				
strong	2					structure	2				
;))	1					tighter	2				
acceptable	1					acidic	1				
airy	1					big	1				
almost	1					bit	1				
also	1					brown	1				
appealing	1					compare	1				
balance	1					comparatively	1				
beyond	1					cooked	1				
bit	1					crisp	1				
bitter	1					crumbiness	1				
bland	1					dark	1				
came	1					denser	1				
chemical	1					different	1				
chew	1					dingy	1				
chewy	1					dryness	1				
crumb	1					favor	1				
darker	1					firmer	1				
denser	1					grains	1				
despite	1					grainy	1				
difference	1					gray	1				
dn	1					grayish	1				
easy	1					light	1				
expectations	1					lighter	1				
felt	1					looked	1				
fermented	1					mouth	1				
firm	1					normal	1				
flavors	1					noticeable	1				
fresh	1					nthing	1				
full	1					prefer	1				
grainy	1					r	1				
gummy	1					remained	1				
heartier	1					salty	1				
identical	1					seemed	1				
lasted	1					slice	1				
light	1					smaller	1				
looked	1					spring	1				
looking	1					still	1				
looks	1					stood	1				
loved	1					strong	1				
make	1					stronger	1				
meets	1					swirl	1				
moist	1					think	1				
natural	1					thought	1				
observed	1					took	1				
overall	1					uneven	1				
preferred	1					weaker	1				
product	1					whole	1				
really	1										
resilient	1										
samples	1										
sample's	1										
sandwich	1										
seemed	1										
since	1										
slice	1										
sweeter	1										
though	1										
toast	1										

Text Explorer for Like Sample=WW

Number of Terms	Number of Cases	Total Tokens	Tokens per Case	Number of Non-Empty Cases	Portion of Non-Empty Cases
104	48	535	11.1458	48	1.0000

Term and Phrase Lists

Term	Count	Phrase	Count	N
texture	25	good texture	5	2
flavor	18	texture and flavor	3	3
good	15	good texture and flavor	2	4
sample	15	texture of the sample	2	4
aroma	12	like the texture	2	3
bread	8	texture was good	2	3
appearance	7	also liked	2	2
liked	7	moister texture	2	2
reference	7			
similar	7			
like	6			
soft	6			
nice	5			
taste	5			
also	4			
better	4			
color	4			
really	4			
tastes	4			
difference	3			
great	3			
softer	3			
603	2			
crust	2			
different	2			
fresh	2			
light	2			
looks	2			
moister	2			
pleasant	2			
slightly	2			
sweeter	2			
j)	1			
914	1			
air	1			
almost	1			
along	1			
amazing	1			
appealing	1			
bad	1			
bought	1			
brighter	1			
chew	1			
chewy	1			
compared	1			
control	1			
crumb	1			
crunchy	1			
everything	1			
except	1			
felt	1			
find	1			
firmness	1			
fluffy	1			
fluffy	1			
full	1			
grain	1			
grainy	1			
gummy	1			
hard	1			
held	1			
inside	1			
less	1			
lighter	1			
look	1			
looked	1			
makes	1			
milky	1			
moist	1			
much	1			
nutty	1			
overall	1			
part	1			
piece	1			
pockets	1			
r	1			
reference	1			
rounded	1			
sandwich	1			
seemed	1			
similar	1			
slight	1			
smell	1			
smells	1			
softness	1			
specifically	1			
spring	1			
store	1			
strong	1			
sweet	1			
tangy	1			
tasted	1			
tasty	1			
tell	1			
textuer	1			
time	1			
toast	1			
together	1			
typical	1			
volume	1			
way	1			
well	1			
whole	1			
without	1			

Text Explorer for Dislike Sample=WW

Number of Terms	Number of Cases	Total Tokens	Tokens per Case	Number of Non-Empty Cases	Portion of Non-Empty Cases
88	48	358	7.45833	44	0.9167

Term and Phrase Lists

Term	Count	Phrase	Count	N
slightly	12	compared to the reference	2	4
reference	9	flavor of the sample	2	4
sample	9	sample was slightly	2	3
n	7	slightly firmer	2	2
crust	6	slightly sour	2	2
flavor	6			
nothing	6			
like	5			
aroma	4			
taste	4			
bread	3			
compared	3			
none	3			
sour	3			
603	2			
914	2			
anything	2			
bit	2			
color	2			
colored	2			
dense	2			
dry	2			
firmer	2			
light	2			
little	2			
loaf	2			
mouth	2			
overall	2			
r	2			
shape	2			
soft	2			
texture	2			
j)	1			
aftertaste	1			
almost	1			
also	1			
apart	1			
back	1			
bite	1			
bitter	1			
bounce	1			
chew	1			
commercial	1			
crisp	1			
crumblier	1			
dark	1			
disintegrating	1			
dislike	1			
disliked	1			
easier	1			
end	1			
especially	1			
expect	1			
expectations	1			
fell	1			
fermentation	1			
fermented	1			
firm	1			
fresh	1			
great	1			
gummy	1			
just	1			
lighter	1			
lovely	1			
mayonnaise	1			
meet	1			
much	1			
near	1			
nitpicky	1			
outer	1			
pleasant	1			
pronounced	1			
quicker	1			
quite	1			
reference	1			
reference	1			
slightly	1			
slight	1			
smelled	1			
smelling	1			
stronger	1			
sweet	1			
thicker	1			
took	1			
verge	1			
yeast	1			
yeasty	1			
yellow	1			