A study on liquid sourdough fermentation as affected by strain and mill fraction composition

by

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Abstract

In the past few years, sourdough has seen a surge in popularity in the United States baking culture. Sourdough utilizes lactic acid producing bacteria, acetic acid producing bacteria, and yeasts to provide pH change, flavor development, and CO_2 production. The objective of this study was to determine the impact different LAB strains have on the fermentation characteristics of experimental milled wheat fractions. Hard red winter wheat was milled to create nine fractions with differing composition. To assess the compositional characteristics of the various fractions, testing was done on moisture, ash, mineral content, starch content, and damaged starch for each fraction. The fractions were then fermented using three purified strains of lactic acid producing bacteria, or LAB. The strains, distinguished as La1, La2, and La3, utilize different metabolic pathways during fermentation, which is hypothesized to result in unique fermentation characteristics. V_{max}, $T_{max,}$ time to pH 4.0, minimum pH, and maximum TTA were collected by monitoring the pH and total titratable acidity during the 48-hour fermentation. The metabolites accumulated in the fermented sourdough were also quantified. The data was analyzed to determine how the various strain and fraction combinations performed, as well as how the fermentation characteristics are correlated to the fraction composition. It was determined that each of the nine fractions had unique compositions, morphology, and functional characterizations. In general, the La2 strain, which is homofermentative, was able to achieve a pH of 4.0 earlier than the other strains. La2 also had the lowest minimum pH and the highest maximum TTA. Specifically, La2 performed best when fermenting those fractions with higher amounts of endosperm. La1 and La3 are both heterofermentative bacteria and performed best when fermenting fractions with higher mineral content. Overall, the shorts fraction resulted in faster acidification and higher final acid production for all strains. It was found that the selection of both substrate and strain has a clear impact on the fermentation characteristics and sourdough characteristics when the process is held constant. These differences can help industry determine what strain or wheat fraction can be utilized to achieve a desired fermentation and sourdough characteristics outcome.

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Dedication

Dedicated to my family who always supported the pursuit of higher education and pushed me to think logically and be curious. Those family members were wildcats before me and provided me with the opportunity to explore my interests and find a home in Manhattan.

Special thanks to the Bakery Science Department in April 2012 during open house. That was my first time in the bake lab, I rolled and shaped pretzels and learned about the use of yeast to ferment bread. This simple interaction sparked an interest that stuck with me through my teenage years, eventually leading to my attendance at KSU earning a major in Bakery Science and Management.

Chapter 1 - Sourdough Acidification

1.1. Introduction

Bread has been a staple in human diets for thousands of years. Nowadays, bread can consist of over ten different ingredients, each added to provide the product with a specific quality. Early bread baking was far more simplistic, consisting of just two ingredients: a ground grain mixed with water. The flavor and leavening of early breads were achieved through the fermentation of yeasts and bacteria inherent in the flour. They metabolize the available sugars producing alcohol, organic acids, and CO_2. These byproducts give bread its most important and recognizable features we still strive to obtain today.

Archeological evidence suggests sourdough bread baking has been present since over 5,000 years ago (Catzeddu et al., 2019). In the late 1800's due to the industrial revolution, yeast leavened breads became common and dominated the bread baking market. As the world became more commercialized, bread became more uniform. It wasn't viable for bakers to continue to use naturally fermented doughs in baking since the time and effort required to make a dough decreased with now readily available yeast (Gobbetti and Gänzle, 2013). Due to this change, naturally leavened breads, or sourdough, became scarcer, until it was more of an artisanal product left to skilled bakers. The art of sourdough has regained popularity in recent years. The 2019 pandemic helped popularize "home sourdough" techniques that spread over social media platforms. A growing number of studies examining the sensory and digestive benefits of sourdough in the baking industry have accompanied this spike in consumer interest.

This review utilized two databases to gather articles related to sourdough acidification and its properties. The search focused on papers produced over the last 30 years, with an emphasis on more recent publications. Keywords included sourdough, sourdough acidification, lactic acid fermentation, lactic acid in bread, sourdough baking, sourdough AND organic acids, acidification of dough, acidification of [flour type] (e.g., wheat, rye, barley), sourdough bread sensory characteristics, sourdough rheology, sourdough flavors AND aromas.

1.2. Sourdough Background

The term sourdough is often used without a complete understanding of what it truly means. To some it may just be a flavor of bread that can be found on grocery store shelves. To experienced bakers, it is a natural leavening technique utilizing natural and lactic acid producing

bacteria to create a baked product. The production from those lactic acid bacteria will cause a decrease in the pH of the dough while simultaneously expelling CO_2, giving the dough rise as well as producing ethanol; this provides the bread a fermented flavor (Calvert et al., 2021).

Sourdough is a technique and a technology – a system and process that produces unique and delicious products. From an ingredient perspective, sourdough is a mixture of grain or grain fractions, and water. The system utilizes inherent lactic acid producing bacteria, acetic acid producing bacteria, and yeasts to provide the pH change, flavor development, and CO_2 production associated with sourdough. Purely based on a microbiological perspective, sourdough is a specific ecosystem containing various carbohydrate concentrations and can be characterized by being within an acidic environment, having limited oxygen, and high counts of lactic acid producing bacteria (De Vuyst and Neysens, 2005).

To better understand how sourdough can be used at different levels of baking, sourdough was broken down into three different "types" (Calvert et al., 2021). Type I is a traditional sourdough method. This type is used for leavening and flavor. It requires frequent backslopping and care to ensure consistent final products (Decock and Capelle, 2005). This is common in smaller bakeries and is considered the most artisan of the sourdough techniques. Some Type I sourdough starters have been around for hundreds of years. Each Type I sour has a unique assortment of yeast and bacteria providing them with unique flavors and characteristics (Gobbetti and Gänzle, 2013). Type I sourdough are backslopped, or "fed", by taking a small part of the sourdough and mixing with water and flour. The bacteria in the sour will begin to consume the fresh endosperm of the flour and begin another fermentation process (Hammes et al., 2005). If the flour used to feed the sourdough is changed, the flavor and characteristics of the final product will change, as different bacteria may be dominant due to the change in flour (Minervini et al., 2012).

Type II sourdough is applied by the large-scale baking industry, typically producing many products per minute. Due to the labor-intensive backslopping process of Type I, and the need to constantly monitor it's fermentation, it is not always the most effective option for an industrial bakery. Therefore, Type II is used as it is a pumpable, liquid sourdough that can be produced in larger quantities. While this type of sourdough can be processed to retain its metabolic activity while providing acidity, it is often supplemented with baker's yeast to reduce the processing time (Arora et al., 2021). Whereas Type I sourdough can contain an exuberant

number of different bacteria from different genus, Type II typically has one or two specialized bacteria to produce replicable end products that do not vary from batch to batch (Di Cagno et al., 2014). Type II commonly has a viscosity such that can be pumped into a mixer directly, eliminating a need to manually scale and add the sour into a batch (De Vuyst and Neysens, 2005).

Type III is a unique sourdough type, as it is the same as Type II, except dried using a spray or drum drying process to improve its shelf stability. Similar to Type II, it is often used in the industrial setting of sourdough production. It differs in that it does not require the storage tanks or pump system needed for Type II. As a product, it is often sold to bakers of any production scale who desire consistent flavor and more control within their sourdough products.

To achieve the flavor and aroma sourdough is so well known for, it requires the use of two major metabolic pathways. During fermentation, *Limosilactobacillus fermentum* and *Fructilactobacillus sanfranciscensis* degrade maltose into a mixture of lactic acid, acetic acid, and/or ethanol, and carbon dioxide via the phosphogluconate pathway (Harth et al., 2016). *Fructilactobacillus sanfranciscensis*' preferred energy source is maltose because of the constitutive expression of a dedicated and energetically efficient maltose phosphorylase (Stolz, et al., 1996). *Fructilactobacillus sanfranciscensis* is maltose positive, while common yeasts found in sourdough are maltose negative (De Vuyst et al., 2014). These activities lower the pH of the cereal matrix and influence the nutritional and organoleptic properties of the sourdoughs and the baked goods. The competitive strength of sourdough LAB species is based on their ability to use alternative external electron acceptors present in the sourdough matrix, such as fructose which is then reduced into mannitol. The expression of several stress responses can be monitored to better understand the impact the fermentation has on a system. It should also be noted in consideration of yeast driven fermentation, that process is carried out via glycolysis and further pyruvate breakdown by yeast into carbon dioxide and ethanol (De Vuyst and Neysens, 2005).

1.3. Microbial Ecology

Sourdough products are rich in various yeasts and bacteria that contribute to the overall characteristics and quality. The most important and recognizable of these yeasts and bacteria are the lactic acid producing bacteria, or LAB. This classification of LAB is given to those bacteria that carry out lactic acid fermentation. In a 2019 paper, Gobbetti describes lactic acid fermentation as "*a traditional, natural, sustainable, and effective tool for improving rheological*

properties, increasing nutritional value, increasing shelf-life, and improving sensory characteristics (Gobbetti et al., 2019). The lactic acid producing bacteria that make up the ecology of a sourdough starter are part of a diverse and unique genus. Recently, more organization and taxonomic distinction has been made to further separate and improve the understanding of these bacteria. In a 2020 reclassification study performed by Zheng et al., new taxonomy for strains within the genus Lactobacillus. This study included many well-known LAB found commonly in sourdough. The taxonomic description is built on work done by Beijerinck in 1901 to first categorize the genus (Zheng et al., 2020). The more these bacteria are classified and understood, the more control bakers will have over the results seen in bakery products.

LAB fermentation is impacted by factors including tolerance to acidic conditions, mechanisms related to carbohydrate metabolism, and nitrogen availability (Minervini et al., 2014). Sourdough microbiomes are extremely complex, and their stability depends on a few key determinants. These include technological parameters such the composition of the flour, the temperature of fermentation, the redox potential, the pH potential, the number of feedings, and the absorption. They also include metabolic activities such as available synthesis of energy from sources, and cofactor regeneration capability (Ercolini et al., 2013). During sourdough fermentation, supported by constant backslopping, the main metabolite produced is lactic acid. The concentration increases quickly during the backslopping. The next most produced metabolite is ethyl alcohol. Its concentration increases slower than that of lactic acid. Finally, acetic acid is produced, but not as quickly or in as large quantities as the previous metabolites mentioned, regardless of which stage it is being produced (Harth et al., 2016). Other metabolites produced during the fermentation include 3-methyl-1-butanol and ethyl acetate (Landis et al., 2021). Acetic acid provides a unique flavor some associate commonly with sourdough however, it has been found that acetic acid producing bacteria, or AAB, have a highly variable abundance over sourdough starters (Landis et al., 2021).

In a review conducted by Minervini et al. (2012), various studies were examined to determine the most prominent LAB strains found in sourdough. It was found that overall *Levilactobacillus brevis, Lactobacillus parlimentarius, Lactiplantibacillus plantarum, Furfurilactobacillus rossiae*, and *Fructilactobacillus sanfranciscensis* dominate in type I sourdoughs that are held at low temperatures and backslopped constantly. Type II sourdoughs most commonly contain more thermophilic and acid tolerant LAB species such as *Lactobacillus*

amylovorus, *Limosilactobacillus fermentum, Limosilactobacillus pontis, Limosilactobacillus reuteri*. Other genera that are less frequently isolated include *Enterococcus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus*, and *Weisella* (Minervini et al., 2012). Sourdough communities exhibit consistent patterns of strong species dominance or co-occurrence. Many communities were dominated by a single yeast and/or bacterial species with a median of three LAB/AAB and one yeast per starter (Landis et al., 2021). Bacteria commonly found in sourdough can be categorized by their oxygen dependency and the products they ferment. Homofermentative LAB degrade hexoses via glycolysis and produce lactic acid as the major end product. Heterofermentative LAB use the pentose-phosphate pathway and yield lactic acid, CO_2, acetic acid, and ethanol (Zuniga, Pardo, and Ferrer, 1993). Figure 1.1 illustrates the pathways that LAB can utilize during carbohydrate fermentation (Gänzle, 2015). The LAB that are considered obligate heterofermentative bacteria include, *Levilactobacillus brevis, Limosilactobacillus fermentum*, *Limosilactobacillus reuteri*, *Furfurilactobacillus rossiae*, and *Fructilactobacillus sanfranciscensis*. Next is the obligate homofermentative bacteria, including *Lactobacillus amylovorus* and *Lactobacillus delbrueckii*. Finally, the facultative heterofermentative bacteria, which includes *Companilactobacillus alimentarius*, *Companilactobacillus paralimentarius*, *Lactiplantibacillus plantarum* (Minervini et al., 2014).

Further examining the Minervini et al. 2014 study, the population dynamics are described to explain the typical cycle of sourdough bacteria during the acidification process. It involves three phases of evolution, beginning with the dominance of LAB belonging to *Enterococcus*, *Lactococcus* and *Leuconostoc*. The next phase involves more sourdough specific bacteria, including *Lactobacillus*, *Pediococcus*, and *Weisella*. The final stage of evolution is dominated by the well adapted sourdough bacteria belonging to the previously mentioned obligate heterofermentative species. This includes *Fructilactobacillus sanfranciscensis*, *Limosilactobacillus fermentum*, and *Lactiplantibacillus plantarum* (Minervini et al., 2014).

While the LAB are the main focus of most sourdough studies, the impact yeasts can have on the system should not be taken lightly. The ratio of yeast to bacteria can range from 1:1 to 1:1000 (Minervini et al., 2014). Yeast are single celled fungi that are capable of rapid multiplication clonally, making them perfect leavening products for bread-baking. Yeasts produce various alcohols, esters, and organic acids that impact the activity of the bacteria in a sourdough, as well as the flavor profile (Bigey et al., 2021). CO_2 production is a key in bread

baking and is important in sourdough products to provide leavening. Traditional sourdoughs require a longer time to produce enough CO_2 to properly leaven a product, while yeasts are seen as a rapid fermenter. This supports the transition from naturally leavened to yeast leavened in the past 150 years.

The most common yeast strain used in different fermentation processes around the globe is *Saccharomyces cerevisiae*. This is referred to colloquially as "baker's yeast" or "brewer's yeast." *S. cerevisiae* comes from a very diverse order of yeasts called *Saccharomycetales.* Yeast and LAB have similar metabolic pathways, resulting in similar byproducts. Figure 1.2 displays the metabolic process of yeast during sourdough fermentation (De Vuyst et al., 2017). In a 2021 study, Landis et al. were able to identify around 70 different yeast variants in 500 different sourdough starters collected from various countries. However, it is more common for a starter to have only a few strains identified, and some have no yeast all together. Other predominant sourdough yeasts besides *Saccharomyces cerevisiae* include *Candida krusei, Kazachstania exigua, Pichia kudriavzevii, Torulaspora delbrueckii*, and *Wikerhamomyces anamalus* (Liu et al. 2018, Ercolini et al. 2013, Minervini et al. 2014). In sourdough, bacteria and yeast interact through the metabolism of nitrogen and carbohydrates. They both create stimulatory and inhibitory compounds (Minervini et al., 2014). LAB do in fact produce CO_2 as a byproduct during the fermentation process, just not in the quantities that the inherent yeasts are able to. It is even common for sourdoughs, regardless of type, to be supplemented with a baker's yeast to provide enough volume to the final product. LAB and yeast are also similar, in that they both produce ethanol, giving bread a distinct "fermented" flavor. In sourdough, this is often masked by the acidic flavors produced by the LAB.

1.4. Sourdough Acidification

Acidification is a key step in the sourdough making process. It gives sourdough products their distinct flavor profile and aroma. Sourdough acidification results in a final pH typically between 3.5-4.3 (Arendt et al., 2007). The final pH is indicative of the bacteria present in the starter culture. Some bacteria are more dominant than others, creating variations in the final product. Many LAB cannot survive in acidic conditions below 4.0, thus the system will maintain a pH at or above after acidification is complete (De Vuyst et al., 2017). There are different factors that impact the time it takes for a sourdough to fully acidify. Those factors include the process used to obtain an acidified sourdough, the bacteria (both LAB and AAB that are found in

the flour), and the type of carbohydrate substrate available. Compared to other chemical and biological agents, acidification proteolysis, activation of several enzymes, and the synthesis of microbial metabolites during fermentation cause changes in a dough's matrix and positively influence a final product's sensory, nutritional, and other functional characteristics (Ercolini et al., 2013).

The starting step in the acidification process is carbohydrate metabolism by the LAB to produce the important metabolites we associate with sourdough products. The process is often limited, as the pH decreases as acidification takes place. A low pH will shift the lactic acid metabolism from hexose fermentation to the utilization of various amino acids including glutamine, glutamate, and arginine. These acids are important in pH homeostasis and the stationary phase survival of LAB (Gänzle, 2015). Amino acids are also released into the medium because of proteolysis. This is essential to the growth of various LABs and yeasts. It also helps in the participation of the Maillard reaction. With that in mind, it is important within the control of acidification, to also consider the management of proteolysis (De Vuyst et al., 2017). Other factors that impact acidification include the length and the temperature of the fermentation. Time of fermentation influences the microbe diversity and the metabolism kinetics of the sourdough. This causes variations in the acidification process and also the nutrient consumption, due to microbial growth (Minervini et al., 2014). Fermentation temperature influences the fermentation quotient: the ratio of lactic acid produced to the amount of acetic acid produced. Higher temperatures will cause a higher level of lactic acid production and lower levels of acetic acid production. This in turn will cause faster sourdough acidification (Arora et al., 2021). The strain of LAB can also impact the rate and level of acidification. *Lactiplantibacillus plantarum* is one of the most robust and available sourdough LAB strains commonly isolated. This is because of its ability to use the main flour carbohydrates, but also the lower concentration carbohydrates including pentoses and sugar nucleotides or amino acids. *Lactiplantibacillus plantarum* is exceptionally adaptive to environmental stresses, more rapid rates of acidification, the synthesis of diacetyl and hydrogen peroxide, and the synthesis of bacteriocins (Minervini et al., 2014).

The production of organic acids inherent in the flour or starter, and from metabolism during acidification, set the stage for the characteristics of sourdough products. The amount of organic acid from the backslopped flour and starter help to set the initial pH and helps modulate the fermentation, the acidification, and the rate of bacterial growth of both LAB and yeasts. It is

also involved with endogenous enzymatic activity that is correlated with the dough yield, or absorption, and the availability of water (Gobbetti and Gänzle, 2013). When acidification is examined in conjunction with glutathione reductase activity, the extracellular generation of oligopeptides and amino acids is possible. This is through the endogenous flour proteases that are activated at lower pH levels and the cleaving of the disulfide bonds in gluten (Arendt et al., 2007). It is important to keep in mind that yeasts form low levels of organic acids, such as acetic acid and succinic acid, that lead to a slight acidification of the leavened dough and affect the flavor (De Vuyst et al., 2017). This level of production is not on the same scale as LAB but still contributes to the overall characteristics of the sourdough.

As discussed in the previous section, different bacteria and LAB strains compete for dominance within the sourdough system as the pH decreases. A distinction should be made between bacteria with a fast acidification capacity and acid tolerance, and those with low acid tolerance and slower acidification capacities. Certain bacterial species, such as enterococci, are involved in the initial stages of backslopped sourdough fermentations but are outcompeted upon backslopping due to their limited acid tolerance (Corsetti et al., 2007). Initially non-sourdough specific LAB such as *enterococci*, *lactococci*, and *streptococci*, can play a role through fast acidification of the flour-water matrix, particularly by activating the flour native enzymes (Harth et al., 2016). In the baking industry, most LAB strains have been selected based on their ability to quickly acidify a dough. They also generate specific flavor compounds desired in products. This is a clear added value, which is reflected in the commercialization of Type III (Gobbetti and Gänzle, 2013). Considering Type I sourdoughs, backslopping practices are based on short fermentation times and a long resting period applied at low temperatures. The other practice is based on long fermentations with adapted temperature profiles, to limit the acidification. Other specific bakery conditions that impact Type I performance include a low value of dough yield, low temperature of dough incubation, and a rather high pH of the dough (De Vuyst et al., 2014).

A sourdough system goes through various changes of differing degrees during the acidification process. Again, the pH of sourdough can range from 3.5-4.3, depending on the ripeness. In a 2007 review, Arendt et al. separated the effects of acidification into two categories, primary and secondary. The primary impacts are as follows. First, structure forming components such as starch, arabinoxylans, and gluten are all impacted by the acidification. The gluten swells in acid, it has also been hypothesized that because of the mild acid hydrolysis of the starch in a

sourdough, the required mixing time is decreased, leading to a less stable dough compared to non-sourdough products (Arendt et al., 2007). The acidification also exerts positive effects on the structure of starch granules, leading to increase water binding capacity (Hammes and Gänzle, 1998). This is important to be aware of because as the surface area of the granule increases, the affinity for water increases, which can lower the stability of a dough system during dough mixing and lower loaf volume after baking (Li et al., 2023). Studies have shown that varying levels of organic acids and salt can impact the uptake of water within a wheat dough system. Increasing the amount of organic acids produced while in the absence of salt will increase the water uptake, reducing the mixing time of a system and weakening the final product. It was also found that the gluten proteins will unfold within acidic conditions. This is due to the positive net charge of the system and the increase in protein solubility. The more acid, the more the reducing of intermolecular and intramolecular disulfide bonds, providing proteolytic enzymes greater access to starch granules, resulting in a more effective proteolysis. This is tied to an improvement of overall flavor in a final product. The enzyme protease's optimal acidic conditions are a pH of 4, which a sourdough system provides (Arendt et al., 2007). Acidification ultimately shifts the pH of the cereal matrix to levels too low for most cereal native enzymes (De Vuyst et al., 2017). Flavor volatiles are enhanced as amino acids are accumulated during the fermentation process (Arendt et al., 2007).

The quality of the end-product made using a sourdough is typically the largest concern for bakers as that is what most directly impacts the consumer. Therefore, it is important to understand how varying levels of acidification will result in different product qualities. One of the most important to consider is the large reduction in the elasticity and firmness of the dough (Arendt et al., 2007). There are variations in acidification properties and metabolism among different LAB; the ecological composition of each sourdough specifically influences the quality of the bread. On a technological level, with respect to processing and the texture, which includes volume, softness, resilience, crumb color, crust color, crust thickness, flavor, aroma, and projected shelf-life, sourdough leads to different advantages depending on the acidification process (De Vuyst et al., 2017).

Texture is a parameter often associated with the overall quality of a baked product. In sourdough, it can be related to the level of microbial acidification and rate of substrate breakdown. One of the most popular uses of LAB in baking is to reduce the amount of staling, or

completely retard the retrogradation process after baking. The anti-staling effect that has been found depends on a particular strain performing the fermentation. The effect involves dynamics other than those associated with acidification. As mentioned before, starch can be affected by the enzymes produced and released by the LAB, causing the properties of the retrogradation process to vary while also slowing the staling rate. Protease is one of the most prominent enzymes in acidified doughs. It prevents staling while also improving the texture. α-amylase is also increased because of the hydrolysis and protease activity, which liberate water from the gluten network (Arendt et al., 2007).

In a 2019 study examining the impact starter cultures and differing fermentation times have on the biochemical characteristics and aromatic compounds in a type II sourdough, Siepmann et al. found the starter has a great impact on the aromatic characteristics of a sourdough bread. They also found that as the pH of the bread is decreased, the hardness of the bread will increase. A sample containing *Limosilactobacillus reuteri*, *Lactiplantibacillus plantarum*, and *Lactobacillus amylovorus* had a pH of 3.59 and required around 400 grams more force to achieve compression, than a sample containing *Levilactobacillus brevis* and *Lactiplantibacillus plantarum* which had a pH of 4.00. *Limosilactobacillus reuteri* is an acid tolerant bacterium that acidifies rapidly, and to a lower level than other less acid tolerant bacteria, causing a loaf with greater hardness (Siepmann et al., 2019). However, if a sourdough starter with a low pH is added to a plain bread dough, the acidification slows down. This results in a loaf with a higher pH and therefore a softer crumb. It would also show an increase in volume but would not have enough strength, causing a volume decrease after cooling. This decrease during cooling would cause an increase in the hardness (Rizzello et al., 2019). Overall, the sourdough would benefit from a lower pH environment during the backslopping. Its short fermentation time will prevent excessive acidification that can negatively impact the sensory qualities and nutrient depletion (De Vuyst et al., 2017).

In a 2016 study, Harth et al. examined how various starter culture bacteria perform while using barley flour as a substrate. *Levilactobacillus brevis* is often found in starter cultures as it is a persistent bacterium. It is common in barley flour sourdoughs due to its potential to breakdown the beta glucans found in barley. Harth (2016) then delves into more characteristics of barley-based sourdoughs in which the *Leuconostoc* and *Weissella* LAB species dominate. This is due to the lower fermentation temperature and a high carbohydrate concentration at the beginning of the

fermentation. This also causes a slower increase in bacterial counts and TTA, which leads to a slower drop in pH, and overall, a higher pH than found in a wheat-based sourdough. The higher pH influences the flour's lipoxygenase activity, resulting in the production of more aroma precursors and a higher capacity to reduce aldehydes, with high flavor impact, into corresponding alcohols with low flavor impact. This is because of the low absorption and higher buffer capacity of the barley flour water mixture (Harth et al., 2016).

There have been significantly more studies delving into the acidification process and ways to control it within the past few years. Banu et al. (2021) prepared blends of wheat, rye, and barley milled into six fractions. The blends were inoculated with a sourdough starter consisting of *Lacticaseibacillus rhamnosus, Levilactobacillus brevis, Lactiplantibacillus plantarum* and were fermented for 20 hours. The authors found that each blend resulted in a unique acidification process and produced different levels of ethanol, glycerol, L-lactic acid, D-lactic acid, and acetic acid. There was a major difference between the pH values produced by each fraction; it was found that the more rye included in a formulation, the lower the final pH would be.

Manini et al. (2016) set out to phenotypically characterize the growth and acidification rate, carbohydrate metabolism, antifungal activity, and exopolysaccharide production of various LAB strains. To accomplish this, thirteen LAB strains were collected from wheat bran sourdoughs. The strains included *Levilactobacillus brevis, Lactiplantibacillus plantarum, Lactobacillus amylovorus*, *Latilactobacillus curvatus, Latilactobacillus sakei, Leuconostoc mesenteroides, Leuconostoc citreum, and Pediococcus pentosaceus.* To obtain microbial counts, the bacteria were plated on MRS sugar and incubated for three days. It was found from the experiment that there was an average increase of 2.7 log $CFUg^{-1}$ in microbial count between all the species. The three *Lactiplantibacillus plantarum, Latilactobacillus curvatus*, *Latilactobacillus sakei*, and the *Pediococcus pentosaceus* had the highest rate of acidification while *Levilactobacillus brevis* had the lowest rate, reaching only around 5.5 on the pH scale.

In a study delving into the differences in acidification based on the strain of bacteria, Boyaci Gunduz et al. (2022) examined eight total sourdough samples collected from bakeries around Turkey. Six were from whole wheat and two were from rye sourdoughs. They conducted MiSeq Illumina technology to determine the relative abundance of the bacterial taxa in the samples. It was found that *L. plantarum* had the quickest acidification in the first 24 hours but slowed after that. *Fructilactobacillus sanfranciscensis* then surpassed *Lactiplantibacillus*

plantarum at the 24-hour mark as it continued to show a decrease in pH. The most effective overall acidifier was a sample that was inoculated with both the *Lactiplantibacillus plantarum* and the *Fructilactobacillus sanfranciscensis* as it quickly acidified because of the *Lactiplantibacillus plantaru*m, and then kept acidifying past 24 hours because of the *Fructilactobacillus sanfranciscensis*. These studies perfectly demonstrate the various ways that acidification can be controlled through strain and substrate selection. Further research into these areas will continue to shed light into the broad topic that is sourdough and acidification.

Sourdough has garnished more interest in recent years for its beneficial attributes regarding gut health and overall health benefits. The acidification process causes and increase in the amount of resistant starch, while liberating peptides, free fatty acids, polyphenols, and water-soluble dietary fibers (Arora et al., 2021). These are all factors that can improve the nutritional attributes of the final products (Gänzle and Zheng, 2019). Certain enzymes can contribute to the overall flavor and quality of a sourdough product, for example, the level of glutaminase activity. Lactobacilli accumulate glutamate, which is an umami active taste component, thus altering the flavor (Zhao et al., 2015). Recent advances in clinical studies show that gastrointestinal and glycemic load responses after bread ingestion also rely on gut microbiome functionality (Arora et al., 2021). The broad carbohydrate fermentation pattern by vertebrate host adapted lactobacilli is used industrially to produce low-FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) bread through degradation of raffinose, mannitol, and fructans (Gänzle and Zheng, 2019). Phenolic compounds, such as phenolic acids, and alkylresorcinols, are considered both an antinutritional factor and a health benefit provider. Some sourdough lactobacilli catalyze the release of bond phenolic acids that are then subjected to further microbial conversions into flavor precursors (De Vuyst et al., 2017).

1.5. Conclusion

Sourdough and its resultant products are traceable throughout human history and will continue to be as new scientific advancements keep the technology relevant. Sourdough is an ecosystem of diverse bacteria and yeasts that impact the end quality characteristics of its products. With a better understanding of the various microorganisms and processes that contribute to those characteristics, they can be better controlled and continuously replicated. As acidification is so complex, it requires constant monitoring to ensure the desired outcome is achieved. Managing the populations of various microorganisms is required, along with having a

full understanding of all factors involved in the system to make consistent end products and achieve results that can be meaningful to research. As more research is conducted within the field of sourdough and lactic acid bacteria fermentation, there should be more focus on end product application. This will be most applicable within the baking industry at various levels of skill. Understanding how the substrate choice as well as availability to specific bacterial strains can impact the rate of fermentation can lead to more precise knowledge of metabolism for those strains, in turn leading to more effective baking.

1.6. Scope of study

In their review exploring the last 30 years of sourdough research, Arora and colleagues found every five years there was an increasing number of articles being published on the subject of sourdough fermentation (Arora et al., 2021). As sourdough continues its roaring resurgence in popularity and demand, the baking industry must find efficient ways to produce a higher volume of sourdough products in a shorter amount of time. The purpose of this study was to contrast the fermentation kinetics of three different LABs inoculated on to experimental wheat milling fractions. It is hypothesized that the different fractions will have differing fermentation rates, enabling the identification of compounds driving acidification. The information gathered can be used on an industrial scale to precisely select certain LAB and tailor the substrate composition to achieve desired fermentation characteristics in the production of Type II sourdoughs. Increased control of fermentation characteristics will decrease the amount of time needed to ferment and subsequently utilize sourdough on an industrial scale.

The methods used in this experiment are designed to emulate what is typically done on an industrial scale to achieve a viable type II sourdough. As mentioned, this study focuses on the use of milled wheat fractions in the sourdough making process. While other studies have attempted to identify how grain selection impacts the fermentation characteristics, this study takes a more novel approach, examining how fraction selection from the same cultivar of wheat will impact those characteristics. This approach can help identify individual mill streams that could be utilized in the industrial production of sourdough. Previous research in the industry indicates those fractions with higher levels of bran inclusion typically support LAB fermentation (Hammes et al., 2005). Presently in the milling industry, the production of white flour containing far more endosperm than bran is desired. Fractions with higher levels of bran are considered a byproduct. The identification of the most favorable mill streams to be utilized in the industrial

production of sourdough has the potential to highlight a new high-value use for fractions currently diverted to low-value uses.

The aim of this study was to research three different areas: 1) preparation and characterization of wheat fractions, 2) fermentation of fractions inoculated with different strains of LAB, and 3) characterization of the fermented liquid sourdoughs produced.

Seeing as each strain has different metabolic capabilities, it is expected that each will result in a unique set of fermentation characteristics depending on what substrate is being fermented, as each substrate has a unique compositional make-up.

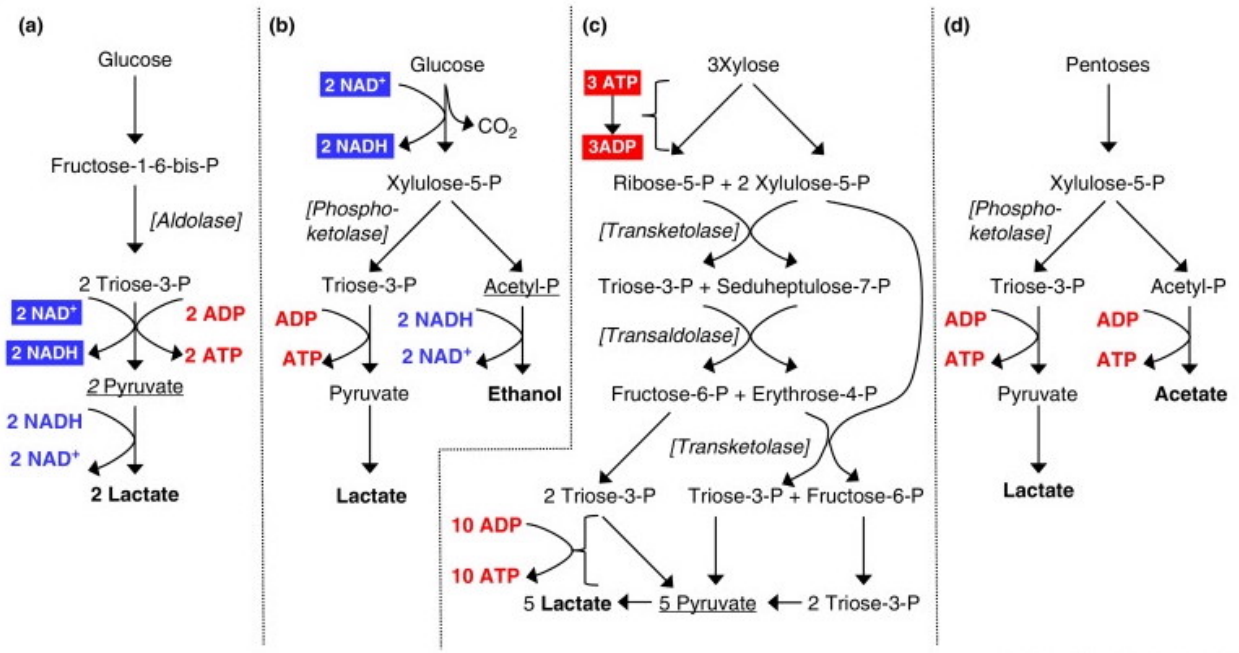


Figure 1.1. LAB metabolic pathways.
(a) Homofermentative metabolism of hexoses by the Emden-Meyerhoff pathway. (b) Heterofermentative metabolism of hexoses by the phosphoketolase pathway. (c) Homofermentative metabolism of pentoses via the pentose phosphate pathway. (d) Heterofermentative metabolism of pentose via the phosphoketolase pathway (adapted from Gänzle, 2015)

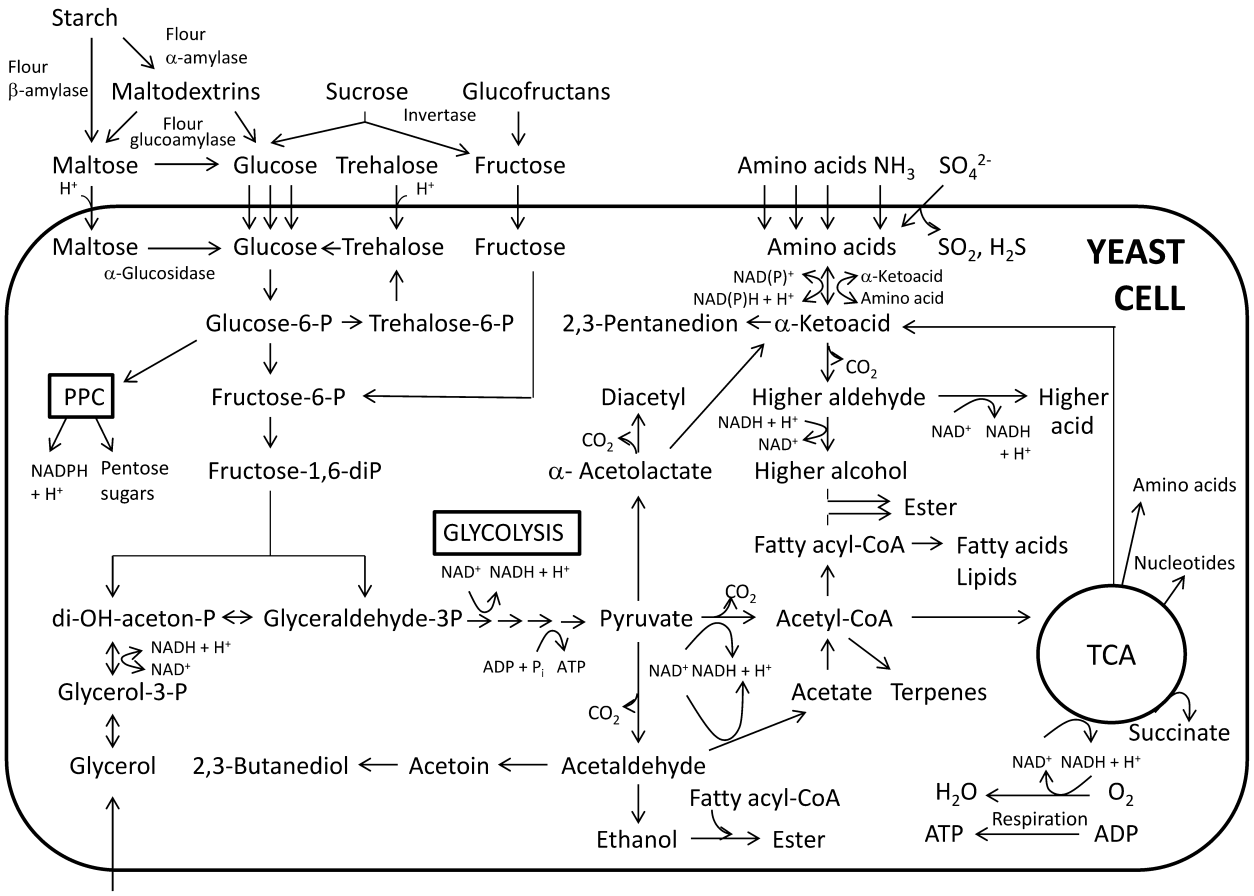


Figure 1.2. Metabolism of a yeast cell.
Major active pathways during sourdough fermentation (adapted from De Vuyst et al., 2017)

Chapter 2 - Methods and Materials

2.1. Materials

2.1.1. Wheat Sample

Twelve 50 lbs. bags of cleaned hard red winter wheat (KanMark 2RL34471), 272.16 kg in total, were obtained from the Foundation Seed Program, through the Agronomy Department at Kansas State University.

2.1.2. LAB Strains

For the study, three purified LAB strains were obtained (Lesaffre International, Lille, Fr.) to be used in the fermentation to observe how different metabolisms impact acidification. The first strain, which is called La1, has heterofermentative metabolic tendencies and is known as one of the more common strains found in typical sourdoughs. The second strain, La2, is another commonly found strain typical in sourdough production, but is homofermentative. The final strain, La3, is heterofermentative but is very different from the other heterofermentative strain, La1. Each of these purified strains were stored at -18 °C in their lyophilized, or freeze-dried, form to ensure maximum viability after addition to the substrate.

2.2. Milling

The wheat was analyzed (Dicky-john GAC2100b) to determine the moisture content of each bag before being tempered to have a consistent 14% moisture content. The moisture content of each 50-pound bag varied from 11.3% to 13.6% and the amount of water added to achieve 14% ranged from one to two kg. The wheat was tempered by a tempering mixer that was powered by a ½ Horsepower Syncrogear motor. The wheat was added, the mixer was powered on, and the wheat began to tumble. The water was then slowly added using a funnel and tube that fed directly into the mixer. Once all water was added, the wheat was mixed for 20 minutes. The hydrated wheat was removed from the mixer and placed in a covered bin. It was then left to temper for a total of 20 hours before milling.

The 272.16 kg of tempered wheat was milled into eight separate wheat fractions using a Buhler MLU 250 experimental mill. The mill flow is shown in Figure 2.1. The fractions included 1st Break (1BK), 2nd Break (2BK), 3rd Break (3BK), 1st Middlings (1M), 2nd Middlings (2M), 3rd Middlings (3M), shorts, and bran. Wheat was fed into the mill at 120 g/min using an FMC Technologies Syntron Magnetic Feeder F-TQ-C to match the mill speed. The milling was

completed in four separate milling periods over three weeks. After milling was complete, the four days' worth of fraction collections for each fraction were blended using a Wenger 500 Pound Flour Blender. This homogenized each fraction before further analysis. After the milling and blending were complete, the extraction rate of each fraction was calculated. Each of the samples were bagged in either 50 lbs. paper sacks that were then sewn shut, or ten lbs. plastic bags sealed using a zip tie. This was dependent on the amount of substrate needed to be stored; some required multiple bags. The bagged samples were then stored at around 4 °C until use.

2.3. Composition Analysis of the Fractions

2.3.1. Moisture

The moisture content of each flour fraction was obtained following the AACCI Method 44-15.02 (AACCI, 2011) for air oven moisture. A 2 g to 2.5 g sample was placed in a metal canister, the actual weight was recorded, and the cup was fired in an oven (ThermoFisher Scientific, Mo. #3511FS) set to 130±1 °C for 60 min uncovered. Once removed from the oven, the canisters were covered with lids and allowed to cool to room temperature for two hours before being weighed. The moisture content (MC) was then calculated using the below equation.

$$MC\ (\%) = \frac{Moisture\ loss\ (g)}{Origial\ sample\ weight\ (g)} \times 100$$

2.3.2. Ash

The ash of each flour fraction was determined using the AACC method 08-01.01. 25 mL crucibles were cleaned and dried before being placed in a Thermolyne muffle furnace (Thermo Fisher Scientific) at 590 °C for two hours to remove any residual moisture. Crucibles were removed and placed in laboratory desiccator to cool to room temperature. Each cooled crucible was handled with nitrile gloves to avoid adding weight to the crucibles. The crucibles were numbered using a wax pencil before being placed on a scale and recording their initial weight. Samples were then scaled into the weighed crucible and the total weight was recorded. The crucibles were then placed in the muffle furnace set at 590 °C and the samples were allowed to incinerate. Samples were left overnight for 24 hours to ensure complete ash. The samples were removed from the oven and allowed to cool in a desiccator for two hours before being weighted. The weight was recorded and subtracted from the initial weight of the crucible and sample to determine the total ash content of each flour fraction. Each fraction was run in triplicate and then averaged for reporting.

2.3.3. Mineral Analysis

Mineral analysis was conducted by the Soils Testing Lab, part of the Agronomy Department at Kansas State University. Samples were sent in triplicate for each of the fractions collected from the mill. The lab utilizes a nitric perchloric acid digestion method followed by induced coupled plasma detection (AOAC 2015.01). The mineral content of the straight grade flour, whole wheat flour, and 2/3BK flour was calculated stoichiometrically using proper mass balance equations.

2/3BK flour : blend of 2BK + 3BK

Straight grade flour : blend of 1BK + 2BK + 3BK + 1M + 2M + 3M

Whole wheat flour : blend of 1BK + 2BK + 3BK + 1M + 2M + 3M + Bran + Shorts

2.3.4. Starch Content

A Total Starch Assay Kit (AA/AMG) testing kit (Megazyme K-TSA-100A) was used to analyze the total starch found in each sample following the AACC Method 76-13.01. Starch content was observed to gather a better understanding of what was available to the bacteria during their metabolism.

Preparation of reagents. The GOPOD reagent buffer was prepared by diluting the contents of the bottle into one liter of distilled water and must be used immediately. The GOPOD reagent enzyme was dissolved in 20 mL of the GOPOD buffer solution, which was then transferred back to the bottle containing the remaining GOPOD reagent buffer bottle. This is known as the Glucose Determination Reagent. The next reagent to be prepared was the 100 mM, pH 5.0 sodium acetate buffer, which will be referred to as [B(a)]. 5.8 mL of glacial acetic acid was added to 900 mL of distilled water and then adjusted to a pH of 5.0 using 1 M NaOH. Then 0.74 grams of calcium chloride dihydrate was added and dissolved before the volume was adjusted to one liter. The next reagent was the 600 mM, pH 3.8 sodium acetate buffer, which will be referred to as [B(c)]. 69.6 mL of glacial acetic acid was added to 1600 mL of distilled water. The pH was then adjusted to 3.8 using 4 M NaOH. 1.48 grams of calcium chloride dihydrate was added and the volume was adjusted to two liters. The final reagent produced was the 1.7 M sodium hydroxide. 68 grams of NaOH were added to 900 mL of deionized water before the volume was adjusted to one liter. It was ensured all NaOH was dissolved in the water before use.

Analysis. 100 mg of flour was accurately weighted into 16×120 mm Corning culture tubes. Tubes were then tapped to ensure all the sample fell to the bottom. Next, 0.2 mL of 80%

v/v aqueous ethanol was added to the flour, as well as a cross type stir bar, and it was mixed using a vortex mixer until completely hydrated. 2 mL of the cold sodium hydroxide solution was added to the tube using a repeater pipette. This was mixed for 15 seconds on a vortex mixer. The tubes were then placed in an ice filled container, which in turn was set on a magnetic stir plate. The stir plate was powered on and allowed to stir for 15 minutes. During that time, tubes were vigorously mixed every 5 minutes using the vortex mixer for three seconds. After 15 minutes on the stir plate, 8 mL of buffer [B(c)] and then mixed on the vortex mixer for five seconds. Immediately, 0.1 mL of thermostable α-amylase was added to each tube using the repeater pipette. Then 0.1 mL of amyloglucosidase was added to the samples using another repeater pipette and 0.2 mL of buffer [B(c)] was added to the reagent blank. The tubes were capped and stirred on the vortex mixer for 3 seconds, then incubated in a water bath at 50 °C for 30 minutes. After 30 minutes, the tubes were removed from the water bath and cooled to room temperature. Tubes were inverted multiple times as they cooled to ensure condensed water on sides of the lids was mixed into the remaining sample. 2 mL of each solution was then added to microfuge tubes before being centrifuged at 13,000 rpm for five minutes. 0.1 mL of the supernatant was removed from the microfuge tube to a 12×120 mm tube containing 9 mL of buffer [B(a)] and then mix for 3 seconds on the vortex mixer. 0.1 mL of each sample was then transferred to the bottom of a 16×120 mm glass test tube and 0.1 mL of blank sample was also added to a 16×120 mm tube. Next, 3.0 mL of GOPOD reagent was added to each tube before being incubated for 20 minutes at 50 °C. The glucose control and reagent blank were incubated concurrently. After the incubation, the absorbance was measured using a spectrophotometer against the reagent blank at 510 nm. The starch content was then calculated using the formula below.

$$Starch\ (\%) = \Delta A \times F \times \frac{EV}{0.1} \times D \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

where	ΔA	Absorbance of sample solution read against reagent blank
	F	Factor to convert absorbance to μg glucose
	EV	Sample extraction volume
	D	Further dilution of sample solution
	100/W	Conversion to 100 mg sample
	162/180	Factor to convert from free glucose to anhydroglucose
	1/1000	Conversion from μg to mg
	0.1	Volume of sample analyzed

2.4. Physiochemical Properties of the Fractions

2.4.1. Damaged Starch

Damaged starch is readily available for enzymatic activity, thus can directly impact the rate of fermentation. The damaged starch was estimated using the amperometric method following the AACC method 76-33.01 using the SDmatic from Chopin. To prepare the test solution, 1.5±0.2 grams of citric acid powder and 3.0±0.2 grams potassium iodide powder was added to a 250 mL sealable plastic container. Next, 120 mL of deionized water was added to the container with the powders and sealed before being mixed. A single drop of 0.1 N sodium thiosulfate was added to the solution and again sealed and swirled to mix. The solution was then poured into a clean and dry SDmatic glass reaction vessel. The reaction vessel containing the solution was placed into the recessed well to hold the sample under the reaction probe. The flour sample was then scaled onto a clean and dry plastic SDmatic sample holder. The target weight was 1.000±0.100 g. The actual weight was recorded to the third decimal place. The device head was lowered so the heating resistor, reaction probe, and the stirrer were submerged in the solution. The plastic sample holder containing the gram of sample was then placed in the vibrating feeder found on the front face of the device head above the vessel. The test was initiated and the exact weight which was earlier recorded to three decimal places was entered on the screen. The weight was confirmed and the SDmatic system automatically began the test. The measurement cycle of the SDmatic utilized is shown in Figure 2.2. After the test was concluded, the Iodine Absorption Percent, Farrand value, UCD, and Iodine Absorption Rate were recorded. The sample holder was removed before the device head was raised up out of the spent solution. The vessel was removed so the spent sample could be discarded before rinsing and cleaning the vessel. The heating resistor, reaction probe, and the stirrer were rinsed using deionized water into a collection container. The rinse water was then discarded, and the next sample was prepared following the same steps as before. Each sample was run in triplicate and each value collected was then averaged.

2.5. Physical Characterization

2.5.1. Particle Size Analysis

The Malvern Morphologi G3 was used to measure the morphologic properties of the flour particles after being milled. Each fraction was analyzed by first opening the Morphologi

program on a computer and turning on the instrument and its connected air pressure supply. The rectangular glass plate to hold the sample was cleaned to eliminate any contaminating particles. Antistatic liquid was sprayed inside the sample chamber to prevent any sticking on the metal interior. Next, the sample was scooped using the 5 mm^3 loading spoon before being transferred into the sample well within the sample entrainment spool. This was then inserted into the dispersion chamber along with an O-ring seal. The spool was secured by screwing the dispersion chamber cap over it. The chamber was then secured by placing it within the clamp attached to the side of the Morphologi G3. The wheat flour standard operating procedure was selected on the computer and the Morphologi G3 went through its calibration phase. Next, the sample was dispersed using 4.0 bar injection pressure for ten milliseconds, and then allowed to settle for 60 seconds. The glass plate, now holding the sample, moved directly under the Morphologi G3 set of microscopic lenses while being illuminated from below the glass. Images of the particle groupings were captured by the camera through the 10× microscopic lens. In total, 10,000 particles were observed, taking up to an hour per sample. After images were fully captured, they were analyzed by the Morphologi G3's wheat flour procedure. The particle size distribution was generated on a both a number and volume basis. The subscripts for those fractions represent the percent of particles less than the specialized values. After the 10,000 particles were analyzed, the Morphologi G3 software provided a data output including circle equivalent diameter, spherical equivalent volume, circularity, convexity, elongation, and area (pixels).

2.6. Functional Characterization

2.6.1. Amylase Activity

A Malt Amylase Assay Kit (Megazyme K-MALTA) kit was also used to analyze both the α-amylase and β-amylase content of the samples following the AACC Method 22-02.01. The reagents and buffers were stored at proper temperature until their use.

Preparation of Buffer Solutions. Multiple buffers had to be prepared before the assay of the samples could be done. The first of these was the stopping reagent, which was prepared by dissolving 1 gram of Tris buffer, 2-amino-2-(hydroxymethyl) propane-1,3-diol ($NH_2C(CH_2OH)_3$), into 900 mL of deionized water. The pH was adjusted to 8.5 using 4.0 M NaOH. The volume was also adjusted to one liter in total using deionized water. The next reagent, Megazyme Ceralpha Buffer A, was produced by adding the 50 mL contents of the Ceralpha Buffer A bottle from the Megazyme kit into 1000 mL of deionized water. The pH was

adjusted to pH of 5.4 using 4.0 M NaOH. The next reagent, Megazyme Betamy-3 Buffer B was produced by adding the 50 mL contents of the Betamyl-3 Buffer B bottle from the Megazyme kit into 500 mL of deionized water. The Ceralpha Buffer A and Betamyl-3 Buffer B were both stored at 4 °C until their intended use. The Megazyme Betamyl-3 Buffer A was produced by adding 2.5 mL of the contents of the Betamyl-3 Buffer A bottle to 50 mL of deionized water. Before use, the Betamyl-3 Buffer A solution had 0.88 grams of cysteine HCL added before its pH was adjusted to 8.0 using the 4 M NaOH. After the addition of the cysteine HCL the solution was only stable for eight hours at 4 °C.

Preparation of Substrate Solutions. The known substrates from the kits were then prepared. 25 mL of deionized water was brought to a boil then allowed to cool. Ten mL of the cooled water was added to a 50 mL polypropylene centrifuge tube, and a tube with ten mL of water was prepared and labeled for each substrate. The contents of the Ceralpha Substrate Solution were added using a 0.1-2.5 µL Eppendorf Reference Pipette with a 0.2 mL pipette tip to the properly labeled tube and mixed for 20 seconds using a vortex mixer set to speed five. The contents of the Betamyl-3 Substrate Solution were added to the properly labeled tube and mixed for 20 seconds using the vortex mixer as speed five. The solutions were kept below -10 °C between uses.

Enzyme Extraction and Malt Extract Filtration. Each of the flour fractions used to ferment were analyzed in duplicate for their enzyme activity. 0.5 g of the samples were weighed into a 13 mL polypropylene tube. Then, 5.0 mL of the Betamyl-3 buffer A was added before being mixed for ten seconds on a vortex mixer at speed five. The enzyme was allowed for one hour to extract at room temperature. During the hour, the samples were vortexed for five seconds every ten minutes, for a total of five mixes during that time to ensure full hydration. At the end of the hour, the samples were again vortexed before being centrifuged at 2,000 g for ten minutes using an Eppendorf Centrifuge 5810 R. Then 0.2 mL of the supernatant extracted during centrifugation was added using an 0.1-2.5 µL Eppendorf Reference Pipette with a 0.2 mL pipette tip to a 12 mL glass tube containing 4.0 mL of Betamyl-3 buffer B. The samples were then mixed for five seconds on the vortex mixer. This resulted in the malt extract that was then used to assay both the alpha (α) and beta (b) amylase.

Assay of β-amylase. After the extract was obtained, 0.2 mL of the malt extract was added to a 12 mL glass tube were pre-incubated at 40 °C for 5 minutes. 12 mL glass test tubes and the

Betamyl-3 substrate solution were also pre-incubated at the same temperature for the same amount of time. After incubation, 0.2 mL of the Betamyl-3 substrate solution was added to the 0.2 mL of malt extract and briefly stirred using the vortex mixer. These were then incubated for exactly ten minutes after the addition of the first aliquot of buffer at 40 °C. After the ten-minute incubation, 3.0 mL of the stopping reagent were added to the sample and stirred using the pipette tip. 2 mL of the sample was then added to a spectrophotometer cuvette. The cuvettes were properly placed into the spectrophotometer and the absorbances were then measured at 400 nm.

$$\frac{Units\ of\ \beta\text{-}amylase}{g\ of\ flour} = \Delta E_{400} \times 19.7$$

Assay of α-amylase. For the assay of the α-amylase, a further dilution was done by adding 0.2 mL of the malt extract to 3.0 mL of Ceralpha Buffer A. The second malt extract was then dispensed into 12 mL glass test tubes and were then preincubated at 40 °C for five minutes. The Ceralpha substrate solution was pre-incubated at the same temperature for the same amount of time. After the preincubation, 0.2 mL of Ceralpha substrate solution was added to the second preincubated malt extract. Samples were stirred briefly on a vortex mixer before being incubated for ten minutes after the addition of the first aliquot of buffer 40 °C. After the ten-minute incubation time, 3.0 mL of stopping reagent was added to the tubes using the repeater pipette and was then mixed using the vortex mixer. The solution was transferred to cuvettes before being placed in the spectrophotometer. The solutions and the reagent blank absorbances read at 400 nm. The spectrophotometer output was then used in the below equation to provide a unit of enzyme activity to compare between the fractions.

$$\frac{Units\ of\ \alpha\text{-}amylase}{g\ of\ flour} = \Delta E_{400} \times 315.6$$

$$\frac{Units\ of\ amylase\ (\alpha\ or\ \beta)}{g\ of\ flour} = \frac{\Delta E_{400}}{Incubation\ time} \times \frac{Total\ vol\ in\ cell}{Aliquot\ assayed} \times \frac{1}{\varepsilon_{mM}} \times \frac{Extraction\ vol}{Sample\ weight} \times Dilution$$

where	ΔE_{400}	Absorbance (sample) – Absorbance (blank)
	Incubation time	10 min
	Total volume in cell	3.4 mL
	Aliquot assayed	0.2 mL
	ε_{mM} *p*-nitrophenol	18.1 (at 400 nm) in 1% Tris buffer solution
	Extraction volume	5 mL per 0.5 g of malt

Sample weight	Grams
Dilution	0.2 mL to volume of 4.2 mL for β-amylase; then further 0.2 mL to 3.2 mL for α-amylase

Measurement. A reagent blank was prepared using the known malt substrate, so the spectrophotometer could be zeroed. A blank with the Ceralpha substrate was prepared to compare to the α-amylase assay, and a blank with Betamyl-3 substrate was prepared to compare to the β-amylase assay. The known malt substrate was diluted using the buffers the same way the other samples were prepared to achieve the malt extract. 0.3 mL of stopping reagent was added to the pre-equilibrated substrate and then mixed for three seconds using a vortex mixer. Then, 0.2 mL of diluted malt extract was added and vortexed again for three seconds. The solution was then transferred to a cuvette where it could be measured and zeroed out before reading the wavelengths of the remaining samples. The calculation from the Megazyme kit provides a value to describe the activity of the enzyme within a sample. The β-amylase value, according to Megazyme, is defined as "one unit of activity is defined as the amount of enzyme, in the presence of excess thermostable Beta-glucosidase is required to release one micromole of p-nitrophenol from PNBBeta-G3 in one minute under the defined assay conditions and is termed as "Betamyl-3 Unit." The α-amylase value is described as "one unit of activity is defined as the amount of enzyme, in the presence of excess thermostable α -glucosidase is required to release one micromole of p-nitrophenol from BPNPG7 in one minute under the defined assay conditions and is termed as "Ceralpha Unit" (Megazyme, 2020).

2.6.2. Mixing and Dough Development

The mixing and gelatinizing properties of the flour fractions were measured using a Chopin MixoLab following the AACC method 54-60.01. A typical MixoLab curve illustrates the changes in dough behavior over time as heat and torque are added to the system. An example chart showing the anticipated changes can be seen in Figure 2.3. Also, displayed in that figure are the data points included in the output of the MixoLab. To begin a test, the MixoLab and water bath attached to it were both powered on. The water bath was allowed 30 minutes to achieve the desired 18 °C. The accompanying computer was powered on as well and the MixoLab program was opened. The Chopin+ protocol was selected for the purposes of this experiment. For each sample, the moisture content was obtained using a Blue Sun Phoenix 5000 near infrared spectroscopy to determine the amount of flour to be used during the test. The

desired absorption was also input. The absorption was held constant at 60% per flour basis (14% basis) so samples were comparable. This value was determined as optimum for a straight grade sample after initially running the sample at 62% and allowing the MixoLab program to calculate the optimum absorption to be 61.2%. The MixoLab mixing carriage was assembled and then locked into place in its designated slot on the MixoLab. The cover was then closed, and the sample addition funnel was placed. The sample was then added to the mixing bowl. The ingredient funnel was then removed from the addition slot and replaced by the water titration nozzle. The MixoLab then began its calibration. Once temperature and torque were acceptable, water was titrated into the mixing bowl as the paddles turned the flour. The test ran for 45 minutes, at which time the carriage could be removed and disassembled to discard the spent dough and clean the carriage. The cleaned carriage was reassembled before starting the next test following the same procedure as before. Each sample was run in duplicate and averaged. Shorts and Bran fractions were omitted from this test as they would not provide accurate results due to a lack of dough forming capacity. For the purposes of this study, the maximum torque value at each significant time was recorded. This includes the dough development value C1, the departure time CS, the protein reduction value C2, starch gelatinization value C3, amylase activity value C4, starch gelling value C5. Specific time values were also collected, including the development arriving time C1, Protein reduction time C2, starch gelatinization time C3, and amylase activity time C4.

2.7. Blending

To gather a better understanding of how common flours available would ferment compared to the separate fractions, fractions were combined to produce a straight grade flour (starchy fractions without the short or bran), and a whole wheat flour (all milled fractions). Also, due to the low yield of 3BK, the fraction was combined with 2BK to ensure there would be enough substrate to analyze and experiment with; this fraction is labeled 2/3BK. The percentage of each fraction needed to produce those three flours is listed in Table 2.1. These composite flours were then stored in the same conditions as the other fractions until their use.

2.8. Fermentation

2.8.1. Experimental Design

The nine flour fractions and combinations were fermented using three strains of bacteria to compare metabolic differences. Fermentation of each flour fraction with each strain was run in triplicate for 27 total samples per strain of LAB. A three-digit number was assigned to each of the samples using the University of Oregon Office of the Registrar Three-Digit Code Number Generator. The assigned numbers were ordered from smallest to largest to determine the order of fermentation.

2.8.2. Inoculation Preparation

A day before planned fermentation, sample preparation materials were dipped in 99.5% ethanol to ensure an uncontaminated bacteria inoculation. The materials dipped included two 15 mL sealable plastic containers and lids, a 50 mL centrifuge tube and its lid, and a 100 g weight boat. These were then set face down on a paper towel to air dry overnight. The sample was prepared the next day. La1 and La2 had concentrations of 1×10^{11} CFU/g, while La3 had a concentration of 9.15×10^{11} CFU/g. For the experiment, the strains were diluted to a concentration of 1×10^{7} CFU/g of dough. For La1 and La2, this was achieved by scaling 1.0 g of freeze-dried purified bacteria into the 100 mL weigh boat. Next, 10 mL of saline solution containing calcium and magnesium that was filtered through a 0.1 μm sieve, was added to the 50 mL centrifuge tube. The bacteria from the weigh boat were added to the saline solution and homogenized using a vortex mixer at speed 5 for ten seconds. For La3, the steps were the same, but involve another dilution. This was achieved by aliquoting 1 mL of the homogenized strain to another 50 mL centrifuge tube containing 10 mL of the saline solution. This was then homogenized using a vortex mixer at speed 5 for ten seconds to achieve a solution of the correct CFU/g. From the solution, 2.33 mL was added to each empty 15 mL container, which were then sealed. The substrate was inoculated as quickly as possible to ensure the sample was viable.

2.8.3. Fermentation Tank Set-up

To ensure constant temperature for the bacteria to thrive, temperature-controlled fermentation tanks were utilized to ensure consistency. The set-up process begins with ensuring fermentation tanks, paddles, and covers were properly sanitized and clear of any debris. The tanks were connected to a VMR MX 7LL R-20 water bath. To ensure consistency between tests, the assembly order was done in the same way each time. First, the water bath's outlet tube was

connected to the A1 tank's inlet connector located towards the bottom of the tank. A short tube connects the outlet connector from the top of the A1 tank to the bottom A2 inlet connector. The A2 outlet was connected to the inlet tube from the water bath to complete the circuit. The fitting locks on the connectors were opened in the same order as their assembly and the water bath was powered on and set to a temperature of 35 °C. Both the A1 and A2 overhead stirrers (Heidolph Hei-TORQUE 400) were powered on using the button on the front face of the stirrer. The stir speed was set at 40 rpms and was confirmed on the digital screen on the front face of the stirrer. The paddle and lid cover were then prepared by inserting the shaft bit part of the A1 paddle through the middle of the A1 cover until the cover was resting on the paddle head. The paddle was then inserted into the stirrer head and tightened by twisting the stirrer head choke clockwise. The locking ring was turned to secure the paddle in place. The tank was then ready for the addition of the sample. This process was repeated on the A2 paddle, lid, and stirrer head. A graphic of this setup once the paddle was submerged in the tank can be seen in Figure 2.4.

2.8.4. Transmitter Calibration

To monitor the pH over the entire fermentation, a Bluetooth transmitter (AMS Alliance) attached to a pH probe (Mettler Toledo InLab Smart Pro-ISM) was submerged in the slurry to spin along with the rotating paddle. This provided constant data collection during the entire fermentation. This was set up by powering on the accompanying computer then ensuring that the USB receiver was properly inserted and blinking to indicate its connection to the transmitter. The iCinac software (iCinac version 2.1.4.1) was then opened on a computer to collect the data. The Bluetooth transmitters, A1 and A2, were powered on by pressing the white button found on the top of the transmitter. A plastic cap was then placed on each transmitter, covering the power buttons to ensure they could not be mistakenly powered off. Each probe was then fitted with its designated transmitter, i.e. A1 with A1 and A2 with A2. Once the transmitters were connected to the Bluetooth receiver recognized by the iCinac software, they were ready for calibration and use. Calibration of the probes was done using buffers solutions whose pH was 4.0 and 7.0 respectively. Beginning with A1, the probe was removed from storage solution and rinsed using distilled water before being submerged in the pH 4.00 buffer solution. On the iCinac software, the A1 tile was clicked, followed by the "Calibration" menu found in the bottom left corner. The pH of the buffer solution was input into the computer and the calibration began. Once the pH was stable for a continuous minute, the calibration was accepted. The probe was then removed

and rinsed before being submerged in the other buffer solution. This process was repeated till the calibration was complete. The probe was removed from the buffer solution and rinsed before being resubmerged in the storage solution. The A2 probe was then calibrated in the same fashion as the A1. Both probes were kept in the storage solution until their intended use.

2.8.5. Sample Preparation

Samples were initially mixed in a stand mixer before being transferred into the prepared fermentation tank to ensure consistent starting texture and end results for each sample. The equipment used to prepare the samples included a Hobart N-50 Mixer, its two 3.5 L Hobart mixing bowls and their accompanying paddles, rubber spatulas, two 250 mL cylindrical beakers, and a 20 mL ladle. Depending on the bran content of the sample, the absorption of the sample was adjusted to achieve a consistent viscosity over the trials, those formulations can be seen in Table 2.2. For the samples without visible bran, the substrate was first added to the mixing bowl followed by the bacteria. The inoculate container was then rinsed into the mixing bowl using the water to ensure all bacteria would be added to the sample. The remaining water was then added to the mixing bowl. The mixer paddle was then used to manually incorporate the water into the flour over a 15 second period. The mixing bowl and mixing paddle were then secured into the Hobart. The sample was mixed on 1st speed for one minute, and then at 2nd speed for four minutes. The bowl was scraped using the rubber spatula after the minute at 1st speed and at the end of mixing. For the whole wheat flour, shorts and bran, a 3000 mL metal bowl was used to hand mix the sample before it was transferred to the fermentation tank. Half of the substrate was added to the bowl, followed by the bacteria in the same fashion as the previous samples. This was mixed manually using a rubber spatula until the sample was hydrated. The remaining substrate was then added, and this was mixed as the remaining water was added slowly. After four minutes of hand mixing the substrate was fully hydrated and ready to be transferred to the fermentation tank. Before transferring any sample to the fermentation tanks, ten grams of sample were removed from the mixing bowl using the ladle and scaled into a 250 mL beaker. This ten-gram sample was used later to measure an initial TTA. The remaining sample in the mixing bowl was then poured into one of the fermentation tanks, using a rubber spatula to ensure the sample was completely transferred. For this experiment, the inoculation amount was not adjusted based on the dry matter but was held constant for all fractions. Due to the high concentration of the

strains being established in each fermentation, it was determined it would not be adjusted depending on dry matter and absorption of the individual fractions.

2.8.6. Real-time pH Data Collection

Once the sample was transferred to the fermentation tank, the stir head with attached mixing paddle was maneuvered to be directly over the center of the fermentation tank. The paddle was lowered to the bottom of the tank before being raised ¼" to avoid scraping. The transmitter and pH probe were secured in the holder of the paddle, so that the probe was submerged 1.5" into the slurry. Information about the sample was then entered into the iCinac software in the following order: the trial number, sample substrate, bacteria type, and date of the test (i.e. 000 Substrate Bacteria 0/0/0000). The specifications of the test were confirmed; Start Mode – Immediate, End Mode – Time, End Value – 48:00:00, Sampling Period – 00:04:00, Offset – 00:00:00. The test was then started on the computer, followed by starting the A1 mixing head, then the A2. During the initial seconds of spin, it was ensured that the paddle was spinning centered in the fermentation tank and was not scraping along the bottom of the tank.

2.8.7. Fermentation Kinetics and Characteristics Calculation

To quantify the results obtained after the fermentation was complete, fermentation kinetics data was computed using the iCinac software. Maximum velocity of acidification, or V_{max} indicates the steepest drop in pH that occurred during the fermentation. This value was calculated for each point by subtracting the later value from the earlier value and dividing that by the amount of time between the two values. The V_{max} unit is reported as the change in pH over four minutes. The point in time at which the V_{max} occurs, known as the T_{max} is reported, indicating how early steep acidification occurs. Minimum pH achieved during each fermentation and time to achieve a pH of 4.0 are also reported. The time to achieve a pH of 4.0 is often considered the point a fermentation needs to achieve to be considered a "sourdough" (Cardinali et al., 2022). The moving average for pH was computed using an average of fifteen pH values. For example, the value collected at 0.93 hours is an average of the fifteen pH values between 0.93 and 2.93 hours. This provides a smooth and generalized curve of acidification, without the lag phase or major deviations in the pH that may occur during the fermentation.

2.8.8. Titratable Acidity (TTA)

To gain a deeper understanding of the changes occurring during fermentation, samples were collected throughout the 48-hour fermentation. TTA is a common parameter used to

determine at which point a sourdough is fully matured. Ten-gram sample was used to analyze the total titratable acid, or TTA. An initial sample was collected after the initial mixing on the Hobart to obtain a baseline acidity. Samples were collected at hour 8, hour 18, hour 24, hour 30, and hour 48. This was achieved by stopping the mixer and sliding the cover up to access the slurry in the fermentation tank. About 20 g of sample were collected using a 25 mL ladle. The ten grams of the collected sample for TTA was added to a 250 mL plastic beaker, followed by 100 mL of water to dilute the sample. A stir bar was placed into the beaker before being mixed on a stir plate until the sample was homogenized. The pH probe attached to the titrator was rinsed using deionized water before being submerged into the sample, and the pH was recorded once stable. The sample was then titrated with 0.1 N NaOH dispensed using a VSI TitroLine 5000 titrator. Total titratable acidity was recorded at pH of 6.6 and 8.5. The pH probe was removed from the sample and rinsed before being placed into its storage solution. The titrated sample was then discarded. The highest value of NaOH to achieve a pH of 8.5 is reported as the fifth fermentation characteristic.

2.8.9. Metabolite Quantification with SEC-HPLC

Analysis using high performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) was utilized to ascertain concentrations of organic acids, saccharides, and alcohols in sourdough samples. Samples were collected at hour 8, hour 18, hour 24, hour 30, and hour 48 and frozen at -18 °C until analysis. Four hours before the preparation of HPLC samples, the frozen ten mL bags of sample were removed from the freezer and allowed to warm to room temperature. After they were fully thawed, the sample was stirred with a spatula to ensure reconstitution of all components of the slurry. The samples were then diluted by adding 1 gram of sample to ten mL of deionized water within a 50 mL centrifuge tube. The tubes were then mixed using a vortex mixer on speed 5 for 1 minute. From the solution 1 mL was taken and then further diluted into another 50 mL centrifuge tube with ten mL of deionized water. The second tube was mixed using a vortex mixer on speed 5 for 1 minute. This was repeated until 16 samples were twice diluted. Those 16 samples were then placed in the ThermoScientific Sorvall ST 16 centrifuge and centrifuged at 4000 rpm for 15 minutes. After the centrifugation, 2.5 mL of supernatant was extracted using a 3 mL plastic medical syringe with Luer lock. The syringe was then affixed with a 0.2 µ PTFE syringe filter. The sample was then dispensed into a 2 mL amber HPLC vial and capped using a PTFE and white silicone septa cap. The vial was then labeled with

the corresponding sample identification. The HPLC utilized an Aminex HPX-87H Column #1250140 for its size exclusion. The column provided a ppm for polysaccharides maltose, fructose, glucose; organic acids citric acid, lactic acid, formic acid, malic acid, succinic acid, glycolic acid, acetic acid, butyric acid, and propionic acid; and alcohols ethanol and methanol. A five-point standard curve was generated for each compound with an R-squared value above 0.99.

2.8.10. Color

At the end of the 48-hour fermentation, the color of the final sample was collected using a Konica Minolta BC-10 Baking Meter. The device provides values on the L*a*b color scale. One liter of sample was extracted from the fermentation tank and then dispensed into a one-gallon plastic bag labeled with the sample information. As much air was removed from the bag as possible before it was sealed and then it was set on a neutral-colored counter. The BC-10 was calibrated using the white calibration cap. The calibrated BC-10 was then placed directly onto the plastic bag of sample to be scanned. The sample was scanned and the L*a*b* value recorded. The value from the three replicates of each substrate were averaged, the La2 values were subtracted from the La1 values, and the difference between them was reported.

2.9. Statistical Analysis

To determine differences between the compositions of the flour fractions, a One-way ANOVA was utilized. To determine differences between the fermentation kinetics and characteristics, a Two-way ANOVA was utilized (fraction × strain) The ANOVA tests were carried out using SAS Studio version 3.82. The same software was used to run pairwise comparisons using Tukey's HSD considering significance at $p < 0.05$. To determine correlations and relationships between the fraction compositions and the fermentation characteristic, principal component analysis and correlation analysis were run using JMP Pro 17, under multivariate analysis.

Table 2.1. Percent of fractions in composite flours.

Fraction	Straight Grade %	Whole Wheat %	2nd/3rd Break%
1BK	7.66%	4.88%	0%
2BK	9.40%	5.98%	73.39%
3BK	3.41%	2.17%	26.61%
1M	38.05%	24.22%	0%
2M	25.15%	16.01%	0%
3M	16.32%	10.39%	0%
Shorts	0%	17.54%	0%
Bran	0%	18.80%	0%

Table 2.2. Sourdough fermentation formulations.

Breaks, Middlings, Straight Grade		
Ingredients	%	Gram
Fraction	100	1060
H_2O	120	1272
Inoculum	0.22	2.33

Whole Wheat		
Ingredients	%	Gram
Whole Wheat	100	1060
H_2O	180	1908
Inoculum	0.22	2.33

Shorts		
Ingredients	%	Gram
Shorts	100	1060
H_2O	260	2756
Inoculum	0.22	2.33

Bran		
Ingredients	%	Gram
Bran	100	707
50% of H_2O	210	1484
Inoculum	0.33	2.33
50% of H_2O	210	1484

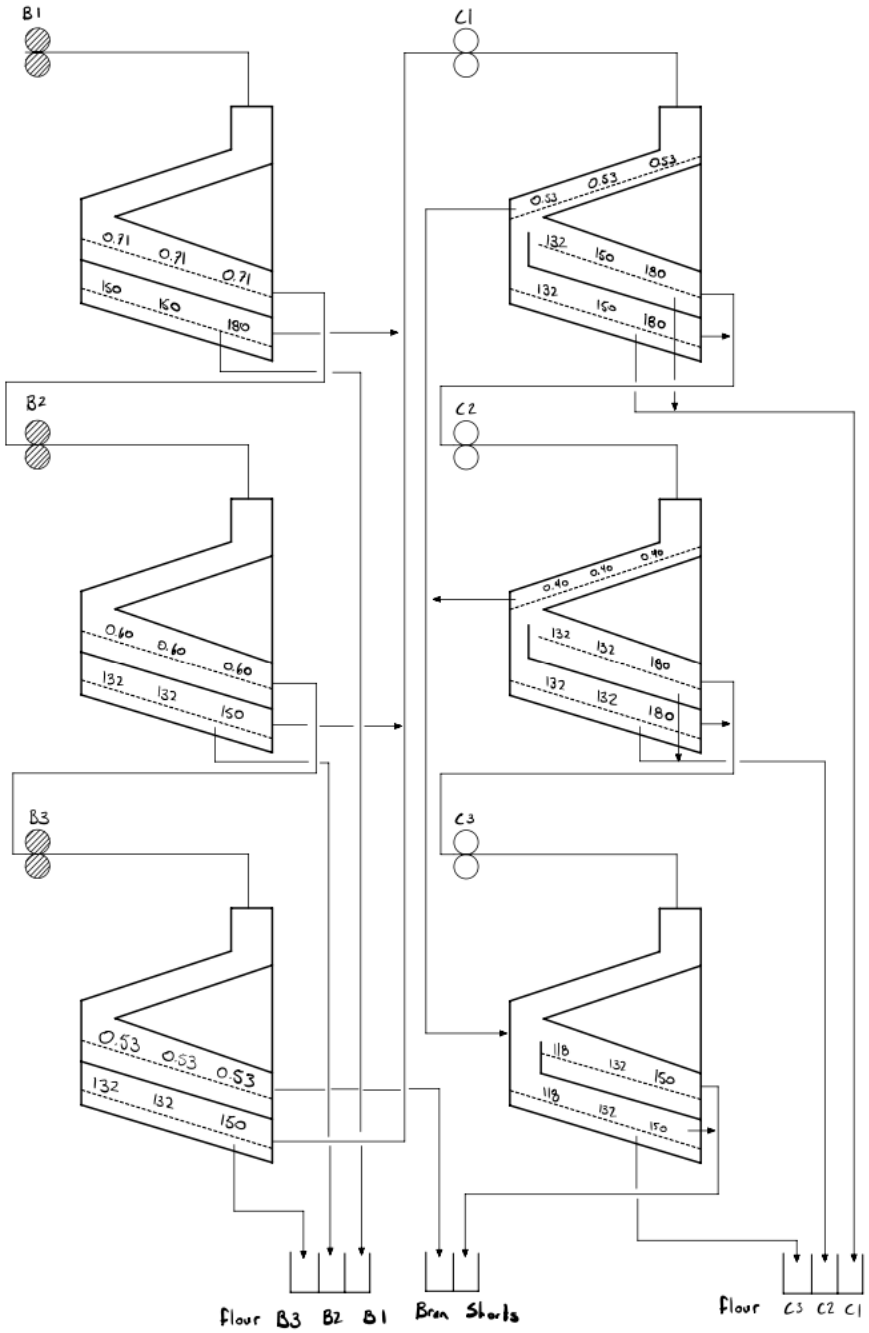


Figure 2.1. Precise milling parameters of Buhler MLU 250 mill.

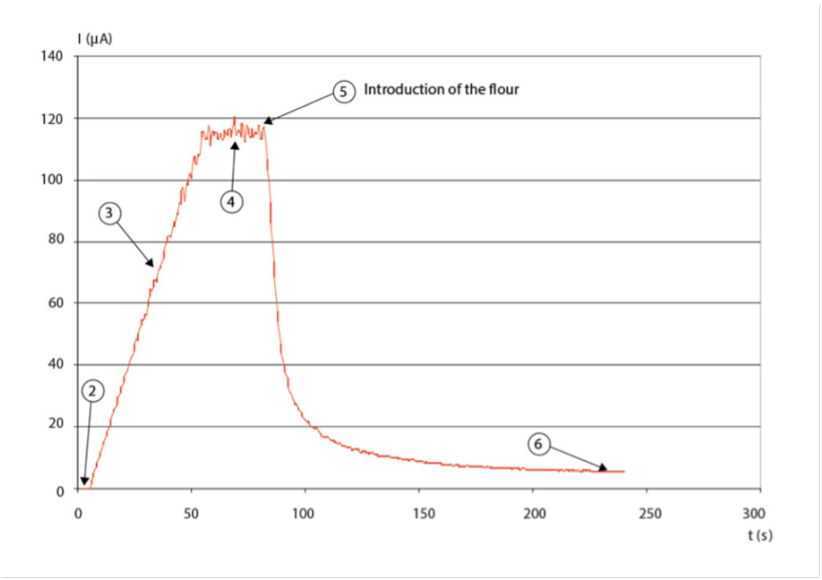
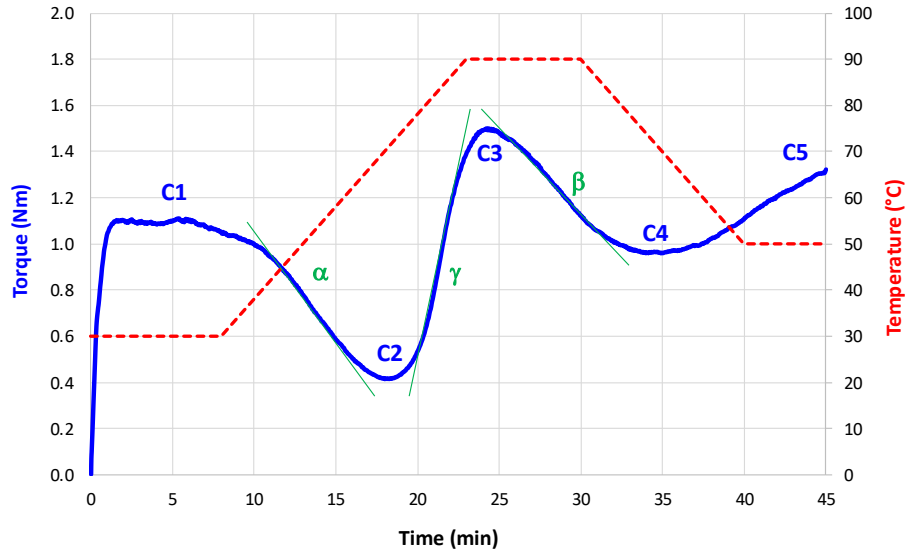


Figure 2.2. SDmatic measurement cycle curve.
(https://www.kpmanalytics.com/products/functional-rheological/sdmatic-2)



- - - - Temperature profile:

Stage-1: Constant temperature of 30°C (8 min)
Stage-2: Heating ramp to 90°C (4°C/min)
Stage-3: Constant temperature of 90°C (7 min)
Stage-4: Cooling ramp to 50°C (4°C/min)
Stage-5: Constant temperature of 50°C (5 min)

——Critical changes:

Initial-C1 Mixing and dough development, gluten network formation
C1-C2 Over-mixing, gluten weakening, starch swelling
C2-C3 Starch gelatinization
C3-C4 Starch rupture, breakdown
C4-C5 Starch re-gelling, amylose and amylopectin re-association

Specific MixoLab parameters:

Parameter	Description	Implication
C1 torque (Nm)	Maximum torque, C1	Dough development
C2 torque (Nm)	Minimum torque, C2	Protein reduction
C3 torque (Nm)	Maximum torque, C3	Starch gelatinization
C4 torque (Nm)	Minimum torque, C4	Amylase activity
C5 torque (Nm)	Maximum torque, C5	Starch gelling (Nm)
C1 time (min)	Time, C1	Development arriving time
C2 time (min)	Time, C2	Protein softening time
C3 time (min)	Time, C3	Starch gelatinization time
C4 time (min)	Time, C4	Amylase activity time
C5 time (min)	Time, C5	End of the test
C1 temperature (°C)	Temperature, C1	Mixing temperature (~30°C)
C2 temperature (°C)	Temperature, C2	Initial pasting temperature; onset of gelatinization
C3 temperature (°C)	Temperature, C3	Final pasting temperature
C4 temperature (°C)	Temperature, C4	Breakdown temperature
C5 temperature (°C)	Temperature, C5	Set-back temperature
Amplitude (Nm)	Band width	Dough strength
Stability (min)	Time C1 torque drops below 1.1	Dough stability
Alpha,α	Slope b/w the end of stability and C2	Protein destabilization and unfolding due to shear and temperature
Gamma, γ (-)	Slope b/w C2 and C3	Viscosity development due to starch swelling and gelatinization
Beta, β (-)	Slope b/w C3 and C4	Breakdown due to prolonged mixing

Figure 2.3. Typical mixing and pasting curve, and specific MixoLab parameters.
(adapted from Rosell et al. 2007)

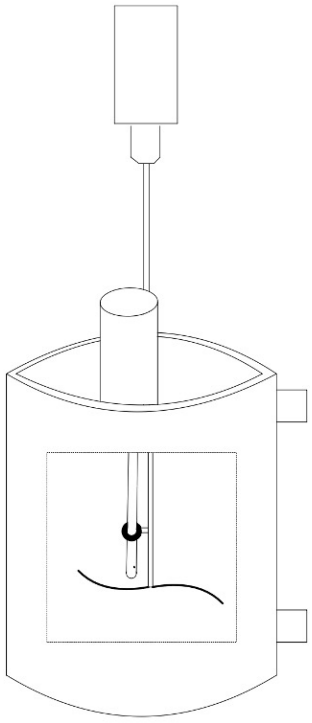


Figure 2.4. Fermentation tank and paddle schematic.

Chapter 3 - Results and Discussion

3.1. Mill Report

The milling process resulted in a total of 274.2 kg of total flour produced. The overall extraction rate after the milling was 63.65%, which is considered just below the industry standard of 70%. This is most likely because of less efficient sifting on an experimental scale, which in turn prevents optimal endosperm extraction. This also led to an increase in the substrate being diverted to shorts. Not enough of 3BK was produced to be fermented so it was combined with 2BK; this resulted in a total weight of 22.3 kg of sample. The totals and extraction percent of each fraction is found in Table 3.1. With the fractions prepared, they were then analyzed to determine differences in characteristics.

3.2. Composition Characterization Analysis

3.2.1. Moisture

Moisture test results are depicted in Table 3.2. Bran had the highest moisture of all the fractions, which is consistent with previous research examining water uptake and holding during kernel tempering of various fractions (Hsu, 1984). The shorts fraction followed the bran in total moisture and was not significantly different from 2M. It also shared similarities with 1M which in turn shared qualities with 2BK. The lowest moisture content belonged to 1BK, 3BK, and 3M, which all had similar values. For the later breaks and middlings, this could be caused by excess time spent being milled and sifted, leading to moisture loss. These are also the most internal fractions that could have the least amount of water uptake during tempering, causing a lower moisture content. 1BK was the first fraction removed from the high moisture bran; the low moisture of that fraction could make that separation easier. Compared to a study done by Czaja and colleagues in which various wheat cultivars were milled and tempered similarly to the wheat used for this experiment, the values collected fell into the expected range (Czaja et al., 2020).

3.2.2. Ash

Bran, as expected, had the highest level of ash content out of all the fractions (Table 3.2). Also as expected, the next highest level of ash was found in the shorts. However, the difference of ash content between the shorts and bran was far greater than that of the difference between the shorts and the other endosperm heavy fractions, which had much lower ash contents compared to the bran. The remaining fractions consisting of the breaks and the middlings had statistically

similar ash contents all far lower than the shorts and bran. The higher levels of ash content are typically associated with higher levels of minerals that have been known to influence the fermentation process (Hammes et al., 2005). The drastic variation between the fractions was expected. From the study conducted by Czaja, bran values for the various whole wheat flours ranged from 0.5-2.5% ash (Czaja et al., 2020). The values for the fractions in this experiment fell in that range, this was expected though as the separated bran should logically have a much higher ash than the whole wheat flour. Similarly, 1BK and 2M were slightly below the expected range, but when recombined with the other fractions, would fall within the range.

3.2.3. Mineral Analysis

Out of all the fractions analyzed, bran had the highest levels of every mineral (Table 3.3). This was expected based on the previously obtained ash content in which bran had the highest level of ash, where the highest mineral content was found (Hammes et al., 2005). Examining the levels of each individual mineral, a clearer understanding of the overall differences between the fractions can be observed. Bran by far has the highest level of phosphorus followed by the shorts. 3BK and 3M were statistically similar which was expected considering the higher level of bran contamination typical in mill streams that have been through more processing. The first two breaks and all the middlings all had the lowest levels of phosphorus and were not significantly different from one another. Potassium was the highest in the bran, followed by the shorts; the breaks and middlings all had statistically similar values and were far lower than the other fractions. Calcium had almost the same statistical output as potassium, with the grouping of bran and shorts versus the middlings and breaks being the same. The level of magnesium was highest in the bran but was statistically grouped with the shorts. 3M, similar to what was observed with the previous minerals, had the highest level of the more endosperm dominant fractions. These remaining fractions were all statistically grouped together. The sulfur concentrations were very similar to previous minerals. bran having the highest concentration followed by 3BK, the shorts, and 2BK. 1BK and the middlings could then be statistically grouped together. The levels of copper, iron, and zinc found in the fractions matched the results of the ash content. These three minerals are often associated with accelerating fermentation in sourdough (Hammes et al., 2005). The bran had the highest level, followed by the shorts, then the remaining samples high in endosperm. The final mineral measured was manganese. Bran had the highest level followed by the shorts, but there were more diverse results found in the higher endosperm samples. 3M and

3BK had similar levels of manganese, followed by 2M. The lowest levels belonged to 1BK, 2BK, and 1M. Overall bran had the highest levels of minerals while 1M had on average the lowest level of mineral content out of all the fractions. Similar to what was discussed in section 3.2.1., if the mineral content of the fractions were combined, they would have similar mineral contents to what was achieved during a 2010 study led by Hussain examining mineral content from various wheat genotypes (Hussain et al., 2010).

3.2.4. Starch Content

When examining the overall availability of starch from each fraction, patterns were discerned to help understand the impact fraction composition has on the fermentation. 1M had the highest starch content followed closely by 2M (Table 3.2). 1BK had a similar starch percent to 2M while not being similar to 1M. The final middlings flour, 3M, had the next highest percent which in turn was statistically similar to 2BK. These were then followed by 3BK which had a significantly lower starch content compared to the previous fraction. Seeing as these samples have the highest endosperm extraction rate during the milling process, their high starch values were as expected. The other fractions, which do not contain such high levels of endosperm and more bran, had much lower levels of starch. The bran had the lowest overall of all the fractions. The lower extraction rate obtained in this work explains the bran's starch content being slightly higher compared to what could be achieved in an industrial mill. The bran from the experimental mill had significantly more residual endosperm after milling was completed. The other sample with lower endosperm, the shorts, had a starch percent that was significantly different from all the fractions. However, the shorts fraction was much more closely related to the middlings and break flours as opposed to the bran while still being low. The starch content helps provide an idea of the available carbohydrates for fermentation (Kim and Kim, 2021).

3.2.5. Damaged Starch

There was a significant difference between the middlings and break flours according to the UCD value measuring the overall damaged starch of each fraction (Table 3.2). 3M had the highest UCD value of all the fractions, followed by 2M and then 1M. The middlings fractions seemed to have higher starch damage overall compared to the other fractions. Shorts was somewhat similar to 1M. The breaks were all similar to each other and had the lowest overall damaged starch. This was as expected because the more the wheat passes through the milling rolls, the starch granules are damaged more than those separated out during the breaks (Yu et al.,

2015). Bran had an iodine absorption of 72.20%, but a negative UCD value, which does not accurately represent the starch damage. To ensure the values collected were accurate and expected from other experimental mill fractions, they were compared to results collected by Wang and their team in 2020 in which they set out to quantify milling damaged starch content in wheat fractions (Wang et al., 2020). Wang and team obtained their damaged starch data using an enzymatic method, reported as a total starch percent. To compare this to the iodine absorption percent collected during this experiment, a graph created by McAllister and colleagues during a 2008 experiment comparing the two values from the same sample can be used to estimate the corresponding starch damage percent value from the iodine absorption percent collected for each fraction (McAllister et al., 2008). Using this method of comparison, it can be determined that the damaged starch values collected during this experiment fall within the expected range of data compared to other similar laboratory scale milling experiments.

3.3. Physical Characterization

3.3.1. Particle Size Analysis

During the course of milling various particle sizes should be produced as the particles have more mechanical energy added to the wheat. The average particle size determined using the microscopic particle imaging and characterization method confirmed that the fractions with the lowest average particle size endured the most processing, going through the rolls more times, thus subsequently enduring the most impact. The smallest was 3M followed by 3BK (Table 3.4). 3BK passes through the break rolls multiple times before being extracted as the “overs,” while the remaining flour, considered the “throughs” go on to be further milled using a smooth roll before being separated using sieves. 3M passes through the break rolls and the reduction rolls multiple times before being extracted as the “throughs.” As it had the most passes through the rolls the most times of the fractions, its small particle size is expected. 3BK, 1M, and 2M had similar average particle size. The particle size of the break fractions decreased as the “throughs” advanced in the milling process, 1BK being larger than 2BK, which was in turn larger than 3BK. The middlings followed the same pattern. The shorts were larger than all the middlings and break flours. This was expected as shorts are collected as an “over” product for all fractions and contains a higher amount of bran. This may explain why the overall extraction rate was lower than the industry standard, as more of the small particles that would normally be separated into the middlings were forced to go over the sieves and not through. Bran had the highest overall

particle size. This was due to most of the bran being collected directly after the initial break. It was not reduced down as much as the other fractions, resulting in the highest diameter.

To determine the circularity and elongation of the particles, the aspect ratio was utilized. The closer the ratio is to 1.0, the more circular it is, and the closer to 0.0 the more elongated it is. Circularity refers to how close a particle is to be a perfect circle, while elongation refers how uncircular or close to a flat line the particle is. Using the aspect ratio, it was determined that the middlings fractions had the most elongated particles. The most circular of the fractions was the bran. This could be due to the way the bran flakes after being removed from the endosperm. If it was not pulverized any further, it would be expected for it to keep its general circular shape. The shorts and 3M had a low level of circularity as they were more elongated. This was most likely due to the fractions being the most processed and going through the rolls more times compared to the other fractions. This causes irregularity in the circular shape, leading to higher elongation. This holds true for the other samples as well. Figure 3.1 shows the cumulative particle size distribution by volume for all flour fraction and byproducts. The bran and shorts, containing higher levels of bran particles, had higher percentage of large particles compared to the other fractions. Typically, according to a study done by Saad et al. (2011), smaller particles have a smoother surface and are mostly starch granules. In the current study, it was found that the middlings had smaller particle sizes and also higher starch values, supporting this claim. Saad also states that as the size increases, more irregularities in the particle shape occur. This increase in irregularity can in turn lead to an increased surface area which helps accelerate fermentation by providing more available substrate for the bacteria to consume. Medium particles are often associated to the bran and can be found in both the shorts and bran fraction. In the current study, it was found that the bran and shorts had varying particle sizes spanning from under 10 μm up to 135 μm. Being milled on an experimental mill explains this difference. It is also typical of the observed large particles to be more elongated. Large particles are commonly starch granules embedded in the protein matrix. The large particles are more elongated than the smaller fractions (Saad et al., 2011). While in this study the largest particles belonged to the shorts and bran, it can still be postulated that the protein and starch matrix causes larger particle sizes in those high endosperm samples.

3.4. Functional Characterization

3.4.1. Mixing and Dough Development Properties

A Chopin MixoLab constant water absorption protocol was utilized to determine the mixing and gelatinization properties of the fractions. This provided information on the interactions between the fraction's polymers in water, in addition to bringing insight into the enzymatic properties, shown in Table 3.5 and Figure 3.2. It was found that within the break flours and the middlings flours, the more later the removal of the fraction from the milling process, the higher the torque required to mix the dough. These more processed fractions typically have less starch and more gluten forming proteins and thus need more torque to properly form a dough. 1BK was not able to reach the target torque of 1.1 Nm and 2BK just barely achieved the target at the end of its mixing phase between the C1-CS data points. 3BK and 2M had very similar C1 and CS values, achieving a torque just above the target. 3M had the highest C1 and CS and had the lowest amount of gluten breakdown at C2. The other C2 values for each fraction had the same statistical grouping as both C1 and C2. 3M, 1BK, and 2BK can all be grouped together for having the highest C3 torque values, meaning they had the most starch gelatinization. 2M, 3BK, and 3M had similar C3 values and can also be grouped together. To determine starch rupture and subsequent breakdown the C4 can be utilized. 3M had the lowest required torque, but overall, the breaks flours had more enzymatic breakdown according to the difference between C3 and C4. The remaining fractions had higher C4 values and were all statistically related to each other. The re-gelling and amylose amylopectin re-association is depicted by the C5. 2BK had the highest overall torque at C5, meaning it has the highest level of starch retrogradation of all the fractions. It was similar to 3BK and 1BK and in turn those fractions were similar to 1M and 2M. 3M had the lowest overall torque value at C5.

3.4.2. Amylase Activity

Both the α- and β-amylase values were measured to determine the enzyme activity of the fractions to be fermented. For the α-amylase activity, none of the fractions were significantly different from one another (Table 3.6), but slight differences can be observed. It was found that 1BK had the highest overall α-amylase activity, while 3BK the shorts and the bran had the lowest levels. Seeing as there was no significant difference α-amylase in each fraction, the differences in torque observed in the MixoLab values C2, C3, C4, and C5 cannot be traced back to the enzymatic activity alone.

Unlike the α-amylase, the β-amylase values of each fraction were significantly different from each other. 3BK had the highest overall β-amylase content by far, followed by 2BK. As 2BK and 3BK values were combined into a single fraction for the fermentation, that fraction still has by far the highest level of amylase activity. The lowest values belonged to the shorts and 3M. The data collected reflects similar patterns seen in a study examining experimental mill stream properties of wheat done by Nkurikiye and others in 2024. Typically, break flours have higher amylase activity than the middlings (Nkurikiye et al., 2024). This result also corroborates what was seen in the MixoLab results as the breaks had the highest enzyme activity. While there was some significant difference between these values, according to the MixoLab data collected, they do not impact the viscosity and therefore would not have an impact on the overall torque (Chen et al., 2021).

3.5. Fermentation

The data points collected during the acidification are displayed as pH versus time curves showing the three replicates and an average acidification curve. Figures 3.3, 3.7 and 3.11 are the acidification curves presented for each flour fraction and by product using three LAB strains, La1, La2 and La3, respectively. Similarly, the acidification curves for two composite flours (straight grade and whole wheat) are presented in Figures 3.4, 3.8 and 3.12. Figures 3.5a, 3.9a and 3.13a depict the moving average of the pH for La1, La2 and La3, respectively. The next set of graphs, Figures 3.5b, 3.9b and 3.13b, portray the progressive increase in TTA during sourdough fermentation of La1, La2 and La3, respectively. Figures 3.6, 3.10 and 3.14 illustrate the rate of change in pH over time (Vmax) during fermentation of the same LAB strains.

Figures 3.15 and 3.16 show the acidification curve of each strain compared to each other by fraction type, showing the exact difference strain selection has on the acidification. Tables 3.7 and 3.8 depict the fermentation characteristic values collected from the acidification data. All fermentations of the various combinations of substrate and strain followed a similar path of acidification. In the early hours, the pH lagged and even increased slightly before beginning its decline. This lag phase was followed by a steep acidification. The time at which this steep acidification occurred was dependent on the strain, but in general it occurred between the fifth and fifteenth hour of fermentation. After the steep acidification, the rate of acidification decreased, and the pH began to stabilize. At this point and for the rest of the fermentation, the rate of acidification was near zero and was even positive at times as some fractions saw a pH

increase after 24-hours of fermentation. This is expected for many LAB strains as their colony concentration increases until anywhere between hour fifteen and twenty-five before conditions become too acidic to sustain their population (Kushkevych et al., 2019). The acidification curves produced are inverse to an expected LAB growth curve, showing the impact that substrate availability, acid production, and buffering effect have on the fermentation.

3.5.1. Fermentation Kinetics and Acidification: La1

Break Flours:

Examining the acidification charts (Figure 3.3 and 3.4) for each fraction, the first seven hours of the 1BK fermentation done by La1, the replicates were all similar in their acidification curves, but after that point, the third replicate began to acidify faster than the others. Around hour twenty they all flattened out and continued to ferment at a very similar rate. On average, 1BK was able to achieve a pH of 4.0 around thirteen and a half hours. It had a V_{max} of -0.0138 occurring around eight hours. The replicates of 2/3BK were far more similar as opposed to the other high endosperm fractions. All replicates had a very smooth acidification curve and achieved a pH of 4.0 on average around fifteen hours. It had an average V_{max} of -0.0117, the same as 1M; this occurred just after the ninth hour of fermentation. The acidification increased around the fourth hour and continued until hour twelve, when the curve began to flatten out.

Middlings:

1M had a lag in acidification over the first four hours before the pH began to decrease. The pH declined at a steady rate for about six hours before a flattening of the curve occurred and the most intense decrease in pH occurred starting around hour eleven. A pH of 4.0 was achieved around the 18^{th} hour when the acidification rate began to plateau for the remaining time. The T_{max} of 1M occurred at 9.02 hours and the V_{max} was -0.0117. 2M followed a similar acidification pattern as 1M but had a more consistent decrease in pH from hour five to hour twenty. Its T_{max} occurred at hour 10.93 and it had a V_{max} of -0.0130. The third replicate of the fermentation had a far more drastic pH decrease starting around hour eight. Neither of the first two replicates had this decrease, in fact they had almost identical acidification curves. The various replicates achieved a pH of 4.0 between fifteen and twenty hours. Similar to 2M fraction, 3M fraction had fairly drastic differences between the three replicates. Its overall acidification could also be described as irregular. 3M had the highest V_{max} of all the fractions fermented using La1. The

value was -0.211 and had a T_{max} of 9.29 hours. Two of the 3M replicates were able to achieve a pH of 4.0 around thirteen hours, while the other replicate took over eighteen hours.

Shorts and Bran:

The replicates of the shorts had very similar acidification curves and also resulted in the most efficient acidification to a pH of 4.0. It took just over ten hours to reach pH of 4.0 and achieved a V_{max} of -0.0162 at 7.64 hours. The acidification continued after a pH of 4.0 was achieved which did not occur in any of the other fractions, despite the compositionally similar whole wheat flour. The bran appeared to have a more severe acidification, but around hour ten the bacteria seemed to run out of substrate to metabolize and the acidification became stunted before it was able to achieve a pH of 4.0. Eventually during the fermentation, the pH dropped below 4.0 but it was well after the thirty-hour mark for all the replicates. It achieved a V_{max} of -0.0141 in just 6.89 hours, the fastest of all the fractions fermented by La1.

Composite Flours:

The straight grade followed a similar acidification trend as the break fractions but was not as efficient in achieving a pH of 4.0. It took almost seventeen hours to do so, which falls between the times it took for the middlings and the breaks to do the same. The replicates of this test did not deviate much, creating a smooth and very understandable average acidification curve. Similar to the time to achieve a pH of 4.0, its V_{max} fell between the values achieved by the middlings and break flours, at -0.0124. However, this occurred much later in the fermentation compared to the other high endosperm fractions. It seemed to keep a high acidification rate for longer than the other fractions, leading to a more gradual and consistent curve. Whole wheat was the second most effective acidifier and performed in a similar fashion as the shorts. The bulk of the acidification occurred from around hour four until hour fourteen when the curve flattened out for the remainder of the fermentation. All replicates were very similar and without much deviation. It took just under eleven hours to achieve a pH of 4.0. Whole wheat also had one of the highest V_{max} values of all the La1 fermented fractions, at -0.0153. However, this value occurred after eight hours, making it more similar to the breaks instead of the shorts.

Overall Findings:

Overall, it was found that the V_{max} and T_{max} values of the fractions fermented using La1 starter was not significantly different. There were differences in the time to pH of 4.0 though. Shorts was the most effective, followed by the whole wheat. The two breaks, 3M, and the

straight grade flour were all statistically similar to each other, but also had some similarities to the previous two fractions that acidified the fastest. They also shared similarities with the slower fermenting fractions including 2M and 1M. Bran was not similar to any of the other fractions, seeing as it did not achieve a pH of 4.0 until over twenty hours after the next slowest fraction.

Figure 3.6 depicts the rate of acidification over time. The fractions with higher levels of endosperm acidified at a steady rate for the initial six hours, and then middlings and straight grade had a drop in acidification around the eight-hour mark. These fractions then hit their peak acidification around hour ten. The breaks increased until their peak around the eighth hour. The shorts, whole wheat and the bran all peaked after six hours and had significantly higher acidification rates compared to those higher endosperm fractions.

Clear groupings between the middlings, the breaks, and some of the higher bran fractions can be seen in the moving pH average (Figure 3.5a). The outlier of the groups was the bran, having its acidification stifled early into the fermentation as there was not enough available substrate for the bacteria to metabolize. The straight grade flour falls between the breaks and middlings groups, which was expected based on its composition. It is expected the whole wheat flour to be closer to the break flours than the shorts because it had a higher bran content due to the inclusion of the shorts and bran in the blend, as well as plenty of endosperm available to be metabolized from the other fractions. From the average acidification it seems that La1 performs best when metabolizing fractions high in minerals, courtesy of the bran, to aid in the metabolism.

A pattern emerged from the data collected when measuring the total titratable acidity for each fraction sampled during the fermentation, seen in Figure 3.5b. 1M had the lowest acidity at any given point during the fermentation, followed by 2M. The straight grade then fell between these and then another group consisting of the remaining high endosperm samples. The next highest TTA values after the straight grade belonged to 1BK. The fraction often intersected the straight grade flour but ended the fermentation with a higher value. The next two fractions had very similar results only deviating from each other at the eighteenth hour. These fractions being 2/3BK and 3M, these fractions' other fermentation values, including the V_{max}, T_{max}, and time to pH of 4.0 were very similar. Following the high endosperm samples, was a five and 8 milliliter gap between the fractions high in bran. The lowest of that group was the bran. It started very high but was quickly overtaken by the shorts and whole wheat flour when examining the total

acidity measured. The shorts and the whole wheat flour had very similar TTA values at each sampled point. They were also the highest, or one of the highest, values at each sampling period.

3.5.2. Fermentation Kinetics and Acidification: La2

There was evident deviation in some of the fractions fermented using La1, however this deviation was not seen in any of the fractions fermented using La2. Seeing as the two strains have different metabolic capabilities, this could play a role in these differences (Calvert et al., 2021). Within the strain, all fractions had very similar acidification curves. Each had varying starting points and lag phases, but after about eight hours they all came together and followed a very similar path for the remaining fermentation. The one outlier was the bran, having a similar problem as seen in the La1 fermentation as it just ran out of substrate to metabolize. The La2 fermented bran curve was still far more similar to the La2 curves of the other fractions than those fermented using La1. The acidification curves for La2 are depicted in Figure 3.7 and 3.8.

Break Flours:

Similar to what was seen in the fractions fermented with La1, there was an initial lag face in fermentation for the first few hours before a drastic drop in pH occurred. For 1BK, this occurred after about three and a half hours and lasted five hours before the acidification curve began to flatten. It achieved a pH of 4.0 after just nine hours, around the same time the flattening begun. The V_{max} occurred at six and a half hours; the value was -0.0260. This is a higher acidification rate than previously observed from what was seen in the La1 results. The 2/3BK acidification curve was very similar to that of 1BK. The V_{max} was -0.0266 which was also very similar to the other break fraction, but it occurred half an hour earlier. It also took longer to achieve a pH of 4.0, at around nine and half hours.

Middlings:

1M was the most effective acidifier for La2 as it took just over eight hours to achieve a pH of 4.0. It had one of the lowest V_{max} values though, at -0.0219, which was only less steep than the bran. The V_{max} occurred just after six hours, making it one of the earliest T_{max} values for this strain. 2M then produced very similar results as 3M. 2M had a V_{max} of -0.0261, while 3M was slightly lower at -0.0256. These values both occurred towards the back half of the sixth hour. On average 2M achieved a pH of 4.0 slightly faster than 3M, but it was by less than half an hour.

Shorts and Bran:

The shorts was able to achieve a V_{max} of -0.0236 after seven hours of fermentation. It then achieved a pH of 4.0 just after nine hours. Unlike what was seen in the results from La1, the shorts and whole wheat had fairly different fermentations, in both the acidification curves and other fermentation values collected. Bran had the lowest V_{max} of all the fractions at -0.0193, as expected. Compared with the fractions fermented with La1, it has the second highest V_{max}. Examining the other values collected, the T_{max} occurred around seven hours, the latest of all the fractions fermented using La2. Continuing the pattern in results, the bran took the longest amount of time to achieve a pH of 4.0, over twelve hours. This was by far the slowest value of the La2 fermented fractions but was significantly faster than the results seen from the La1 fermented bran.

Composite Flours:

Straight grade had one of the highest V_{max} of all the fractions, while also having one of the earliest T_{max} values. It achieved its V_{max} of -0.0284 just six hours, similar to what was seen from 1M. The whole wheat had a similar acidification as the shorts but achieved a pH of 4.0 just before the nine-hour mark. This was the second fastest time to acidify, behind only 1M. It had a V_{max} -0.0208 and was able to achieve that at the seventh hour.

Overall Findings:

Overall, it took all the fractions fermented with La2 between eight and ten hours to achieve a pH of 4.0, except for the bran, which took over twelve hours. The fastest was 1M taking only 8.28 hours. The bran also had the lowest $V_{max.}$ Statistically, the straight grade had the highest V_{max} and was followed by the remaining fractions that all had similar results. All the of samples had related T_{max} values, 2M having the earliest at 6.13 hours and bran having the latest at 7.20 hours. As well as being the first fraction to achieve a pH of 4.0, 1M had the lowest average pH achieved during the fermentation at 3.3861. This was similar to the straight grade fraction, the remaining middlings, 1BK, and the shorts; the bran had the highest minimum pH.

Unlike the fractions fermented using La1, there was no clear grouping of acidification rates within La2, seen in Figure 3.10. Peaks can be discerned from the figure showing similar results to what was seen in the overall acidification curves. These peaks were not as clear as seen in the La1 graph. 2M and 2/3BK had the highest peaks around the six-hour mark, followed by

shorts having its peak shortly after. The lowest peak belonged to bran, showing a similar curve to that of the bran fermented with La1.

The average acidification graph, Figure 3.9a, shows just how similar these fractions perform to each other. Each of the fractions had different starting points followed by a lag phase that lasted under three hours before a drastic acidification began for all the fractions. This lasted until the ninth hour when the curves began to flatten out. The fractions had very similar rates of acidification from the ninth hour till around twenty-four hours into the fermentation. After that point, 1M, 2M, and 1BK continued to decrease in their pH until the forty-eight-hour mark at the end of the fermentation. These were the fractions with the most available substrate allowing them to continue their acidification after the other fractions that contained higher amounts of bran leveled out. The bran fraction again was an outlier compared to the other fractions similar to what was seen in La1. Unlike La1, the bran achieved a pH of 4.0 but still did not acidify to the same extreme as the other fractions.

The total titratable acidity results of La2, shown in Figure 3.9b, were very similar to the samples fermented using La1. The biggest difference was the acidity produced by the bran. The highest acidity at any sampling period belonged to the bran. The next highest was the shorts and the whole wheat, similar to what was seen from La1. This was followed by the breaks, the straight grade flour, and the middlings fractions. 3M started lower than the breaks but increased as time went on. The results support the concept that higher total acid production was related to the amount of available minerals in each fraction; 3M has more bran particles than the other middlings.

3.5.3. Fermentation Kinetics and Acidification: La3

Break Flours:

The Fermentation curves for La3 are depicted in Figure 3.11 and 3.12. The first break fraction fermented using La3 had a very similar lag phase to that of La1. At around five hours, clear differences between the strains became evident as La3 acidified in a much more similar fashion to La2. It was able to achieve a V_{max} of -0.0249, which occurred slightly before seven hours into the fermentation. After about nine hours the acidification began to flatten out but continued to slightly decrease in pH for the remaining hours of fermentation. 2/3BK had fairly drastic increases and decreases in pH during the initial ten hours of fermentation, after a short lag phase of three hours. It had a high V_{max} of -0.0365 which occurred before the six-hour mark of

fermentation. Two of the replicates had very similar acidification curves that saw continuing acidification throughout the fermentation, but the other replicate saw an aggressive plateau after about twenty-four hours. This difference caused the averaged curve to not have as steep of a decline over the back half of the fermentation. Of all the replicates fermented using La3, 2/3BK portrayed the largest variation between its replicates.

Middlings:

1M had a similar lag phase to what was seen in the break flours, followed by an acidification curve similar to what was seen in the fractions fermented using La2. It's V_{max} was -0.0263 which occurred at around five and half hours into the fermentation. The V_{max} was statistically similar to 1BK. This fraction also had a similar slow and steady acidification over the entire fermentation that was seen with other La3 and La1 fermented fractions. Overall, 2M was very similar to 1M fermented with La3. Its V_{max} was -0.0261 and occurred around the five and half hour mark as well. This fraction's acidification began to flatten out before seven hours but continued to acidify during the remaining hours of fermentation. 3M had a more drastic acidification compared to the other middlings fractions, reflected in its V_{max} of -0.0308, which occurred close to six hours into the fermentation. The replicates of this fraction acidified very quickly during the first ten hours before they began to plateau approximately 24 hours after the fermentation began.

Shorts and Bran:

The shorts fraction had similar curves as seen in the other two strains. All replicates had very smooth curves with very similar pH changes between hours three and nine where a majority of the acidification occurred. It had a V_{max} of -0.0201 which occurred almost seven hours into the fermentation. Bran was the most unique fraction that was fermented using La3. Similar to what was seen with the bran fermented using La1, the fraction had difficulty achieving a pH of 4.0. Only one of its replicates was able to acidify to that low of a pH, and the average curve did not even drop below the 4.0 point. Bran had a V_{max} of -0.0117 which occurred around the eighth hour of the fermentation.

Composite Flours:

The straight grade had a lag phase that lasted four hours before the major pH drop occurred. This was very similar to what was seen in the straight grade fraction that was fermented using La2. The fraction had a V_{max} of -0.0239 that occurred around five and a half

hours. The curves do not reflect what would be expected from its composition, being a blend of the breaks and middlings flour as the V_{max} was more statistically similar to the fractions containing higher levels of bran. Whole wheat had fairly different replicates, similar to what was seen in the whole wheat fraction that was fermented using La1. The fraction had a V_{max} of -0.0179 that occurred at just after seven hours.

Overall Findings:

As seen within the previous heterofermentative, La1, stark differences between the fractions were observed during fermentation with La3. The time to achieve a pH of 4.0 varied with the most efficient fraction being the shorts, taking 9.51 hours. As two of the replicates of the bran were not able to achieve the 4.0 value, it was by far the least effective to achieve high acidification. Interestingly, 2/3BK had the highest average V_{max} out of all the fractions. This same fraction fermented using La1 had one of the lowest V_{max} values, while when fermented using La2 had one of the highest. The 2/3BK V_{max} was similar to the remaining break and middlings fractions. The next highest V_{max} belonged to straight grade, then the shorts, and finally the bran. The T_{max} for the fractions were achieved between five and eight hours. The earliest belonged to 2M, however there was no statistical difference between any of the T_{max} values including the bran which had a T_{max} of almost eight hours. Shorts not only achieved a pH of 4.0 quickest, but it also had the lowest minimum pH recorded during the fermentation of any of the fractions. 2/3BK, 2M, 3M, the straight grade, and whole wheat flours all had similar minimum pH values to the shorts. 1BK, 1M, and the bran did not have such low minimum pH values, but 1BK and 1M were at least able to acidify below the 4.0 point. The TTA data collected shows a clear separation of total acidity depending on the composition of the fractions. All of the high endosperm fractions had statistically similar maximum TTA values. The shorts had the highest acid produced, followed by the whole wheat flour, the bran, then all the high endosperm fractions.

The high endosperm samples had very consistent acidification rates, seen in Figure 3.14, the most active being 2/3BK. This fraction was closely followed by the three middlings flours, then the straight grade, and finally 1BK. The high bran fractions did not fall into any discernable groupings. Shorts peaked next after the high endosperm fractions; it was a much more consistent curve. Since it had the lowest overall acidification, this makes sense as there was no spiking during the fermentation, instead it consistently decreased. The next highest peak on the

acidification rate graph belonged to the whole wheat flour. While not as high as the shorts, it was fairly similar, which supports the pH data. The bran fraction had the shortest but widest curve. It continued to acidify at a higher rate than the other fractions longer into the fermentation, but still did not reach the low pH values as the others did.

When examining the moving average graph, Figure 3.13a, as with the other strains, the bran appeared as the outlier of the fractions, not acidifying as much as the other fractions. The whole wheat started at the second highest pH behind the bran, acidified at a similar rate as the middlings flours, but then continued to acidify beyond where the pH the other endosperm heavy samples plateaued around. Shorts had the third highest starting pH but had the most drastic drop in acidity, as well as the lowest overall pH achieved. It was the only fraction to reach a pH below 3.5, which was easily accomplished using La2. Like the previous strains, the middlings, breaks, and straight grade had similar acidification curves. They had comparable lag phases, major acidification periods, and leveling off periods. 1BK had the least amount of acidification out of those high endosperm samples, followed by 1M. The two remaining middlings flours, 2/3BK and the straight grade flour can be grouped together, having very similar acidification patterns. The groupings of the fractions were akin to what was seen in those fractions fermented using La1, but the curves themselves appear to have more in common with the fractions fermented using La2.

The shorts had the highest total acidity production out of all the fractions, seen in Figure 3.13b, as well as having the lowest pH. The shorts by far had the highest values at every stage of the sampling. The next highest total acidity levels belonged to the remaining high bran containing samples, the whole wheat, and the bran. The high endosperm samples were grouped together with a significantly lower level of acidity compared to the previous fractions. Out of that group, 2/3BK had the highest level of total acidity. It was followed by 3M and the straight grade flour. The last three fractions had very similar acid production; in that group the highest amount of acid produced belonged to 2M, then 1BK, and finally 1M. This was similar to what was observed in the other strains of LAB used to ferment.

3.5.4. Comparisons between fermentation parameters of La1, La2, and La3

Comparing the various fermentation parameters from the La1 and La2 fermentations, clear differences emerge. La2 was able to achieve a pH of 4.0 far more rapidly regardless of which fraction was being fermented. This held true for the V_{max} and T_{max} values as well, they

were steeper for La2 in the case of each fraction. La2 produced more acid overall compared to La1, regardless of the fraction being utilized.

The groupings of curves were very similar between these two heterofermentative strains of lactic acid producing bacteria (La1 and La3). The fractions fermented using La3 achieved a T_{max} earlier than those fermented using La1. This is most likely because of the inability for La3 to achieve as low of a pH as La1. La3 also had steeper V_{max} values, which is indicative of an effective fermentation. However, La1 had higher max acidity and lower minimum pH than La3. The fractions of both strains took similar amounts of time to achieve a pH of 4.0, but on average, La3 took slightly longer to achieve that value.

The differences in results between La2 and La3 show the distinctions between metabolic properties of the strains. La2 had more severely negative V_{max} values and was able to achieve those values far quicker than La3. It also had lower minimum pH by far, and higher maximum total acidity. Additionally, it was able to achieve a pH of 4.0 much faster than La3. When comparing the moving average graphs for the two bacteria, they have similar acidification after the initial lag, but the La2 acidification was far more aggressive. La2 and La3 had very similar T_{max} values. Overall fractions fermented using La3 were able to achieve the V_{max} earlier than La2, except for 1M and the bran. They also had nearly identical T_{max} values for whole wheat flour. With the fermentation of all fractions using different LAB strains complete, the fermented liquid sourdoughs were characterized.

3.6. Sourdough Characteristics

3.6.1. Metabolite Quantification

While many metabolites were quantified, most were below the detection limit, therefore this discussion will focus on the disaccharides, lactic acid, and acetic acid. These metabolites are extremely useful in identifying differences between the fermentations. The disaccharide level shows the amount of available substrate for the bacteria to ferment and metabolize. It is assumed that maltose molecules make up the majority of the disaccharide content. The other metabolite commonly analyzed is ethanol. However, almost no ethanol was recorded from La2, but some fractions fermented with La1 and La1 did produce very small amount of ethanol towards the end of the 48-hour fermentation. Due to the lack of data, ethanol was omitted from this study.

As seen in Figure 3.17, middlings flours fermented with La1 had the highest levels of disaccharides throughout the 48-hour fermentation. It was observed that the samples collected at

hour 8 had an initial increase in available disaccharide level. This was due to the cleaving of maltose molecules from the larger starch granules in each fraction. After the hour 8 samples, the middlings flours slightly decreased in disaccharide level until the end of the 48-hour fermentation. The straight grade flour followed a similar pattern as the middlings flours, an initial uptick followed by a slight decline by the end of the fermentation. Interestingly, the final level of disaccharides was higher than the starting value, meaning there was still substrate available for fermentation, but the conditions were likely too acidic to sustain optimum metabolism (Minervini et al., 2014). The breaks followed a similar pattern as the aforementioned fractions, but at much lower concentration. The shorts and whole wheat flour had an initial spike in available disaccharides at hour 8 before decreasing at each sampling time until it ran out by hour 30. Bran had very low levels of detectable disaccharides throughout the first few hours, until at hour 18 it was fully used up. This tracks with the results seen in the acidification curves.

The disaccharide values in fractions fermented with La2 were very similar to fractions fermented with La1. Following the same pattern of an initial increase from hour 0 to hour 8 before a gradual decrease and leveling out until hour 48. At that point at the end of the fermentation, a unique result was seen as all the fractions dropped below 10000 ppm, indicating the disaccharides being almost fully utilized.

Overall, as seen in Figure 3.18, the lactic acid levels seemed to increase more in the fractions containing higher levels of bran compared to those with more endosperm. The middlings flours fermented with La1 had lower levels compared to those fermented with La2. The most unique fraction result within the two trains belonged to the bran. La2 produced a far higher concentration of lactic acid until hour 48 when the two bacteria ended up having similar final acid content. The La2 fermented fractions produced lactic acid at a more accelerated rate during the early stages of the fermentation. Once the fermentable substrate became less available the amount of lactic acid produced by the La1 bacteria increased to a similar level seen being produced by La2. This was as expected, being as the La2 bacteria only produces lactic acid during a majority of its fermentation regardless of the substrate being fermented.

As expected, La1 produced acetic acid alongside lactic acid. The acetic acid values, seen in Figure 3.19, were far lower than the lactic acid values. Fermentations inoculated with the homofermentative La2 did result in minimal acetic acid production. The fractions containing bran produced some acetic as well as the straight grade flour blend. Within La1, those higher

bran fractions resulted in more acetic acid than their more endosperm heavy counterparts. While this could impact the end product aromas and overall flavor of baked goods produced using the various fractions, it does not seem to have a significant impact on the overall acidification or amount of acid produced during fermentation.

Very similar results were achieved for the fermentations using La3 as those fermented using La1 and La2. Being as La3 is a heterofermentative bacteria, it is expected to produce metabolites in a more similar manner to La1 than La2. This is especially true for acetic acid production. Like La1, bran as a substrate resulted in the most acetic acid production compared to the other fractions. A unique outcome was seen in the La3 results as 2/3BK resulted in a much higher level of acetic acid compared to the other strains. At the 48th hour, that same fraction had the second highest overall production, only behind the bran. This final value was followed closely by the whole wheat, 2M, and the straight grade flour. This was a very different result from what was seen from the previous two strains as no samples high in endosperm had acetic acid levels that high. The shorts and 1BK had similar levels to each other following the straight grade and 2M. The remaining fractions, 1M and 3M, had half the concentration of acetic acid that the shorts and 1BK had. Overall, it was lower than what was observed in the other two strains.

The amount of lactic acid produced by La3 was very similar to what was produced by the fractions fermented using La1. The high endosperm fractions had lower acid production from their counterparts fermented using La1, but the higher bran samples had very similar values throughout the fermentation of all strains. Overall, La3 had a very gradual production of lactic acid compared to the others. It appeared the fractions with higher amount of bran contamination had higher levels of acid production compared to those fractions that had higher levels of pure endosperm. For the samples high in bran, they followed the same pattern as seen with the other strains, shorts having the highest level, then the whole wheat, and then the bran. There was also a sizable gap between the high bran and the high endosperm samples, similar to what was seen with the other strains.

The levels of disaccharides examined within La3 had similarities to both La1 and La2 strains. As more maltose was cleaved from the starch in each fraction, the disaccharide values increased and held steady during the first 30 hours of fermentation. Unlike La2, at the 48-hour mark of fermentation, there were still disaccharides left to be fermented. This was similar to

what can be seen with La1, but there was about 5000 ppm less of disaccharides in La3. The final values of La3 were intermediate to the other two strains. The differences shown provide insight into how each fraction can be most effectively utilized for fermentation.

3.6.2. Fermentation Quotient

The molar ratio of lactic acid to acetic acid was calculated for the fractions fermented with the different bacteria over the 48-hour fermentation (Table 3.9). The homofermentative bacteria, as expected, did not produce much acetic acid. Within that strain, the fractions with higher levels of bran resulted in some acetic production, but it occurred towards the end of the fermentation. On the other hand, the heterofermentative bacteria produced acetic acid by the 8th hour of fermentation. The same held true as before, the samples higher in bran had the most acetic acid production compared to those fractions with higher endosperm content. In Table 3.19 the ratio between lactic and acetic acid production is shown. In those fractions with acetic acid production, it was typical to see a higher lactic ratio halfway through the fermentation that would decrease towards the end of the fermentation. This is because more lactic acid was produced early into the fermentation while acetic acid was produced towards the end of the fermentation. Shifts in rate and type of metabolite are common responses to acid production itself and substrate availability (Minervini et al., 2014; Brandt et al., 2004).

3.6.3. Color

The color values are depicted in Table 3.10. Within the color measurement for each strain, there were slight differences showing the impact different strains can have on the fractions. For all but two of the fractions La1 produced a brighter sourdough than La2 according to the L*. While the differences were often slight, the straight grade fraction was much brighter when fermented by La1 than La2. All of the values collected from both strains had more red and yellow characteristics measured by *a*, and *b. Similar to what was found for lightness, La1 resulted in more fractions that had more red and yellow hues than La2. Fractions 1BK, 3M, the bran, and the straight grade that were fermented using La1 had higher L*a*b values than La2.

3.7. Multivariate Analysis of Fermentation Characteristics

3.7.1. Principal component Analysis (PCA)

To gain an understanding of how the end characteristics are correlated to the compositional characteristics of the various fractions principal component analysis, or PCA, was utilized. This is a dimension reduction technique that utilizes vectors to visually depict the complex relationships observed in the results. The PCA reduces the number of dimensions into two components to better analyze those dimensions.

Figure 3.20 is a scatterplot divided into four quadrants to help distinguish correlations and groupings from the data collected. Here, more relationships can be established such as the time to pH 4.0 for La1 was correlated to the circularity of the particles in a fraction. This grouping also supports the previous evidence found about the impacts the minerals have on La2 and La3's time to reach 4.0, meaning as mineral content increases, the time required varies in the same way. Starch can be found in its own quadrant, displaying a lesser correlation to the fermentation parameters. Calcium was a unique mineral as it seems to have the most minimal impact on the fermentation parameters. Parameters that do not share strong relationships to others do not appear on the scatterplot.

3.7.2. Correlations

Pairwise correlations were computed between fermentation parameters and fraction composition to elucidate driving factors. The results are depicted in Tables 3.11, 3.12 and 3.13. La1 maximum TTA was most highly correlated to the maximum diameter particle size. As the largest particle belonged to the bran, this shows the importance mineral content has on the acid production for La1. This is supported by the significant correlation the maximum TTA value also has with magnesium, copper, zinc, and the overall ash content. Starch content and α-amylase were negatively correlated with maximum TTA. This is explained by the fractions with the higher amounts of starch and α-amylase activity were those with lower bran content. Thus, bran components are stronger drivers of acid production as compared to starch availability and enzyme activity. Similar to the maximum TTA of La1, the availability of minerals had the most impact on the minimum pH achieved by each strain. Potassium and phosphorus had the strongest positive correlation to the minimum pH. This was also true for the La1 T_{max} value, as it occurred later in the fermentation than observed in the other two strains. The V_{max} of La1 was not highly correlated to any fraction characteristic. It was somewhat positively correlated to the average particle size,

which again can be explained by bran having a high particle size and the strain's preference for fractions high in bran.

The maximum TTA achieved by La2, similar to what was seen with the previous strain, was highly correlated with all minerals, except for calcium. It was also correlated with the maximum particle size. The minimum pH was again correlated to the minerals but not calcium. Sulfur and iron were the most impactful of the minerals for La2 acidification. The time to pH 4.0 was positively correlated with all the minerals except magnesium and calcium. This is in contrast to La1, for which magnesium had high correlations to acidification. The T_{max} was strongly positively correlated to the level of magnesium. This was expected seeing the weak correlation it had with the time to pH 4.0, it causes the V_{max} to occur later, and subsequently the time to achieve pH 4.0 is extended. The correlation between V_{max}, starch and damaged starch were weaker as compared to minerals.

Similar to La1, the maximum TTA for La3 was most correlated to the maximum particle size. Again, it is presumed this is a function of composition of the largest particles and not size itself. Magnesium was the mineral with highest positive correlation to maximum TTA, while iron, phosphorus, potassium, and calcium had no correlation. The minimum pH for La3 was not significantly correlated to any parameter or compound. Time to pH 4.0 however had a strong positive correlation with most minerals, except for calcium and magnesium. This is interesting as the fractions fermented using La3 took longer to achieve pH 4 than most of the other fermentations. In agreement, the T_{max} was very positively correlated with most minerals. La3 had some of the earliest T_{max} values and very strong positive correlation with iron and strong negative correlation with starch. Manganese and zinc were the two minerals most correlated to the V_{max} of La3. The defining fermentation characteristic of La3 was the short amount of time it required to reach V_{max}, apparently driven by starch availability in combination with manganese and zinc.

In summary, the results collected indicate that the driver of acidification speed for La1 was the elongation of particles, presumably pointing to bran content. Total acid production for the same strain was driven by the maximum particle size and the overall starch content, which is also attributed to higher bran content. La2 had different drivers for both acidification speed and acid production. The starch content was the main driver of the speed, while the overall acid production was driven by magnesium and copper content. The main driver behind the

acidification speed of La3 was the amount of starch and the alpha amylase content, and for the total acid production it was the maximum particle size and magnesium content. Besides these drives, it can be stated that fermentation characteristics of La2 are heavily influenced by the amount of available starch. This could be due to the metabolic pathways utilized by La2, resulting in more lactic acid production. It can also be stated that fractions with higher mineral contents aid in fermentation of both La1 and La3. While La3 achieved a steeper V_{max} than La1, it was not able to acidify as effectively regardless of what fraction was being fermented. It can be hypothesized that while a high mineral content allowed La3 to acidify earlier in the fermentation, and then buffering prevented the pH from decreasing to a similar value achieved by the homofermentative La2.

The metabolite differences and the correlation statistics show how the characteristics of the liquid sourdoughs can be impacted by the composition of the fractions, and also the different strains paired with those fractions. A better understanding of these connections can lead to more informed decisions about substrate and strain selection for liquid sourdough fermentation.

Table 3.1. Milling yield and extraction percentages

Fraction	Total (kg)	Extraction %
1BK	13.4	4.9%
2BK	16.4	6.0%
3BK	5.9	2.2%
1M	66.4	24.2%
2M	43.9	16.0%
3M	28.5	10.4%
Shorts	48.1	17.5%
Bran	51.6	18.8%
Total	**274.2**	100.0%

Table 3.2. Moisture, ash, starch, and damaged starch content of flour fractions and byproducts.

Fraction	Moisture (%)	Ash (% db)	Starch (% db)	Damaged Starch Ai (%)	Damaged Starch UCD
1BK	12.55 ± 0.04 ^{d}	0.440 ± 0.01 ^{c}	76.18 ± 0.07 ^{bc}	93.44 ± 0.16 ^{c}	19.4 ± 0.47
2BK	13.09 ± 0.02 ^{c}	0.555 ± 0.03 ^{c}	75.97 ± 0.52 ^{c}	93.26 ± 0.11 ^{c}	18.9 ± 0.28
3BK	12.34 ± 0.05 ^{d}	0.656 ± 0.00 ^{c}	72.02 ± 0.19 ^{c}	93.98 ± 0.26 ^{c}	20.8 ± 0.68
1M	13.84 ± 0.29 ^{bc}	0.502 ± 0.01 ^{c}	78.83 ± 0.52 ^{a}	96.04 ± 0.30 ^{ab}	26.3 ± 0.85
2M	14.18 ± 0.18 ^{b}	0.471 ± 0.01 ^{c}	78.79 ± 0.18 ^{ab}	96.56 ± 0.20 ^{ab}	27.7 ± 0.51
3M	12.74 ± 0.55 ^{d}	0.639 ± 0.01 ^{c}	76.11 ± 0.45 ^{c}	97.16 ± 0.23 ^{a}	29.3 ± 0.65
Shorts	14.11 ± 0.11 ^{b}	1.975 ± 0.09 ^{b}	55.72 ± 0.13 ^{e}	95.46 ± 0.13 ^{b}	24.8 ± 0.34
Bran	15.60 ± 0.05 ^{a}	5.912 ± 0.08 ^{a}	15.52 ± 0.30 ^{f}	72.20 ± 1.08 ^{d}	-

Table 3.3. Mineral composition of the flour fractions and byproducts

Fraction	P (ppm)	SO4-S (ppm)	Ca (ppm)	Mn (ppm)	Cu (ppm)
1BK	1177.3 ± 39.21 ^{d}	1513.1 ± 47.12 ^{e}	233.9 ± 13.1 ^{cd}	8.5 ± 0.06 ^{cde}	1.4 ± 0.45 ^{c}
2BK	1169.6 ± 33.3 ^{d}	1796.7 ± 63.56 ^{d}	265.5 ± 11.5 ^{cd}	7.3 ± 0.48 ^{de}	1.4 ± 0.61 ^{c}
3BK	1627.5 ± 47.5 ^{c}	2182.7 ± 46.1 ^{b}	365.0 ± 11.4 ^{c}	11.0 ± 0.23 ^{cd}	2.5 ± 0.06 ^{c}
1M	1021.4 ± 23.2 ^{d}	1532.0 ± 50.59 ^{e}	208.9 ± 11.6 ^{d}	6.8 ± 0.06 ^{e}	1.1 ± 0.59 ^{c}
2M	1223.5 ± 11.7 ^{d}	1530.3 ± 13.45 ^{e}	229.2 ± 6.7 ^{cd}	8.9 ± 0.11 ^{cde}	1.5 ± 0.70 ^{c}
3M	1582.8 ± 6.6 ^{c}	1556.1 ± 6.59 ^{e}	247.3 ± 6.6 ^{cd}	12.9 ± 0.11 ^{c}	2.2 ± 0.52 ^{c}
Shorts	5324.6 ± 147.4 ^{b}	1983.2 ± 47.05 ^{c}	714.1 ± 48.5 ^{b}	86.6 ± 1.80 ^{b}	7.6 ± 0.17 ^{b}
Bran	15023.7 ± 176.9 ^{a}	2559.2 ± 42.71 ^{a}	1670.6 ± 139.7 ^{a}	213.7 ± 3.79 ^{a}	16.5 ± 0.59 ^{a}

Fraction	K (ppm)	Mg (ppm)	Fe (ppm)	Zn (ppm)
1BK	1471.6 ± 113.2 ^{cd}	483.0 ± 342.7 ^{d}	35.1 ± 10.7 ^{c}	7.5 ± 2.4 ^{c}
2BK	1385.0 ± 115.4 ^{cd}	646.4 ± 314.2 ^{cd}	32.7 ± 14.2 ^{c}	9.3 ± 4.6 ^{c}
3BK	1635.1 ± 65.9 ^{c}	737.7 ± 154.9 ^{cd}	39.5 ± 9.3 ^{c}	16.3 ± 5.7 ^{c}
1M	1238.0 ± 67.0 ^{d}	874.3 ± 371.1 ^{cd}	18.1 ± 7.4 ^{c}	9.3 ± 4.7 ^{c}
2M	1359.4 ± 67.3 ^{cd}	916.6 ± 136.1 ^{cd}	20.5 ± 2.8 ^{c}	18.2 ± 13.1 ^{c}
3M	1598.0 ± 0.0 ^{c}	1286.0 ± 135.2 ^{bc}	20.9 ± 2.5 ^{c}	13.7 ± 3.6 ^{c}
Shorts	5122.8 ± 201.7 ^{b}	1672.7 ± 71.1 ^{ab}	71.8 ± 4.3 ^{b}	68.1 ± 4.0 ^{b}
Bran	14178.5 ± 68.4 ^{a}	2180.1 ± 35.5 ^{a}	174.0 ± 5.2 ^{a}	147.6 ± 3.7 ^{a}

Table 3.4. Particle size analysis

Fraction	Ave PS (µm)	Max PS (µm)	Aspect ratio
1BK	110.74	208.47	0.699
2BK	94.21	219.18	0.702
3BK	82.64	187.76	0.694
1M	87.90	169.67	0.682
2M	84.96	190.36	0.673
3M	74.04	163.72	0.676
Shorts	135.10	283.74	0.662
Bran	136.90	315.55	0.685

PS = particle size
Max PS refers to the largest particle size observed within the sample.

Table 3.5. Mixing and dough development characteristics of the fractions.

Fraction	C1 (Nm)		CS (Nm)		C2 (Nm)	
1BK	0.995 ± 0.012	d	0.950 ± 0.013	d	0.425 ± 0.003	d
2BK	1.042 ± 0.005	c	1.019 ± 0.008	c	0.504 ± 0.014	c
3BK	1.218 ± 0.016	b	1.212 ± 0.013	b	0.602 ± 0.015	b
1M	1.068 ± 0.029	c	1.061 ± 0.024	c	0.512 ± 0.007	c
2M	1.220 ± 0.006	b	1.209 ± 0.016	b	0.597 ± 0.008	b
3M	1.373 ± 0.008	a	1.339 ± 0.006	a	0.649 ± 0.011	a

Fraction	C3 (Nm)		C4 (Nm)		C5 (Nm)	
1BK	1.854 ± 0.001	a	1.731 ± 0.016	ab	2.647 ± 0.045	abc
2BK	1.852 ± 0.005	a	1.750 ± 0.015	a	2.809 ± 0.042	a
3BK	1.771 ± 0.002	bc	1.629 ± 0.008	bc	2.736 ± 0.018	ab
1M	1.838 ± 0.017	a	1.673 ± 0.066	abc	2.563 ± 0.087	bcd
2M	1.790 ± 0.006	b	1.675 ± 0.006	abc	2.485 ± 0.060	cd
3M	1.728 ± 0.021	c	1.614 ± 0.004	c	2.397 ± 0.005	d

Note:
Only the torque values were reported as the time values were not significantly different from each other

Table 3.6. Amylase activity of the fractions.

Fractions	α-amylase (units of α-amylase/g of flour)	β-amylase (units of β-amylase/g of flour)
1BK	13.70 ± 0.34 [a]	33.72 ± 0.87 [bcd]
2BK	12.97 ± 0.95 [a]	39.07 ± 2.40 [b]
3BK	11.55 ± 1.74 [a]	50.27 ± 0.81 [a]
1M	12.56 ± 0.02 [a]	33.97 ± 2.82 [bcd]
2M	12.79 ± 0.97 [a]	38.06 ± 0.81 [bc]
3M	13.60 ± 0.62 [a]	30.22 ± 0.54 [d]
Shorts	10.69 ± 1.68 [a]	30.83 ± 0.66 [d]
Bran	10.86 ± 0.72 [a]	33.24 ± 0.94 [cd]
Straight Grade	11.84 ± 0.49 [a]	33.35 ± 1.24 [cd]
Whole Wheat	11.59 ± 0.42 [a]	34.45 ± 0.42 [bcd]

Table 3.7. Fermentation characteristics of La1, La2 and La3.

Fraction	V_{max} (ΔpH/time)	T_{max} (h)	Time to pH 4.0 (h)	Min pH	Max TTA (mL)
La1:					
1BK	-0.0138 ± 0.0021 [a]	7.91 ± 2.30 [a]	13.71 ± 1.81 [abc]	3.59 ± 0.03 [b]	13.706 ± 0.745 [bcd]
2/3BK	-0.0117 ± 0.0010 [a]	9.29 ± 0.28 [a]	14.95 ± 0.70 [abc]	3.58 ± 0.06 [b]	15.189 ± 0.239 [bc]
1M	-0.0117 ± 0.0023 [a]	9.02 ± 2.83 [a]	17.27 ± 1.90 [ab]	3.60 ± 0.03 [b]	12.203 ± 0.553 [d]
2M	-0.0130 ± 0.0015 [a]	10.93 ± 3.60 [a]	18.75 ± 2.58 [a]	3.60 ± 0.09 [b]	12.472 ± 1.034 [cd]
3M	-0.0211 ± 0.0089 [a]	9.29 ± 3.62 [a]	14.98 ± 3.12 [abc]	3.54 ± 0.06 [b]	15.410 ± 1.879 [b]
Shorts	-0.0162 ± 0.0019 [a]	7.64 ± 0.28 [a]	10.31 ± 0.69 [c]	3.53 ± 0.04 [b]	22.707 ± 0.647 [a]
Bran	-0.0141 ± 0.0015 [a]	6.88 ± 1.21 [a]	18.41 ± 6.54 [a]	4.01 ± 0.09 [a]	21.497 ± 0.726 [a]
Straight Grade	-0.0124 ± 0.0012 [a]	12.84 ± 1.50 [a]	16.86 ± 0.54 [abc]	3.57 ± 0.05 [b]	12.615 ± 0.872 [cd]
Whole Wheat	-0.0153 ± 0.0009 [a]	8.35 ± 0.20 [a]	10.92 ± 0.15 [bc]	3.56 ± 0.05 [b]	22.871 ± 0.970 [a]
La2:					
1BK	-0.0260 ± 0.0031 [ab]	6.58 ± 0.43 [a]	9.13 ± 0.23 [b]	3.44 ± 0.03 [bcd]	15.132 ± 0.872 [cd]
2/3BK	-0.0266 ± 0.0027 [ab]	6.13 ± 0.13 [a]	9.45 ± 0.28 [b]	3.55 ± 0.02 [b]	15.420 ± 0.066 [c]
1M	-0.0219 ± 0.0003 [ab]	6.18 ± 0.56 [a]	8.28 ± 0.41 [b]	3.39 ± 0.04 [d]	13.323 ± 0.632 [d]
2M	-0.0261 ± 0.0073 [ab]	6.53 ± 0.26 [a]	9.29 ± 0.56 [b]	3.39 ± 0.02 [cd]	15.197 ± 0.882 [cd]
3M	-0.0256 ± 0.0029 [ab]	6.76 ± 0.20 [a]	9.55 ± 0.47 [b]	3.48 ± 0.07 [bcd]	16.707 ± 0.301 [c]
Shorts	-0.0236 ± 0.0003 [ab]	7.16 ± 0.15 [a]	9.02 ± 0.77 [b]	3.51 ± 0.03 [bcd]	22.730 ± 0.497 [ab]
Bran	-0.0193 ± 0.0007 [a]	7.20 ± 1.35 [a]	12.26 ± 1.59 [a]	3.76 ± 0.08 [a]	24.109 ± 1.002 [a]
Straight Grade	-0.0284 ± 0.0029 [b]	6.18 ± 0.34 [a]	8.96 ± 0.43 [b]	3.49 ± 0.05 [bcd]	15.635 ± 0.795 [c]
Whole Wheat	-0.0208 ± 0.0003 [ab]	7.02 ± 0.43 [a]	8.92 ± 0.38 [b]	3.50 ± 0.02 [bc]	22.051 ± 0.807 [b]
La3:					
1BK	-0.0249 ± 0.0061 [bcd]	6.84 ± 0.73 [a]	20.09 ± 4.93 [b]	3.81 ± 0.09 [ab]	11.452 ± 1.164 [c]
2/3BK	-0.0365 ± 0.0093 [d]	5.73 ± 0.46 [a]	12.09 ± 3.55 [bc]	3.70 ± 0.15 [bc]	14.398 ± 2.209 [c]
1M	-0.0263 ± 0.0049 [bcd]	5.64 ± 0.34 [a]	14.40 ± 1.87 [bc]	3.73 ± 0.06 [b]	10.955 ± 0.505 [c]
2M	-0.0261 ± 0.0020 [bcd]	5.42 ± 0.15 [a]	11.96 ± 3.05 [bc]	3.63 ± 0.07 [bc]	12.883 ± 0.430 [c]
3M	-0.0308 ± 0.0025 [cd]	5.87 ± 0.35 [a]	11.87 ± 1.51 [bc]	3.68 ± 0.04 [bc]	14.328 ± 1.440 [c]
Shorts	-0.0201 ± 0.0010 [abc]	6.76 ± 0.08 [a]	9.51 ± 0.78 [c]	3.44 ± 0.08 [c]	24.378 ± 0.292 [a]
Bran	-0.0117 ± 0.0012 [a]	7.96 ± 0.28 [a]	44.93 ± 5.31 [a]	4.06 ± 0.09 [a]	20.240 ± 1.442 [b]
Straight Grade	-0.0239 ± 0.0022 [abc]	5.47 ± 0.40 [a]	11.29 ± 2.81 [bc]	3.66 ± 0.09 [bc]	12.160 ± 1.400 [c]
Whole Wheat	-0.0179 ± 0.0019 [ab]	7.02 ± 1.00 [a]	13.07 ± 4.27 [bc]	3.62 ± 0.16 [bc]	20.679 ± 1.546 [ab]

Table 3.8. Comparison of strains within fractions in regard to their fermentation parameters.

Fractions	Strain	V_{max}	T_{max}	Time to pH 4.0	Min pH	Max TTA
1BK	La1	-0.015 $_{a}$	8.36 $_{a}$	10.98 $_{a}$	3.56 $_{a}$	22.872 $_{a}$
	La2	-0.021 $_{b}$	7.02 $_{a}$	9.11 $_{a}$	3.52 $_{a}$	22.051 $_{a}$
	La3	-0.018 $_{ab}$	7.02 $_{a}$	13.07 $_{a}$	3.62 $_{a}$	20.679 $_{a}$
2/3BK	La1	-0.012 $_{a}$	9.29 $_{a}$	14.67 $_{a}$	3.57 $_{a}$	15.189 $_{a}$
	La2	-0.027 $_{b}$	6.13 $_{b}$	9.51 $_{a}$	3.55 $_{a}$	15.420 $_{a}$
	La3	-0.037 $_{b}$	5.73 $_{b}$	12.09 $_{a}$	3.70 $_{a}$	14.398 $_{a}$
1M	La1	-0.012 $_{a}$	9.02 $_{a}$	17.29 $_{a}$	3.59 $_{b}$	12.203 $_{ab}$
	La2	-0.022 $_{b}$	6.18 $_{a}$	8.58 $_{b}$	3.38 $_{c}$	13.323 $_{a}$
	La3	-0.026 $_{b}$	5.64 $_{a}$	14.40 $_{a}$	3.73 $_{a}$	10.955 $_{b}$
2M	La1	-0.013 $_{a}$	10.93 $_{a}$	18.40 $_{a}$	3.57 $_{a}$	12.472 $_{b}$
	La2	-0.026 $_{b}$	6.53 $_{ab}$	9.42 $_{b}$	3.40 $_{b}$	15.197 $_{a}$
	La3	-0.026 $_{b}$	5.42 $_{b}$	11.96 $_{b}$	3.63 $_{a}$	12.880 $_{b}$
3M	La1	-0.021 $_{a}$	9.29 $_{a}$	15.07 $_{a}$	3.53 $_{b}$	15.410 $_{a}$
	La2	-0.026 $_{a}$	6.76 $_{a}$	9.64 $_{b}$	3.48 $_{b}$	16.707 $_{a}$
	La3	-0.031 $_{a}$	5.87 $_{a}$	11.87 $_{ab}$	3.68 $_{a}$	14.328 $_{a}$
Shorts	La1	-0.016 $_{a}$	7.64 $_{a}$	10.40 $_{a}$	3.52 $_{a}$	22.707 $_{b}$
	La2	-0.024 $_{c}$	7.16 $_{b}$	9.16 $_{a}$	3.51 $_{a}$	22.730 $_{b}$
	La3	-0.020 $_{b}$	6.76 $_{b}$	9.51 $_{a}$	3.44 $_{a}$	24.378 $_{a}$
Bran	La1	-0.014 $_{a}$	6.89 $_{a}$	44.22 $_{a}$	3.99 $_{a}$	21.497 $_{ab}$
	La2	-0.019 $_{b}$	7.20 $_{a}$	12.31 $_{b}$	3.75 $_{b}$	24.109 $_{a}$
	La3	-0.012 $_{a}$	7.96 $_{a}$	44.93 $_{a}$	4.06 $_{a}$	20.240 $_{b}$
Straight Grade	La1	-0.012 $_{a}$	12.84 $_{a}$	16.98 $_{a}$	3.57 $_{ab}$	12.615 $_{b}$
	La2	-0.028 $_{b}$	6.18 $_{b}$	9.02 $_{b}$	3.49 $_{b}$	15.635 $_{a}$
	La3	-0.024 $_{b}$	5.47 $_{b}$	11.29 $_{b}$	3.66 $_{a}$	12.160 $_{b}$
Whole Wheat	La1	-0.015 $_{a}$	8.36 $_{a}$	10.98 $_{a}$	3.56 $_{a}$	22.872 $_{a}$
	La2	-0.021 $_{b}$	7.02 $_{a}$	9.11 $_{a}$	3.52 $_{a}$	22.051 $_{a}$
	La3	-0.018 $_{ab}$	7.02 $_{a}$	13.07 $_{a}$	3.62 $_{a}$	20.679 $_{a}$

Note: Letters indicate significant difference within each fraction

Table 3.9. Fermentation quotients (lactic acid: acetic acid ratio).

Time (h)	1BK	2/3 BK	1M	2M	3M	Shorts	Bran	Straight Grade	Whole Wheat
La1 Lactic: Acetic									
8	0.1: -	-: -	-: -	2.3:1	0.1: -	0.3: -	2.3:1	0.4: -	0.2: -
18	0.5: -	0.4: -	0.3: -	0.3: -	15.4:1	4.8:1	2.7:1	0.4: -	5.9:1
24	0.5: -	0.5: -	0.4: -	0.4: -	0.6: -	6.7:1	2.6:1	0.4: -	5.8:1
30	4.9:1	0.6: -	0.6: -	9.3:1	7.4:1	6.7:1	2.6:1	0.6: -	5.8:1
48	4.2:1	5.0:1	0.6: -	6.1:1	5.2:1	5.8:1	2.9:1	0.7: -	5.1:1
La2 Lactic: Acetic									
8	0.2: -	0.4: -	0.3: -	0.3: -	0.3: -	0.4: -	0.4: -	0.3: -	0.5: -
18	0.6: -	0.7: -	0.6: -	0.6: -	0.5: -	1.1: -	1.0: -	0.6: -	1.0: -
24	0.7: -	0.8: -	0.7: -	0.8: -	0.6: -	1.2: -	1.1: -	0.7: -	8.5:1
30	0.8: -	0.9: -	0.8: -	0.8: -	0.7: -	8.8:1	1.2: -	0.7: -	8.5:1
48	0.9: -	1.0: -	0.9: -	4.8: -	0.8: -	8.0:1	7.7: 1	6.0:1	7.5:1
La3 Lactic: Acetic									
8	0.2: -	0.2: -	0.2: -	0.2: -	0.2: -	0.2: -	0.2: -	0.1: -	0.3: -
18	0.3: -	0.4: -	0.3: -	5.2:1	0.3: -	18.5:1	3.9:1	0.4: -	5.6:1
24	0.4: -	0.5: -	0.4: -	4.7:1	0.3: -	7.8:1	3.7:1	0.4: -	5.5:1
30	10.3:1	6.3:1	10.2:1	3.0:1	0.3: -	7.7:1	3.5:1	3.7:1	5.1:1
48	2.6:1	5.3:1	2.7:1	2.4:1	4.6:1	7.3:1	3.1:1	2.5:1	4.8:1

Note:
All values presented as mol/L
"-" indicates both or either acid was not produced and should not be taken as a ratio.

Table 3.10. La1 and La2 color difference.

	La1			La2			Color difference (La1 - La2)		
Fractions	**L***	**a***	**b***	**L***	**a***	**b***	**ΔL**	**Δa**	**Δb**
1BK	84.1 bc	2.6 de	12.1 c	83.7 a	1.9 c	11.7 b	0.4	0.7	0.5
2/3BK	84.4 abc	2.9 de	12.9 bc	85.0 a	2.6 c	12.9 ab	-0.6	0.3	0.0
1M	86.3 a	2.2 e	13.4 bc	85.6 a	2.2 c	13.2 ab	0.7	-0.1	0.2
2M	85.0 abc	2.5 de	13.1 bc	85.3 a	2.4 c	13.2 ab	-0.3	0.1	-0.1
3M	83.4 c	3.3 d	14.1 bc	83.1 a	3.0 c	13.4 ab	0.3	0.3	0.8
Shorts	67.8 e	6.6 b	15.5 ab	67.4 c	7.0 ab	16.3 a	0.4	-0.4	-0.8
Bran	53.8 f	9.5 a	17.8 a	53.3 d	8.6 a	16.3 a	0.5	0.9	1.4
Straight Grade	85.7 ab	2.7 de	13.9 bc	84.5 a	2.1 c	13.3 ab	1.3	0.6	0.6
Whole Wheat	72.5 d	5.5 c	13.5 bc	72.4 b	5.3 b	13.6 ab	0.1	0.2	-0.1

Table 3.11. La1 composition and fermentation characteristic correlations.

Fermentation Characteristic	by Variable	Correlation	*p-value*
Vmax	Elongation	-0.84	0.0042**
Tmax	Average particle size	-0.71	0.0317*
Min pH	Ash	0.89	0.0013**
	Potassium	0.89	0.0015**
	Phosphorus	0.88	0.0016**
	Iron	0.88	0.0019**
	Starch content	-0.86	0.0027**
	Damaged starch	0.86	0.0030**
	Manganese	0.85	0.0038**
	Copper	0.83	0.0052**
	Zinc	0.83	0.0054**
	Absorption	0.82	0.0064**
	Sulfur	0.79	0.0107*
Max TTA	Magnesium	0.76	0.0171*
	Copper	0.75	0.0209*
	Average particle size	0.75	0.0210*
	Zinc	0.74	0.0226*
	Sulfur	0.73	0.0244*
	Manganese	0.73	0.0252*
	Absorption	0.73	0.0252*
	α-amylase	-0.73	0.0266*
	Starch content	-0.72	0.0301*
	Iron	0.70	0.0351*
	Phosphorus	0.69	0.0379*
	Potassium	0.69	0.0395*
	Ash	0.69	0.0416*

Note: *p-value** = moderate correlation: *p-value*** = strong correlation

Table 3.12. La2 composition and fermentation characteristic correlations.

Fermentation characteristic	by Variable	Correlation	*p-value*
Vmax	Ash	0.74	0.0227*
	Manganese	0.74	0.0232*
	Potassium	0.73	0.0242*
	Phosphorus	0.73	0.0245*
	Zinc	0.73	0.0255*
	Starch content	-0.73	0.0264*
	Copper	0.73	0.0269*
	Absorption	0.71	0.0310*
	Iron	0.71	0.0329*
	Magnesium	0.69	0.0410*
	Damaged starch	0.68	0.0425*
Tmax	Magnesium	0.82	0.0074**
	Copper	0.77	0.0156*
	Zinc	0.77	0.0162*
	Manganese	0.76	0.0187*
	Absorption	0.75	0.0189*
	Starch content	-0.73	0.0248*
	Phosphorus	0.72	0.0273*
	Potassium	0.72	0.0287*
	Average particle size	0.72	0.0287*
	Ash	0.71	0.0321*
	Iron	0.71	0.0322*
Time to pH 4.0	Ash	0.88	0.0019**
	Phosphorus	0.88	0.0020**
	Potassium	0.88	0.0020**
	Iron	0.87	0.0021**
	Starch content	-0.86	0.0028**
	Copper	0.85	0.0041**
	Manganese	0.85	0.0041**
	Zinc	0.83	0.0052**
	Damaged starch	0.83	0.0056**
	Sulfur	0.83	0.0059**
	Absorption	0.82	0.0068**
	Magnesium	0.70	0.0367*
Min pH	Sulfur	0.94	0.0002**
	Iron	0.90	0.0009**
	Ash	0.89	0.0013**
	Starch content	-0.89	0.0014**
	Phosphorus	0.89	0.0014**
	Potassium	0.89	0.0015**
	Copper	0.88	0.0019**
	Manganese	0.87	0.0022**
	Zinc	0.86	0.0032**
	Absorption	0.85	0.0036**
	Magnesium	0.73	0.0252*
	Damaged starch	0.72	0.0278*

Max TTA	Magnesium	0.87	0.0021**
	Copper	0.87	0.0022**
	Zinc	0.87	0.0023**
	Manganese	0.86	0.0030**
	Absorption	0.86	0.0031**
	Starch content	-0.84	0.0043**
	Phosphorus	0.83	0.0056**
	Iron	0.83	0.0060**
	Potassium	0.83	0.0061**
	Ash	0.82	0.0067**
	Sulfur	0.82	0.0070**
	Average particle size	0.79	0.0105*
	α-amylase	-0.79	0.0120*

Note: *p-value** = moderate correlation: *p-value*** = strong correlation

Table 3.13. La3 composition and fermentation characteristic correlations.

Fermentation Characteristic	by Variable	Correlation	*p-value*
Vmax	Zinc	0.81	0.0086**
	Manganese	0.80	0.0092**
	Water absorption	0.79	0.0109*
	Potassium	0.79	0.0113*
	Copper	0.79	0.0113*
	Phosphorus	0.79	0.0117*
	Ash	0.78	0.0125*
	Starch content	-0.78	0.0136*
	Iron	0.77	0.0158*
	Average particle size	0.74	0.0219*
	Magnesium	0.73	0.0263*
	Damaged starch	0.71	0.0306*
	α-amylase	-0.70	0.0366*
Tmax	Iron	0.87	0.0022**
	Average particle size	0.86	0.0032**
	Starch content	-0.85	0.0039**
	Manganese	0.85	0.0039**
	Copper	0.85	0.0041**
	Potassium	0.84	0.0042**
	Phosphorus	0.84	0.0046**
	Ash	0.83	0.0052**
	Absorption	0.83	0.0054**
	Zinc	0.83	0.0059**
	Sulfur	0.77	0.0158*
	Magnesium	0.67	0.0486*
Time to pH 4.0	Iron	0.87	0.0022**
	Ash	0.87	0.0023**
	Potassium	0.87	0.0023**
	Phosphorus	0.86	0.0027**
	Starch content	-0.85	0.0040**
	Manganese	0.83	0.0056**
	Copper	0.81	0.0075**
	Damaged starch	0.81	0.0085**
	Zinc	0.80	0.0091**
	Absorption	0.80	0.0095**
	Sulfur	0.75	0.0200*
Max TTA	α-amylase	-0.79	0.0110*
	Magnesium	0.78	0.0125*
	Average particle size	0.75	0.0209*
	Zinc	0.72	0.0282*
	Water absorption	0.72	0.0286*
	Copper	0.72	0.0290*
	Sulfur	0.71	0.0316*
	Manganese	0.70	0.0361*
	Starch content	-0.68	0.0435*

Note: *p-value** = moderate correlation: *p-value*** = strong correlation

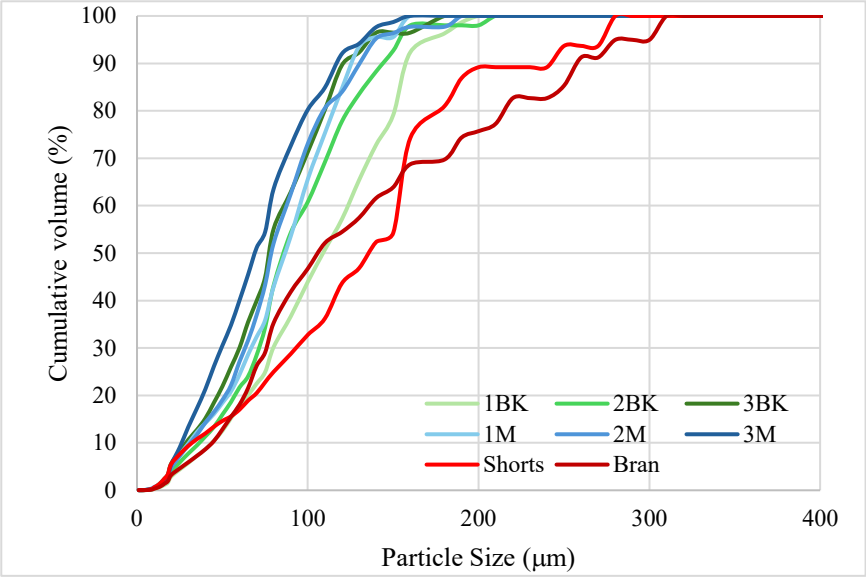
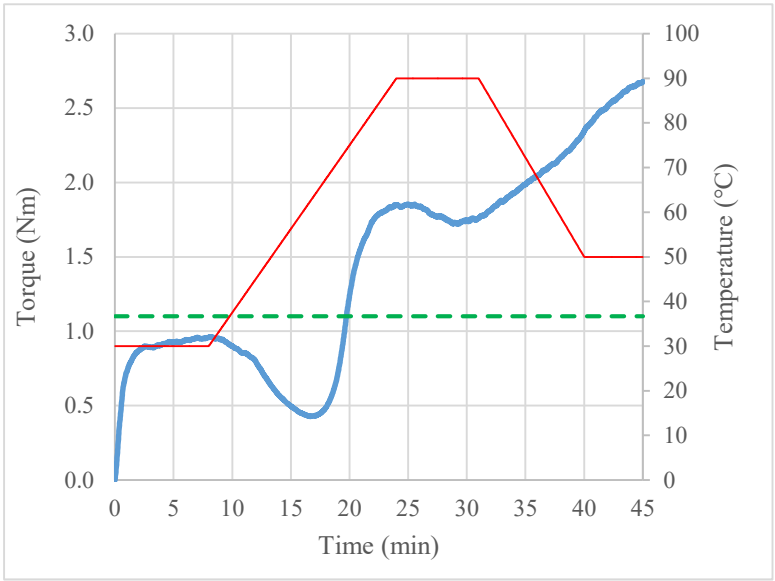
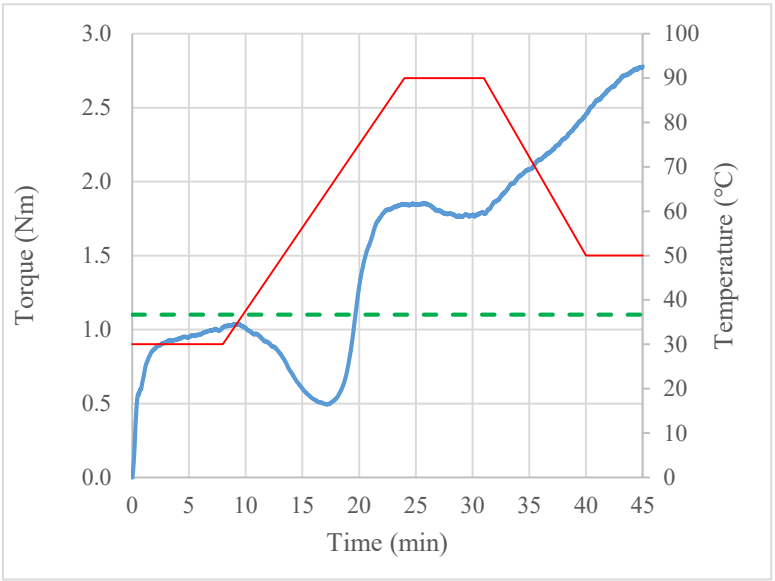


Figure 3.1. Cumulation particle size distribution by volume.

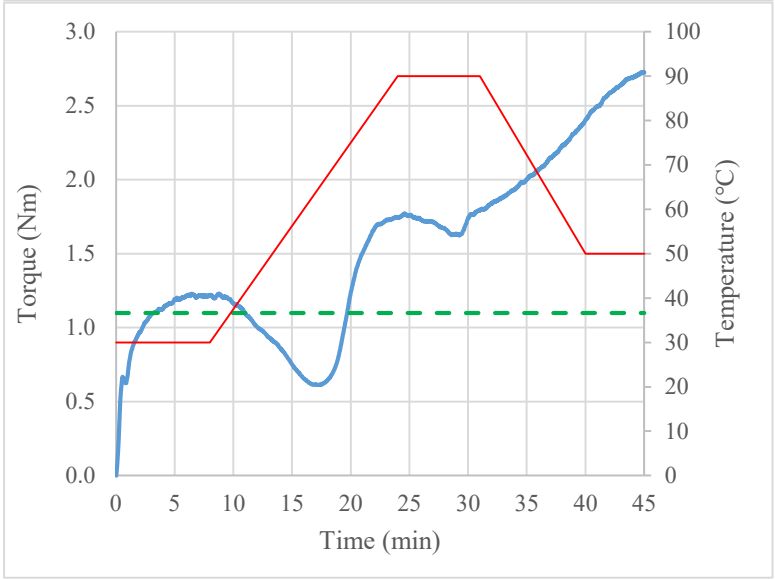
(a)


Torque (Nm)
Temperature (°C)
Time (min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45

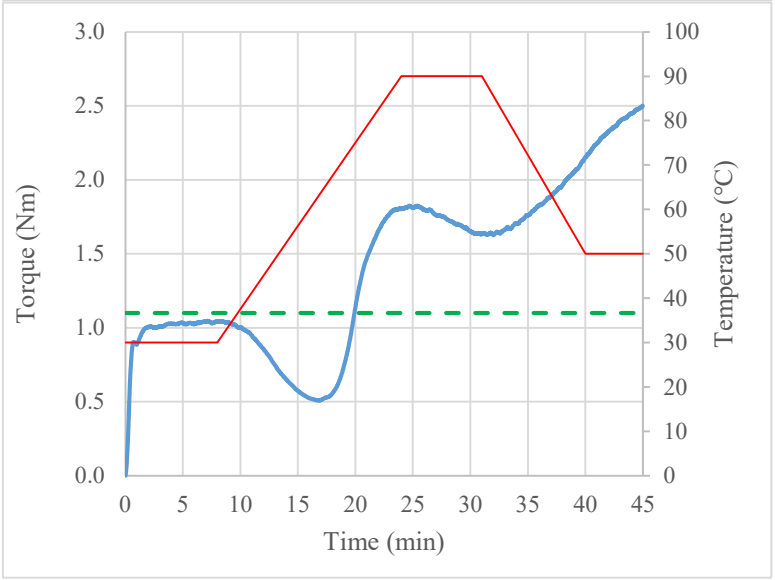
(b)


Torque (Nm)
Temperature (°C)
Time (min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45

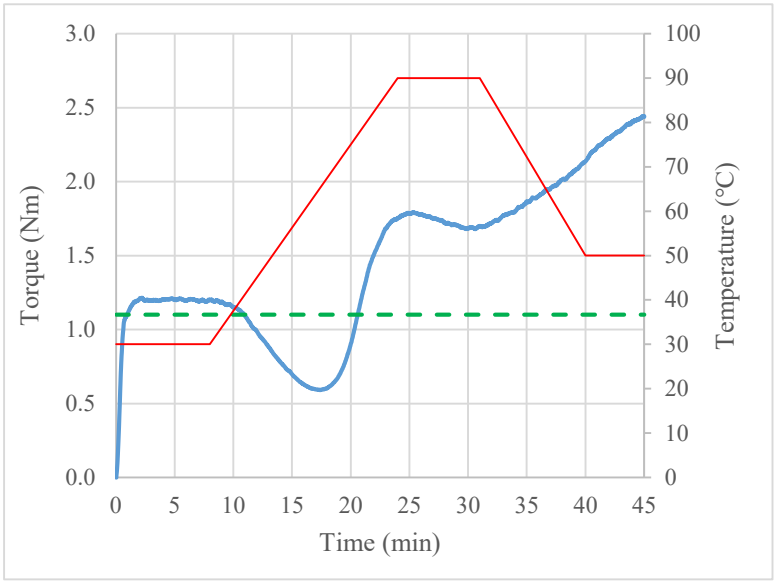
(c)


Torque (Nm)
Temperature (°C)
Time (min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45

(d)


Torque (Nm)
Temperature (°C)
Time (min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45

(e)



(f)

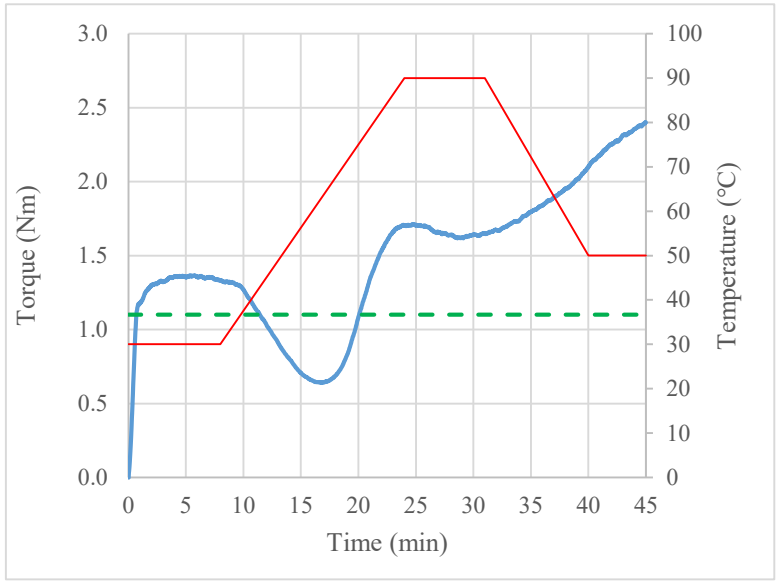
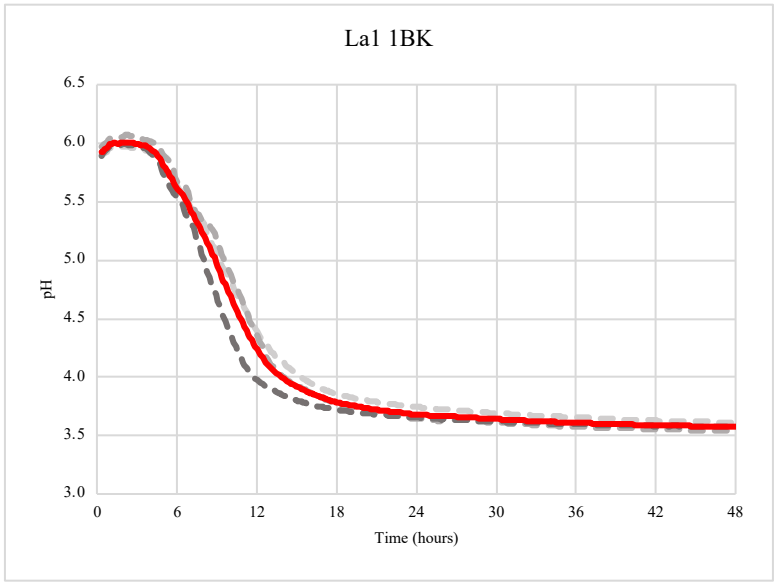


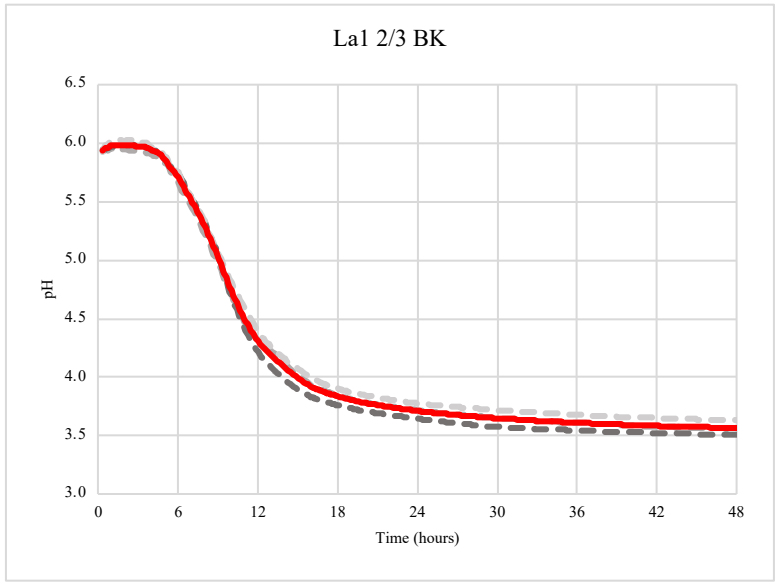
Figure 3.2. MixoLab curves of all flour fractions (a) 1BK, (b) 2BK, (c) 3BK, (d) 1M, (e) 2M, (f) 3M.

Dotted green line: Target toque of 1.1 Nm
Red line: Dough temperature (°C)
Blue line: Measured torque value (Nm)

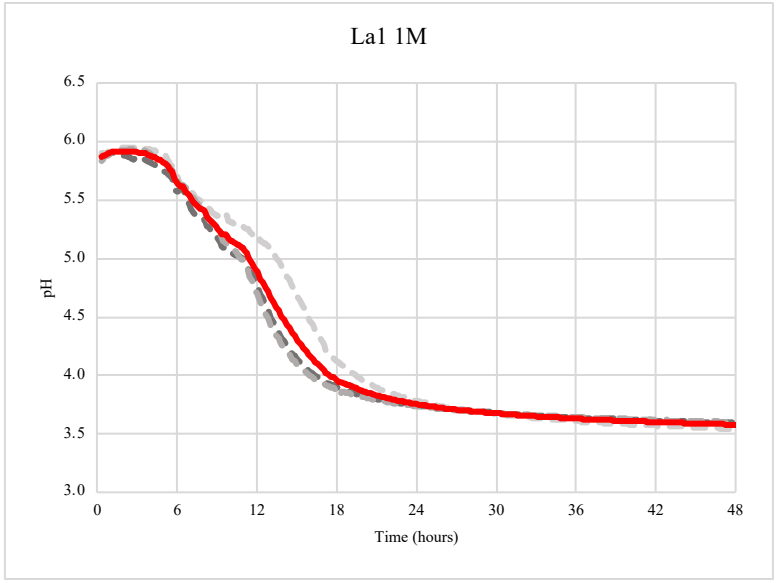
(a)


La1 1BK
pH
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
6
12
18
24
30
36
42
48
Time (hours)

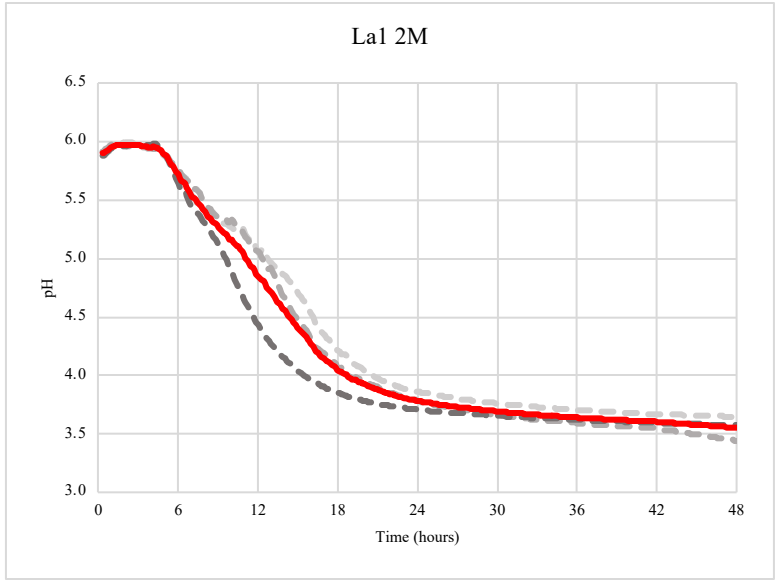
(b)


La1 2/3 BK
pH
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
6
12
18
24
30
36
42
48
Time (hours)

(c)


La1 1M
pH
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
6
12
18
24
30
36
42
48
Time (hours)

(d)


La1 2M
pH
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
6
12
18
24
30
36
42
48
Time (hours)

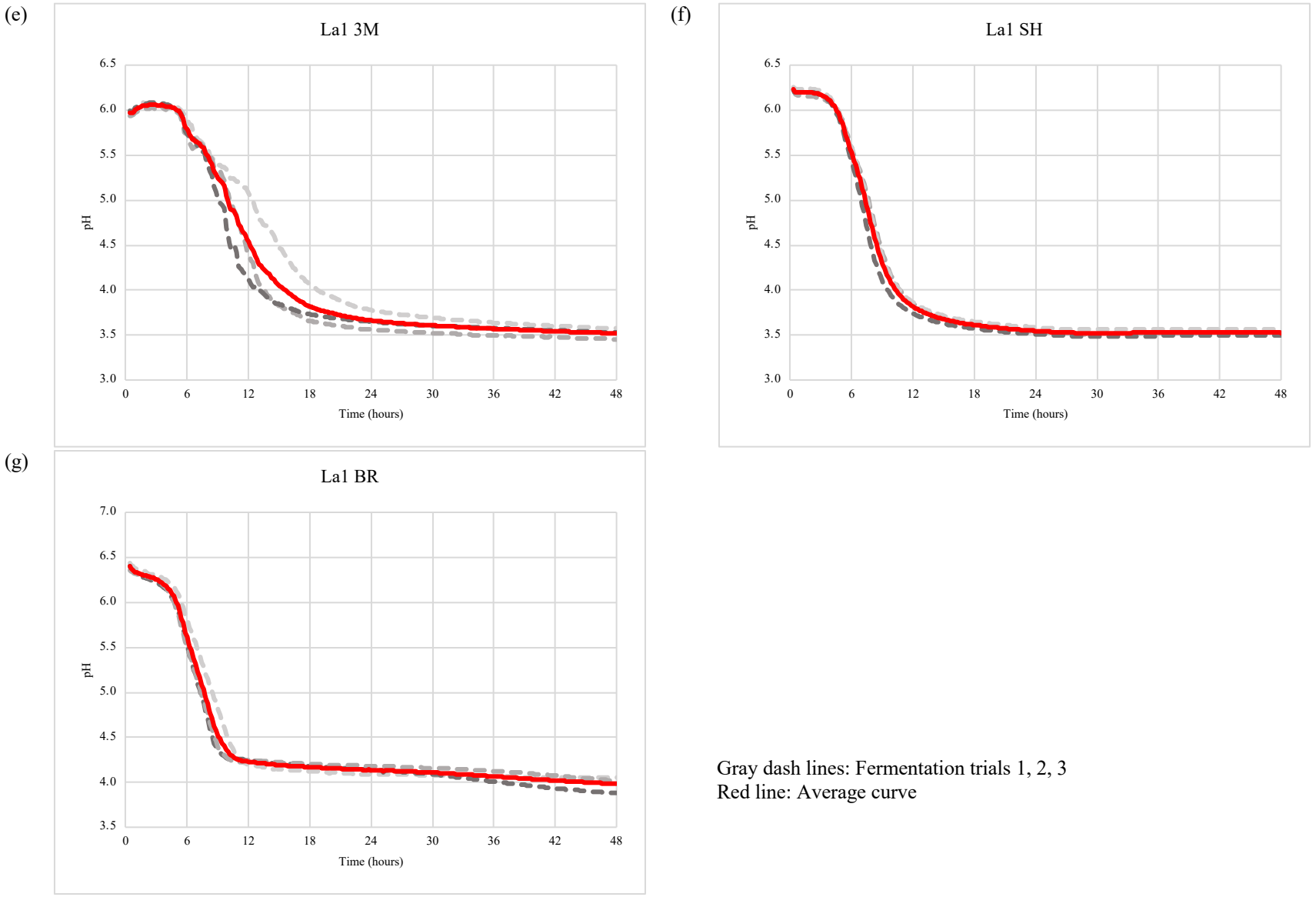


Figure 3.3. La1 acidification curves of flour fractions and byproducts. (a) 1BK, (b), 2/3BK, (c) 1M, (d) 2M, (e) 3M, (f) shorts, (g) bran.

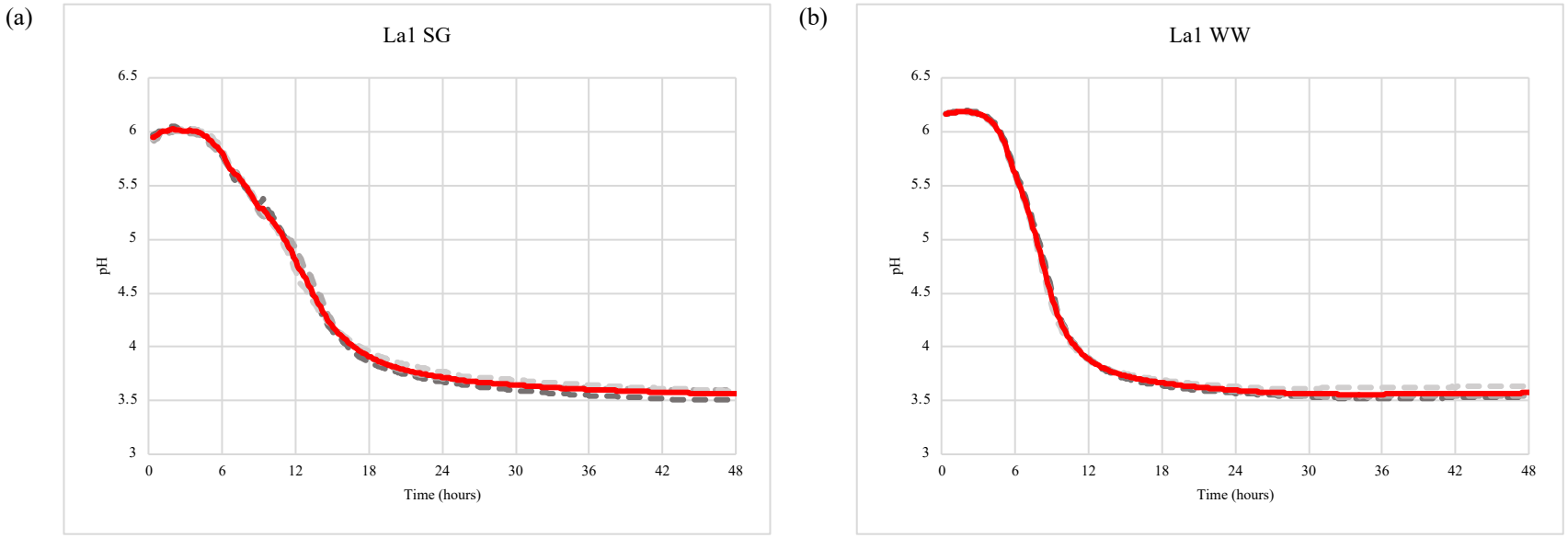
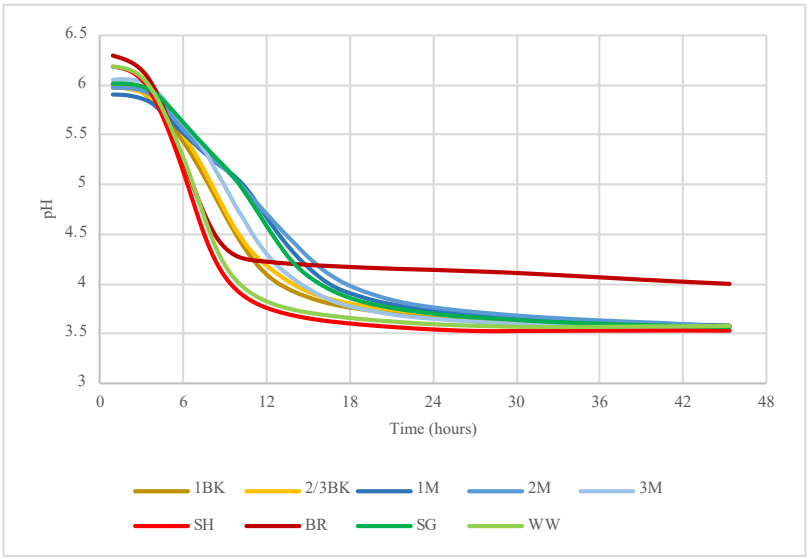


Figure 3.4. La1 acidification curves of composite flours (a) straight grade, (b) whole wheat.

Gray dash lines: Fermentation trials 1, 2, 3
Red line: Average curve

(a)



(b)

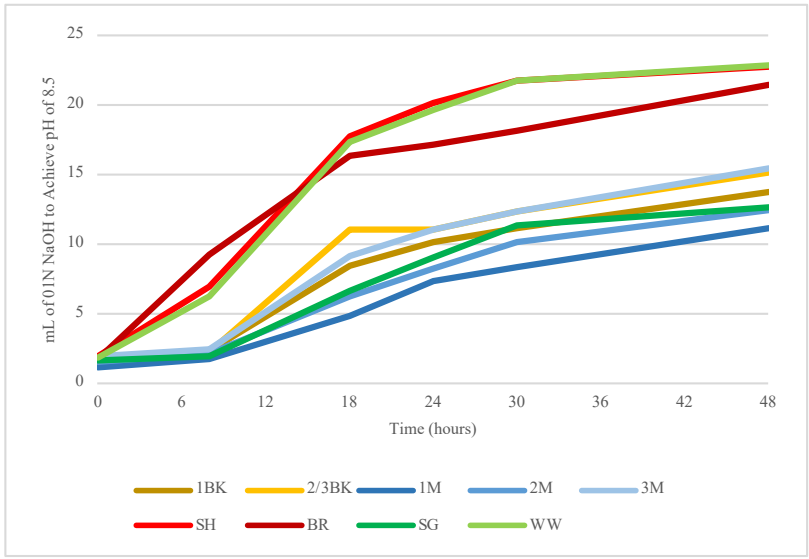


Figure 3.5. La1 (a) moving average curves in comparison, (b) TTA progression.

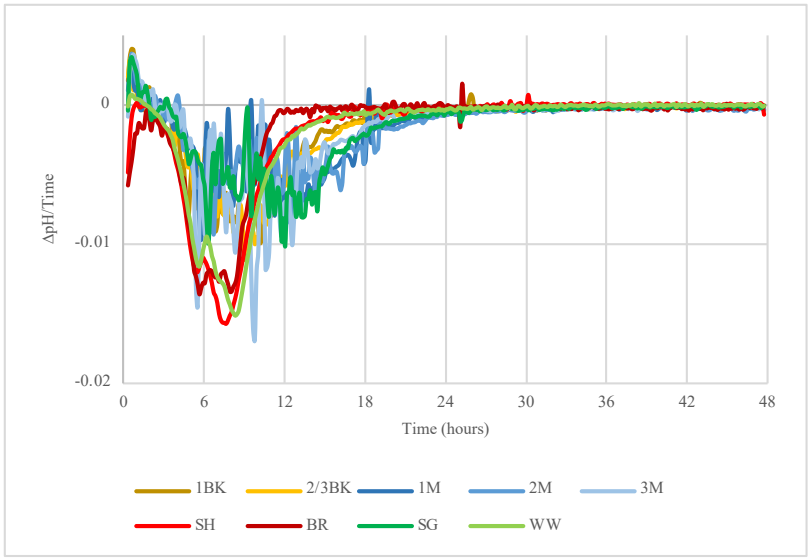
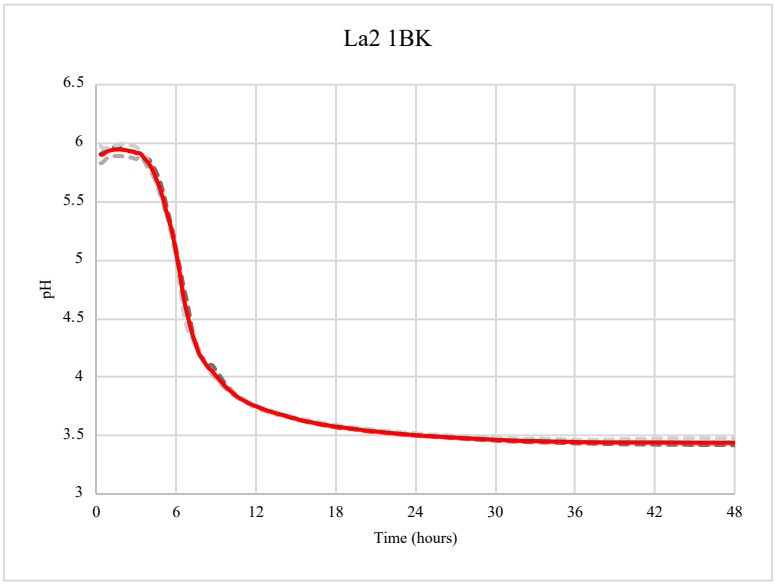
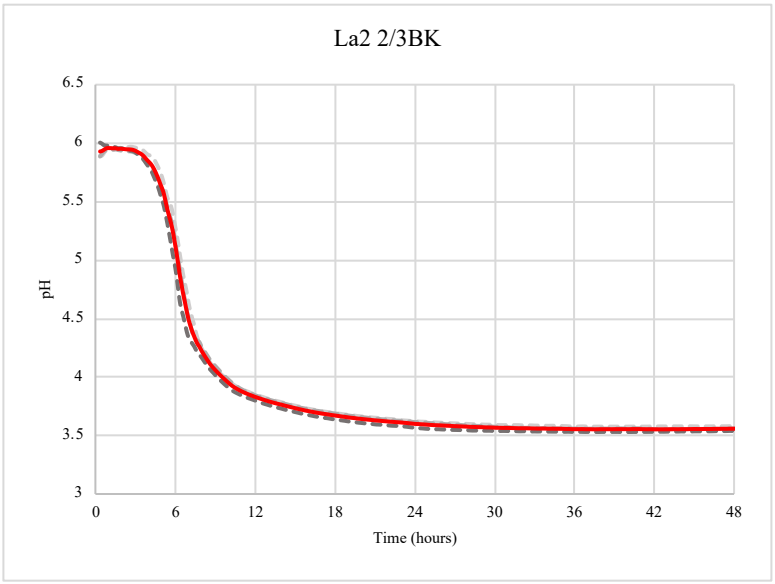


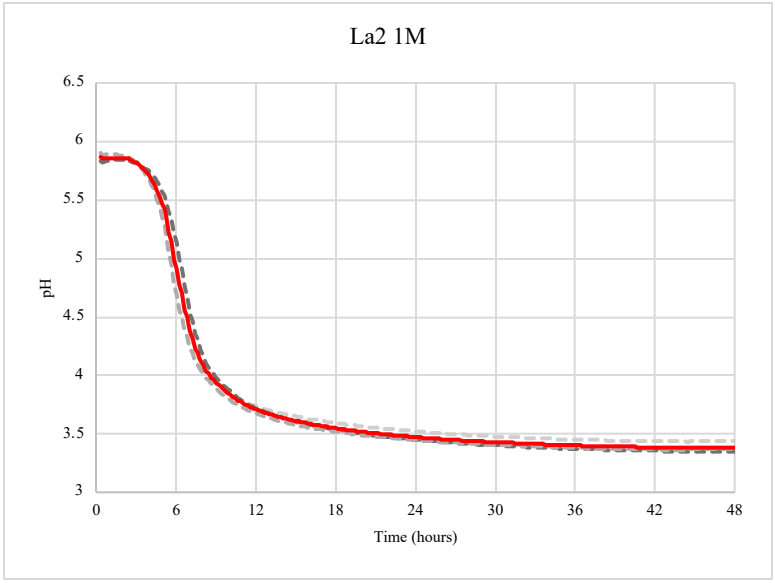
Figure 3.6. La1 change in acidification rate over time.

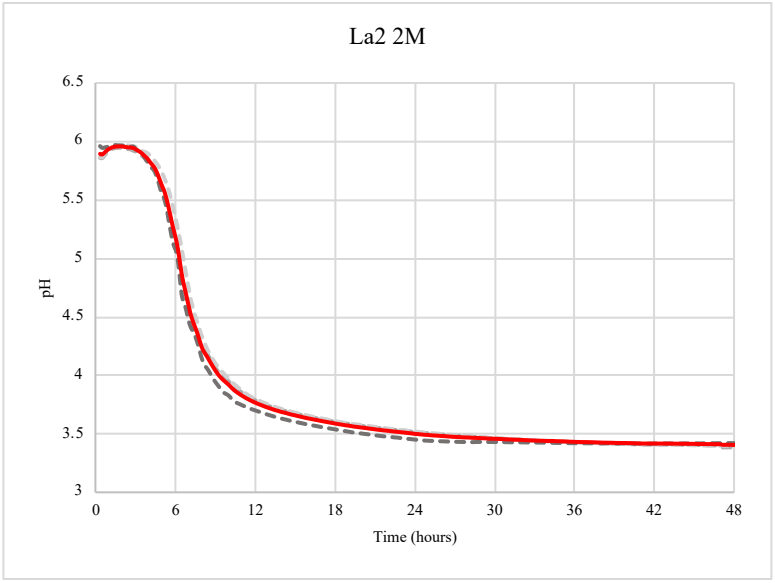
(a)


La2 1BK
pH
Time (hours)

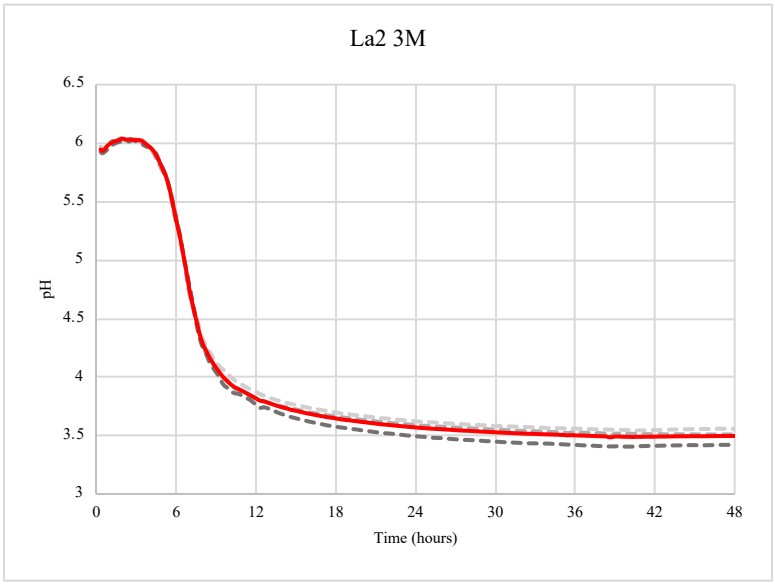

La2 2/3BK
pH
Time (hours)

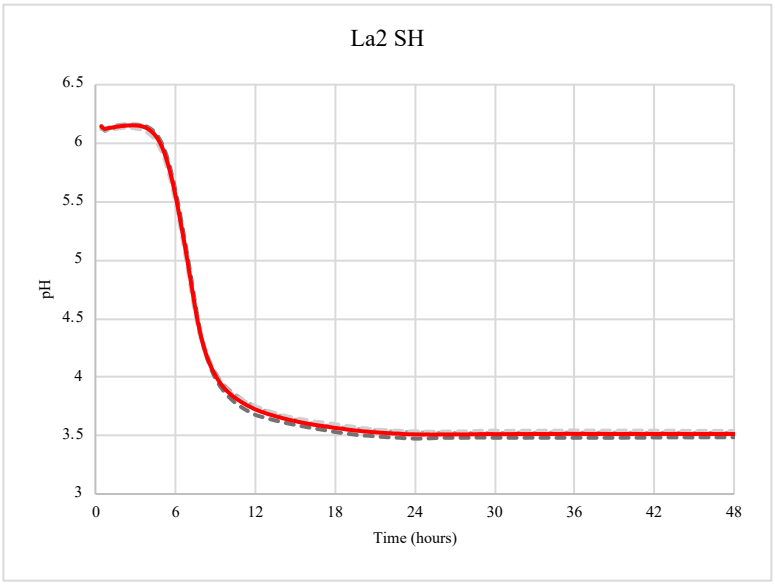
(c)


La2 1M
pH
Time (hours)

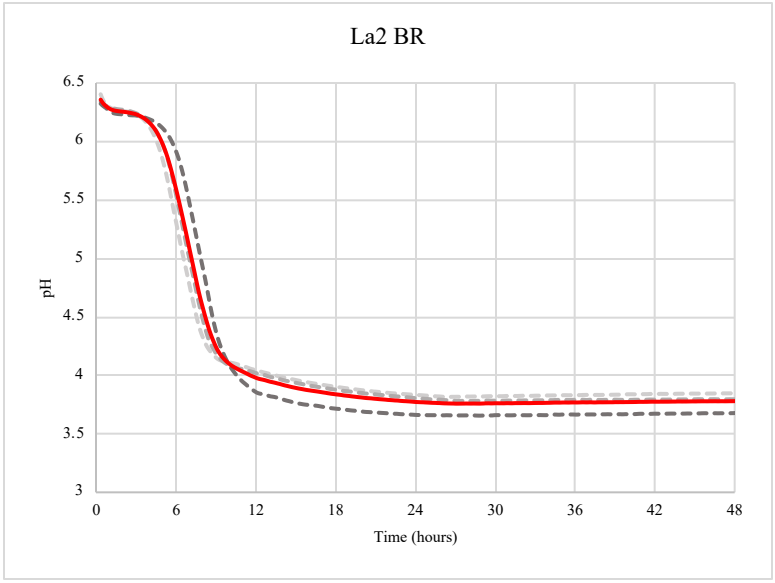

La2 2M
pH
Time (hours)

(e)





(g)



Gray dash lines: Fermentation reps 1, 2, 3
Red line: Average curve

Figure 3.7. La2 acidification curves of flour fractions and byproducts. (a) 1BK, (b), 2/3BK, (c) 1M, (d) 2M, (e) 3M, (f) shorts, (g) bran.

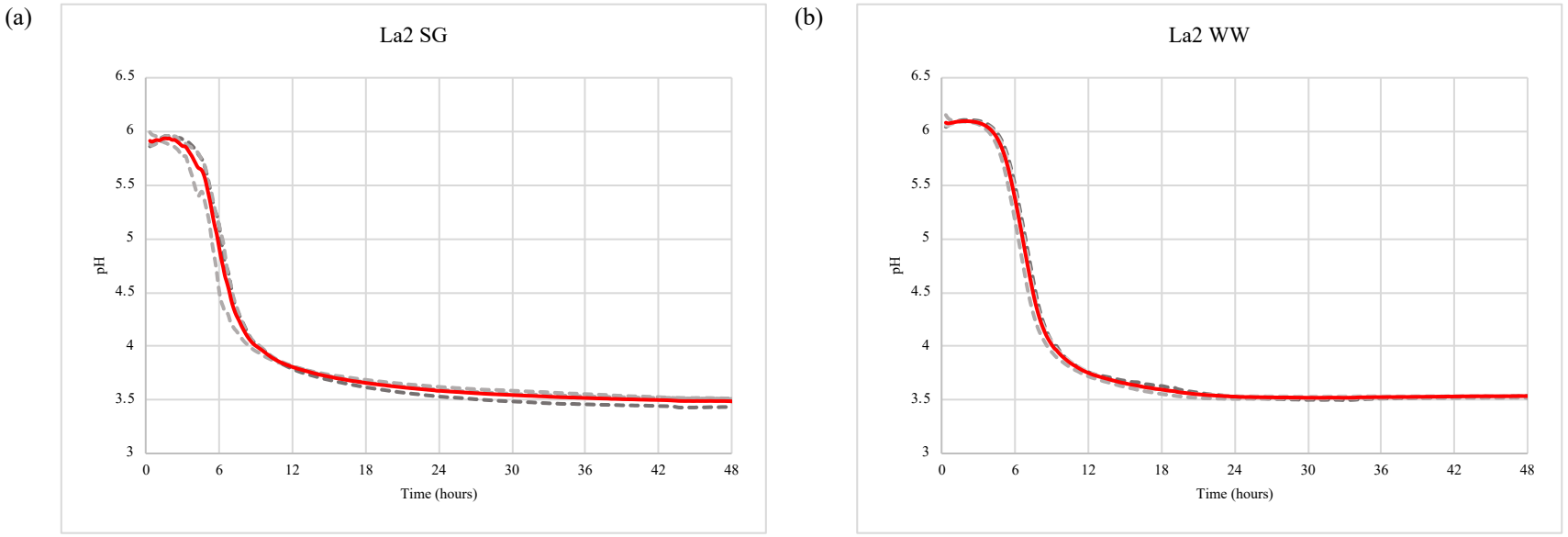
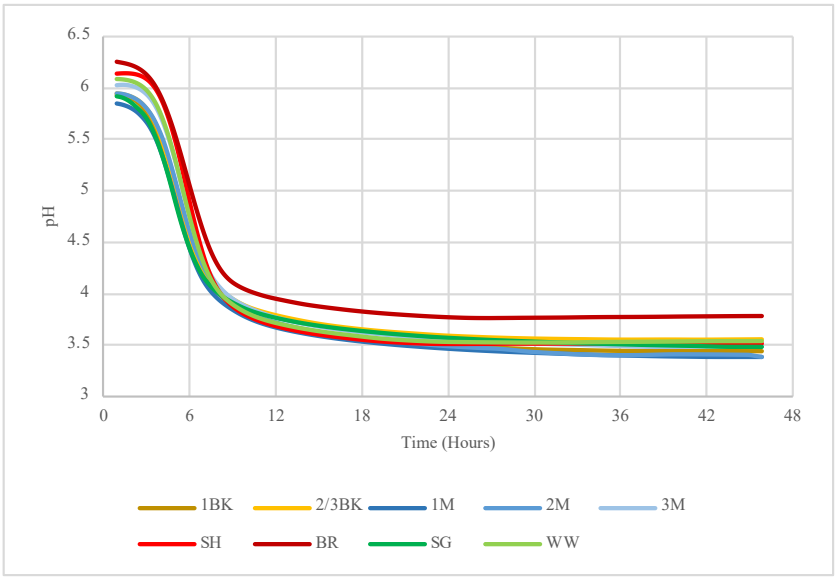


Figure 3.8. La2 acidification curves of composite flours (a) straight grade, (b) whole wheat.

Gray dash lines: Fermentation reps 1, 2, 3
Red line: Average curve

(a)



(b)

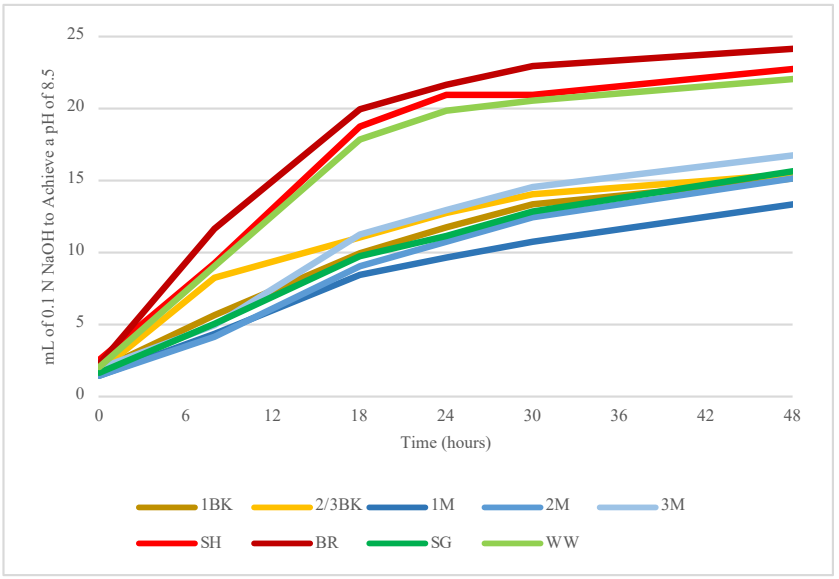


Figure 3.9. La2 (a) moving average curves in comparison, (b) TTA progression.

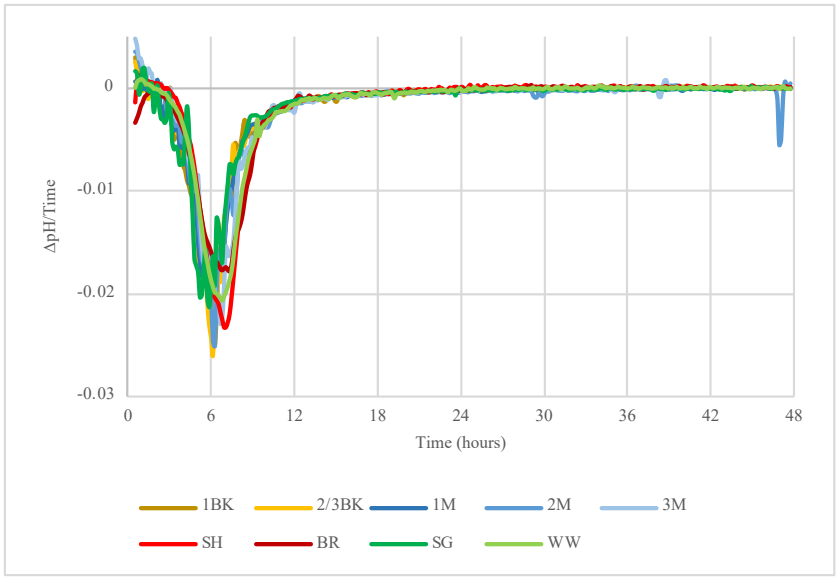
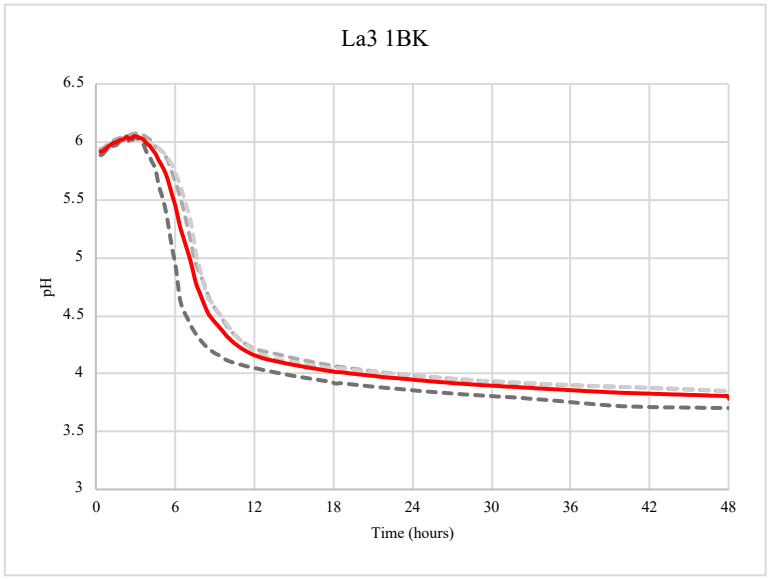
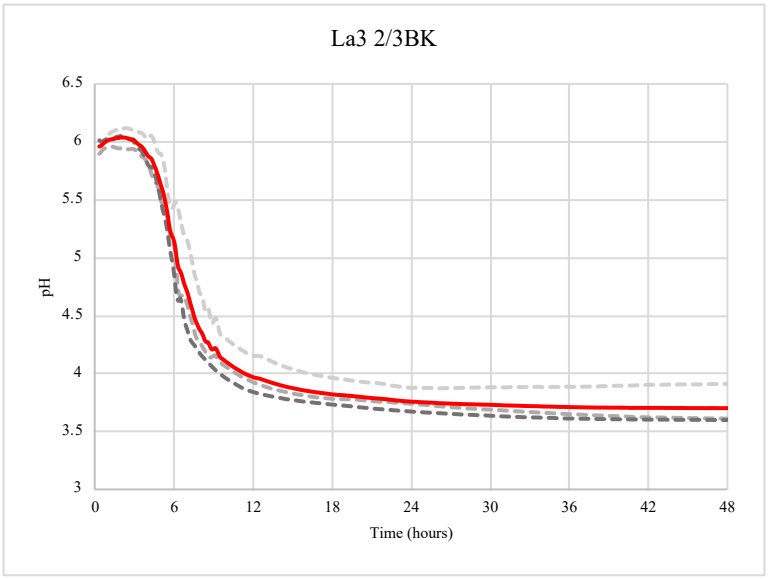


Figure 3.10. La2 change in acidification rate over time.

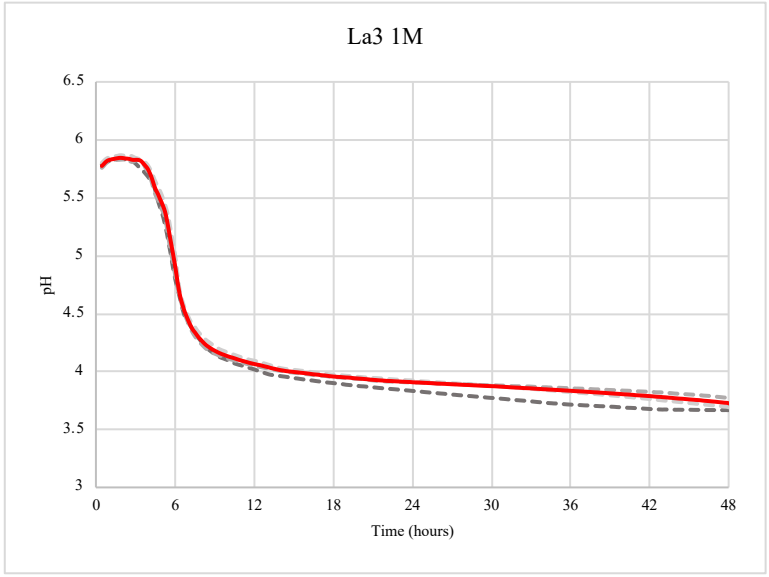
(a)


La3 1BK
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)

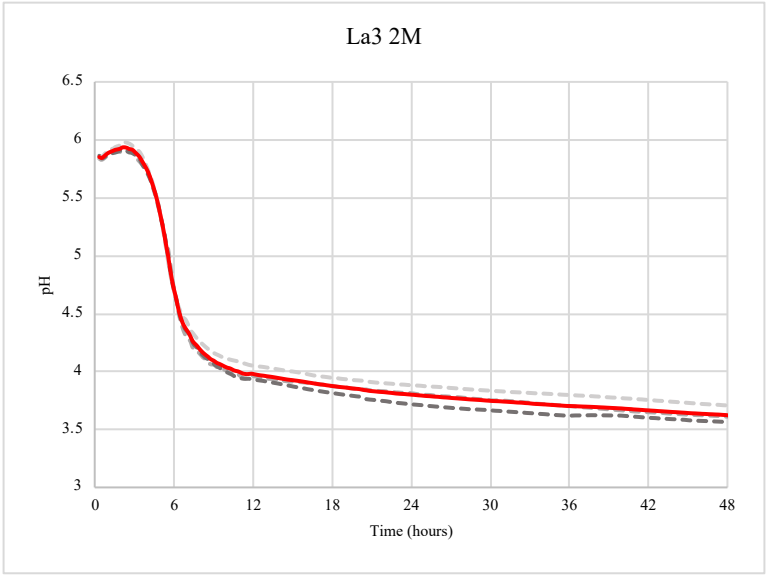
(b)


La3 2/3BK
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)

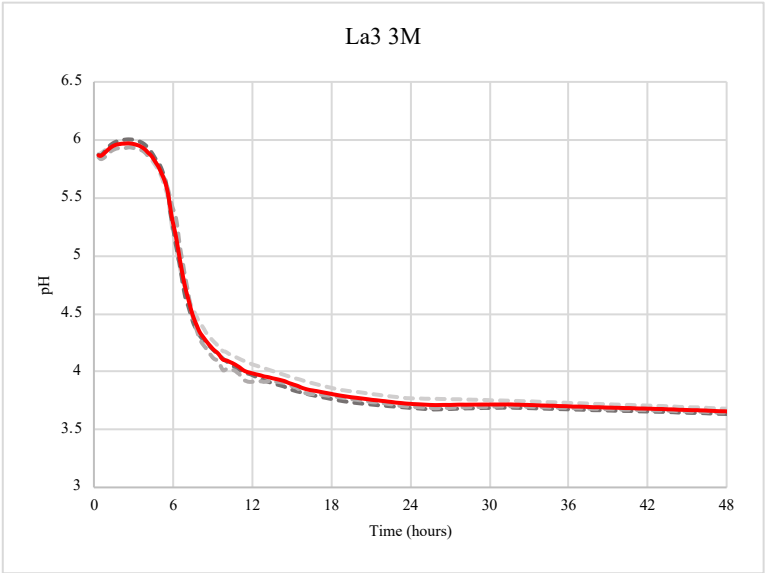
(c)


La3 1M
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)

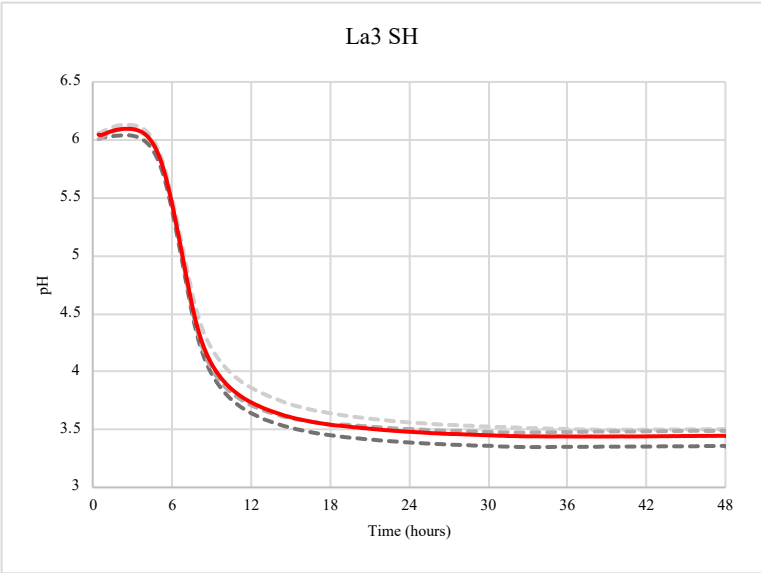
(d)


La3 2M
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)

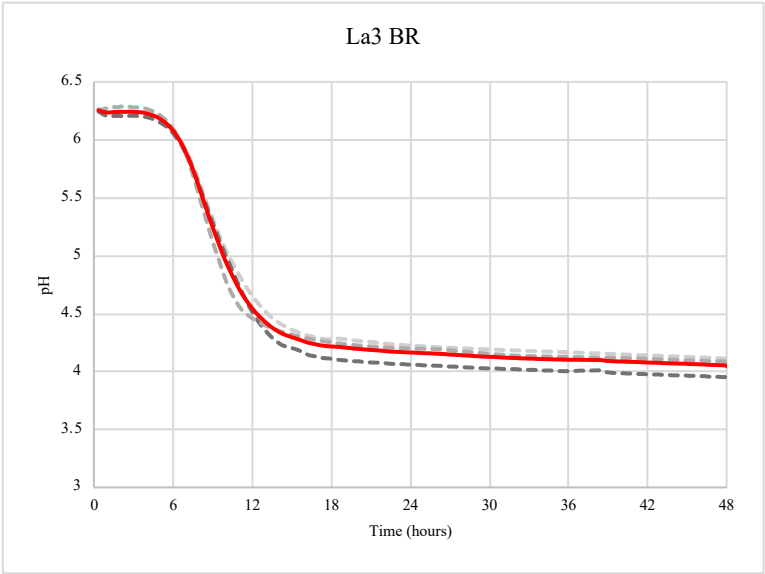
(e)



(f)



(g)



Gray dash lines: Fermentation reps 1, 2, 3
Red line: Average curve

Figure 3.11. La3 acidification curves of flour fractions and byproducts (a) 1BK, (b), 2/3BK, (c) 1M, (d) 2M, (e) 3M, (f) shorts, (g) bran.

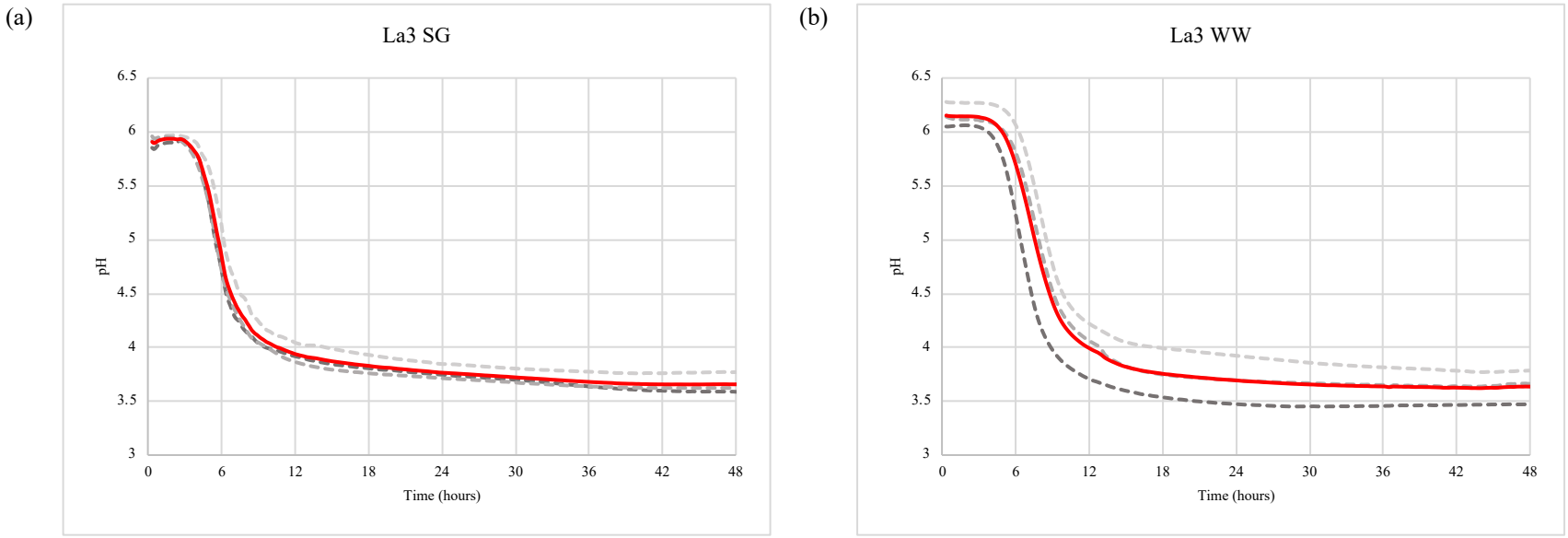
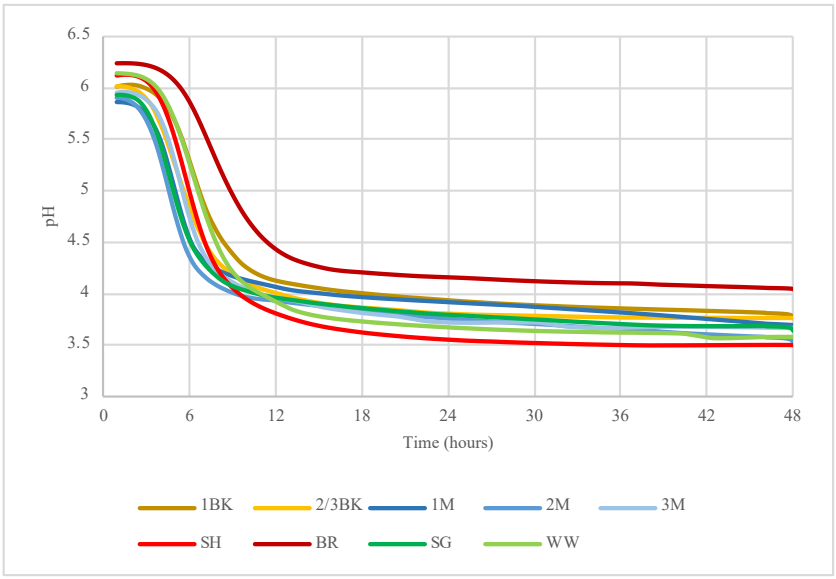


Figure 3.12. La3 acidification curves of composite flours (a) straight grade, (b) whole wheat.

Gray dash lines: Fermentation reps 1, 2, 3
Red line: Average curve.

(a)



(b)

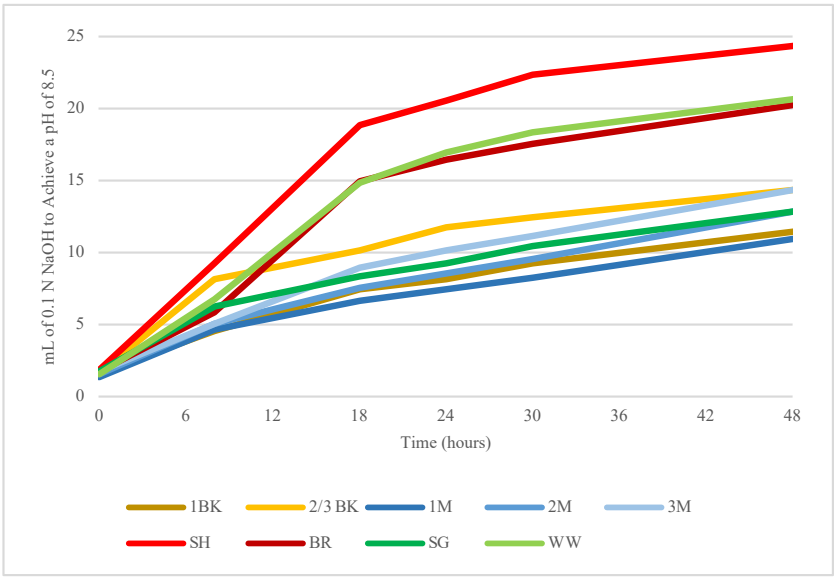


Figure 3.13. La3 (a) moving average curves in comparison, (b) TTA progression.

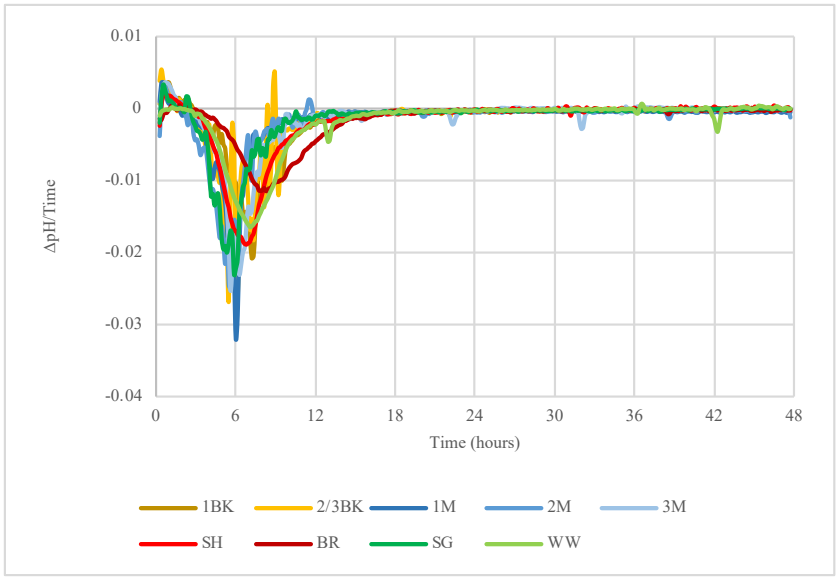
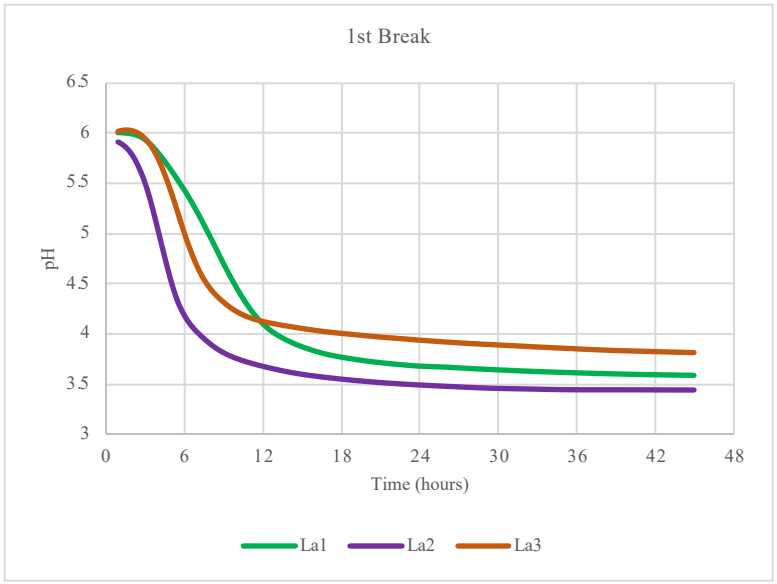
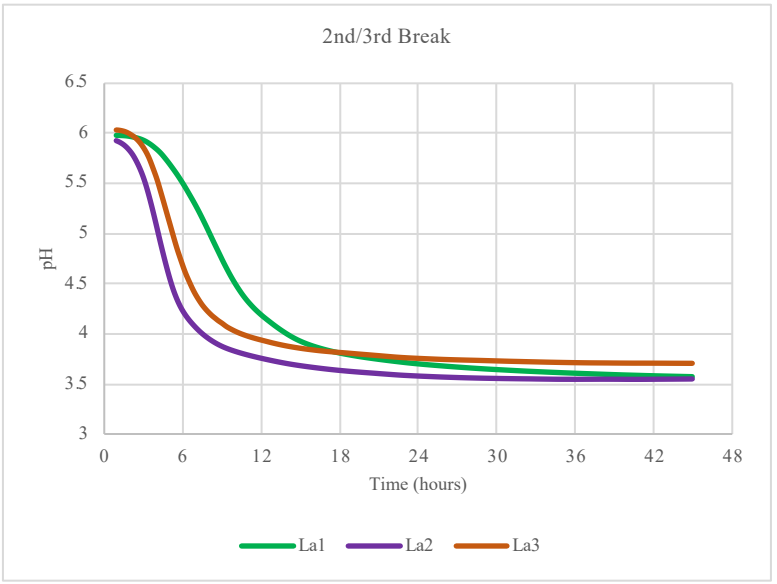


Figure 3.14. La3 change in acidification rate over time.

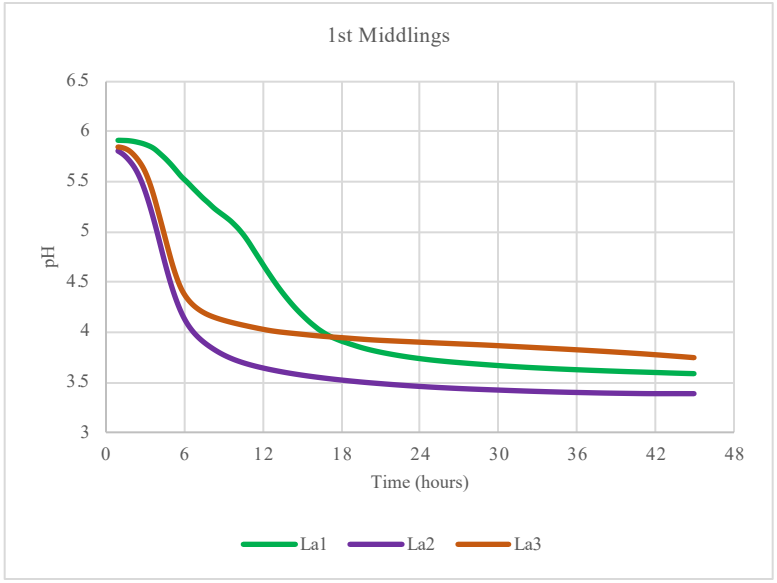
(a)


1st Break
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)
La1
La2
La3

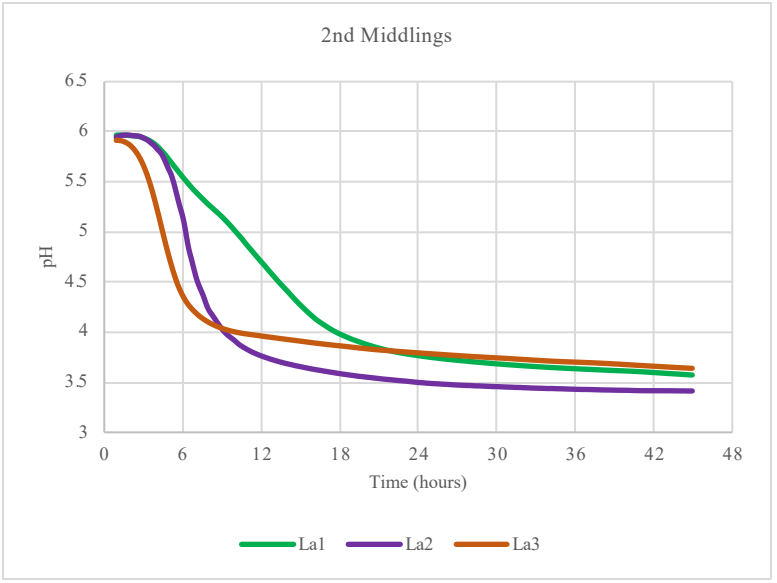
(b)


2nd/3rd Break
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)
La1
La2
La3

(c)


1st Middlings
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)
La1
La2
La3

(d)


2nd Middlings
pH
6.5
6
5.5
5
4.5
4
3.5
3
0
6
12
18
24
30
36
42
48
Time (hours)
La1
La2
La3

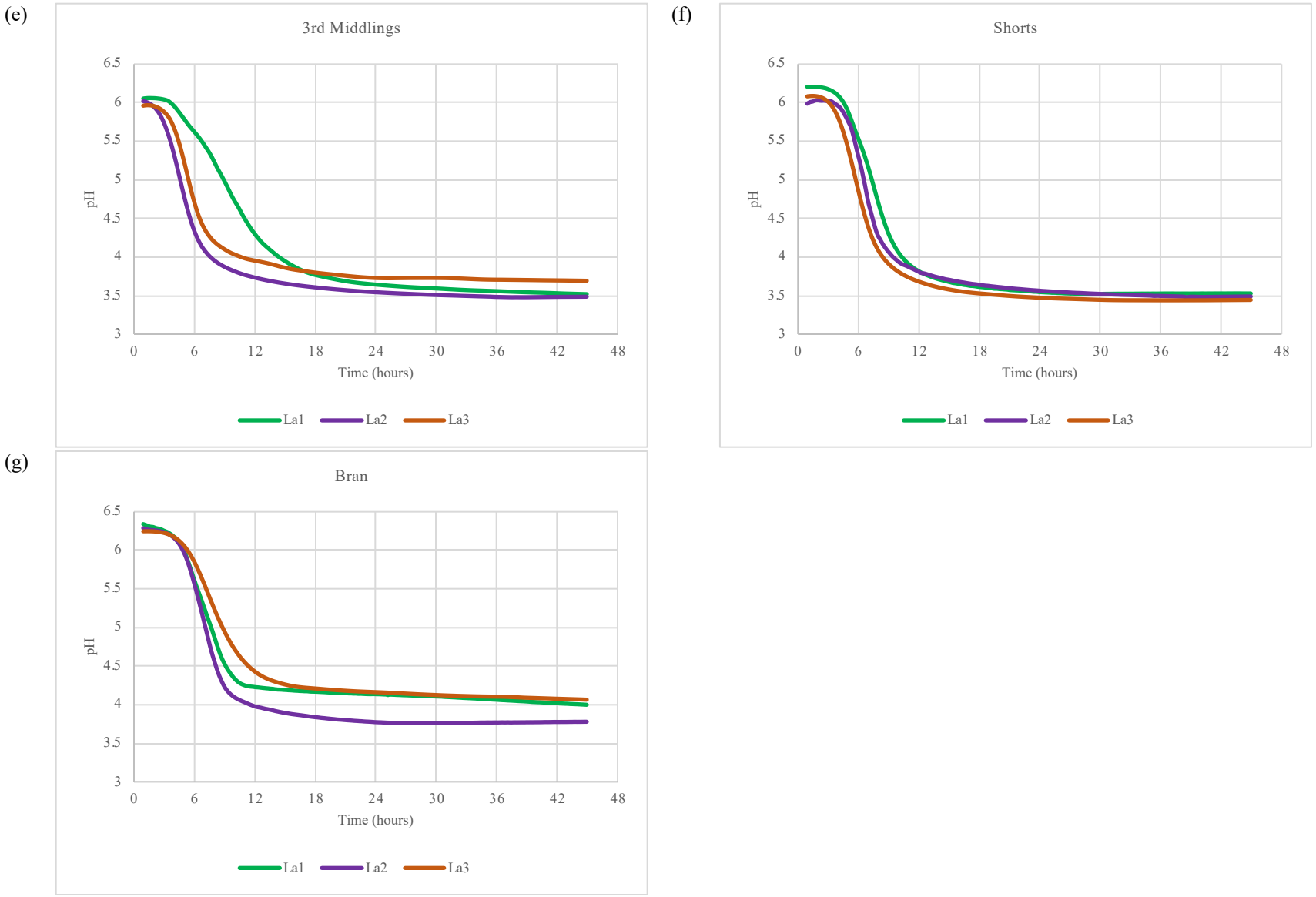


Figure 3.15. Acidification curve comparisons with respect to three strains (a) 1BK, (b), 2/3BK, (c) 1M, (d) 2M, (e) 3M, (f) Shorts, (g) Bran.

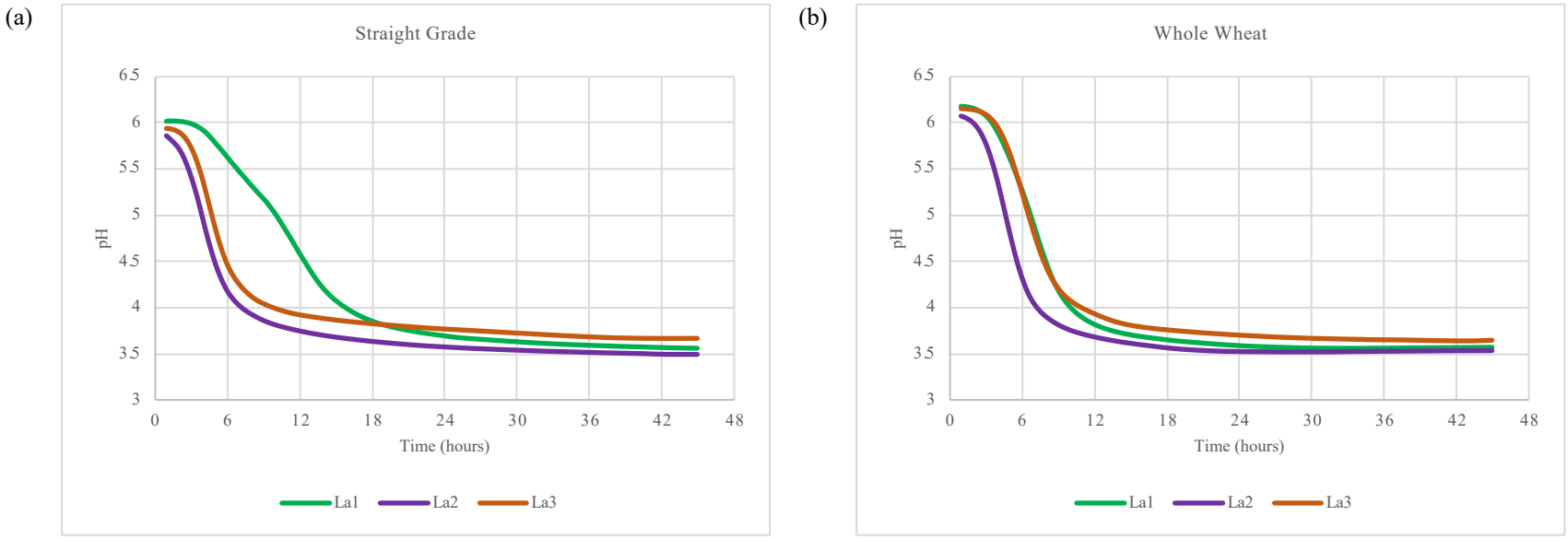


Figure 3.16. Acidification curve comparisons of composite flours with respect to three strains (a) straight grade flour, (b) whole wheat flour.

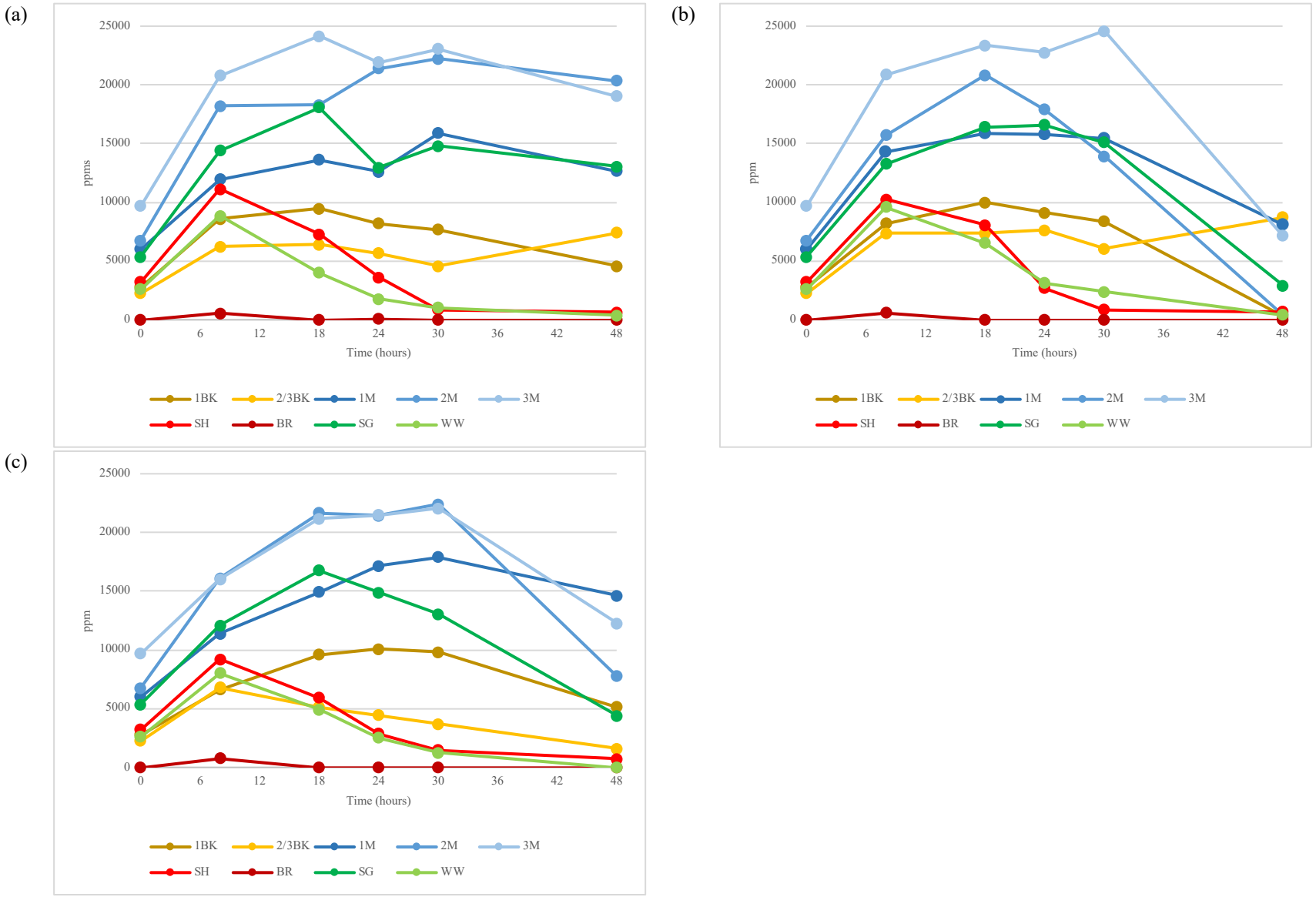


Figure 3.17. Disaccharide availability (a) La1, (b) La2, (c) La3.

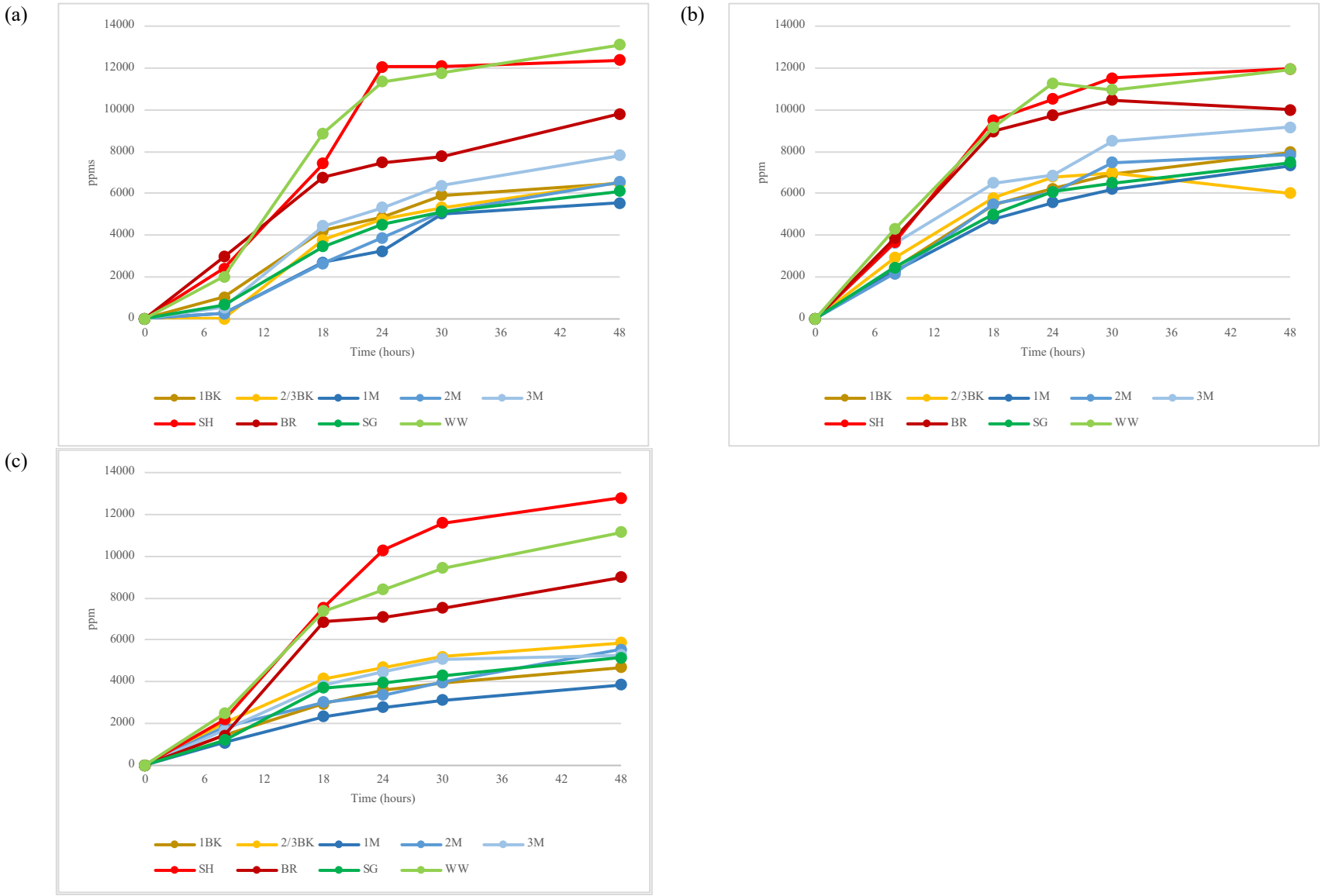


Figure 3.18. Lactic acid production (a) La1, (b) La2, (c) La3.

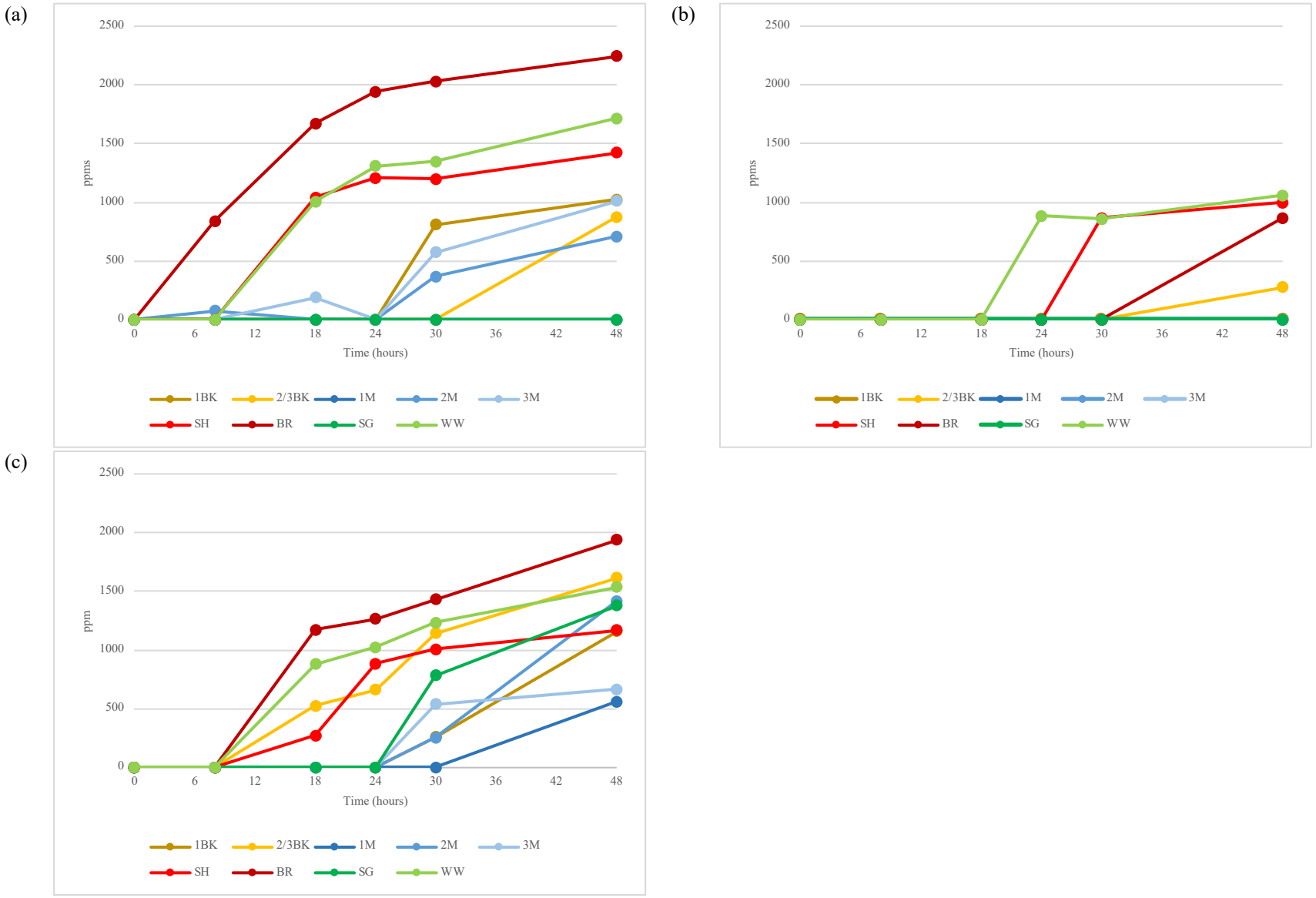


Figure 3.19. Acetic acid production (a) La1, (b) La2, (c) La3.

Figure 3.20. Principal component analysis (PCA) scatter plot.

Chapter 4 - Conclusions and Future Work

4.1. Milling

The experimental mill yielded 274.2 Kg of total flour to be used for analysis and fermentation. The flour extraction was 63.65%, resulting in more endosperm in the fractions typically with higher levels of bran. The 2BK and 3BK were combined (2/3BK) due to an insufficient amount of 3BK being milled.

4.2. Composition Characterization Analysis

The bran fraction had the highest amount of moisture, ash content, and every mineral measured, while the middlings were the lowest. The middlings were the highest percent starch, while bran was the lowest. From this it can be ascertained that the mineral content and starch content were negatively correlated. Due to the importance both mineral content and starch availability have on fermentation, the selection of strain to ferment these fractions is extremely important.

4.3. Physical Characterization

The bran had the highest average particle size or diameter, as well as the highest maximum particle size measured. This was expected as the particle size of the endosperm samples is continuously reduced as they go through various rolls, whereas the bran is often removed early in the milling process and remains intact. Relevant to fermentation, smaller particle sizes would increase substrate availability, so would an increase in surface area. Most of the particles were far more circular than they were elongated, thus reducing exposure to enzymatic activity. 2BK was characterized by the highest circularity, while the middlings were more elongated than the breaks. This was expected as they pass through smoother rollers compared to the breaks during their reduction in the mill.

4.4. Functional Characterization

The torque required to mix the fractions at different times at different temperatures varied from fraction to fraction. A pattern emerged between the middlings and the breaks. As the number of passes through rolls increased, so did the torque associated with protein strength, while the torque associated with starch gelatinization and retrogradation was reduced. The C4 value, measuring the enzyme activity, did not produce the same results as the enzyme testing

done on the same samples. From this, it can be concluded that the variation between the values at that point is not only due to the enzyme activity but is also likely due to starch content.

While the α-amylase did not have any significant difference between the fractions, the β-amylase did. β-amylase was the highest in 3BK and 2BK, followed by 2M. The remaining fractions all had non-significant differences. This makes observing differences between the fermentations caused by the amylase content difficult to perceive. Theoretically the higher the value of β-amylase, the more maltose units available for the LAB to easily consume, but this was not necessarily observable in the data collected.

4.5. Fermentation

The fermentation results elucidated the impact the strain selection and substrate availability have on the fermentation characteristics. It was found that each strain and substrate combination produced a unique set of fermentation characteristics. In general, La2 was the most effective strain at acidifying and producing acid. It had the fastest time to pH 4.0, lowest minimum pH values, and the highest maximum TTA values. La3 had the earliest decrease in pH, reflected in it having the steepest V_{max} as well as the earliest T_{max}, but was not able to ferment to a low enough pH like the other two strains. Finally, compared to the other strains, La1 lagged in all the fermentation characteristics, taking longer to acidify and not producing as much acid as the others. These clear differences can be used to more effectively select LAB strains to be used in industrial fermentation. The most effective fermentation of the La2 fractions was 1M, which achieved a pH of 4.0 just after eight hours. While La2 was overall the most effective at acidifying the sourdough, it does not mean it would produce a desired product. It had very low levels of acetic acid which gives sourdough its recognizable flavor. Similar to La1, La3 was able to achieve its fastest time to pH of 4.0 using the shorts. It was the only fraction to achieve that point under ten hours. To achieve a pH of 4.0 using a heterofermentative bacteria such as La1 or La3, a balanced fraction with both bran and endosperm is required to accelerate the acidification. For the homofermentative La2, the amount of available endosperm was more important than bran to accelerate the acidification.

Each of strains acidified in a unique way when observing them by fraction. For 1BK, La2 acidified earliest and had the steepest decline of the strains, followed by La3, and finally La1. This was a typical pattern seen in 2/3BK, 1M, 3M, straight grade fraction, and the whole wheat fraction. 2M was unique as La3 was the fastest to acidify but was not as steep as La2. It also did

not acidify to as low of a pH like La1 and La2 did. The shorts had a similar outcome but all three of the strains performed in a very similar way at each point of the fermentation. The bran was the only case in which La1 was faster at acidifying than another strain. In this case La3 took longer to acidify but the two heterofermentative bacteria had very similar acidification curves after 16 hours. The whole wheat had similar results to the shorts, but the extra endosperm available in the sample to the homofermentative La2 to consume caused it to have a more aggressive acidification compared to the other strains. This information shows the clear importance of strain selection when it comes to achieving desired fermentation characteristics during acidification.

Of all the fractions fermented, the most effective was the shorts. While it did not always have the fastest acidification for each strain, it was the most consistent acidifier. It provided La1 with the fastest time to a pH of 4.0, which was the only value comparable to the other time to pH 4.0 values from the other strains. The shorts' ability to consistently ferment regardless of strain and metabolic tendencies shows the balance needed between minerals provided from the bran, and substrate available for fermentation from the endosperm. Shorts has a starch content around 50-60% and has higher mineral content compared to the high endosperm samples, especially manganese, copper, zinc, and iron. The shorts milled for this experiment had a higher starch content than what would be seen in the industry, meaning if some of a shorts fraction from a different mill was fermented it might not result in the same consistent acidification. However, knowing the starch and mineral content could be used to blend a flour with similar specifications to achieve that consistent fermentation. The roll gaps in a mill could also be widened reducing the efficiency of the endosperm extraction, resulting in a high starch and mineral content shorts to be used for fermentation. Shorts, being considered a byproduct of the milling process, could be an optimum pairing with any of the strains to create a value-added product. Overall, La2 had the lowest dependency on substrate variation, while La1 and La3 were highly dependent on the substrate selection. La1 and La3 seemed to prefer the fractions with higher mineral content, La2 preferred those fractions with a starch content over 50%.

4.6. Sourdough Characterization

The usage of disaccharides during fermentation, as well as the production of acids and other metabolites helps in perceiving the metabolic activity each strain is experiencing depending on the fraction being metabolized. The production of acid not only decreases the pH impacting the structure and strength of the dough, but also the flavor and aroma of the final product. For

each strain, the fraction high in bran had the most overall lactic acid production. This is extremely important for La2, being homofermentative, it is the only metabolite it produces enough of to be recorded for each fraction. La2 did not produce an exorbitant amount of lactic acid compared to the other strains though. In fact, it produced the same amount or less for every fraction. La1 and La3, the heterofermentative strains, while producing lactic acid, also produced acetic acid. The high bran fractions produced the most out of all the fractions. This was also true for La2, the high bran fractions produced some acetic acid even though it is a homofermentative LAB strain. All three strains saw a similar usage of disaccharides during fermentation, beginning with an initial increase as they were cleaved from the starch molecules, and a gradual decrease as they were metabolized.

The metabolic pathway of each LAB studied can shed additional light into the results. La2 is homofermentative in nature, thus produced a larger amount of lactic acid over other metabolites compared to the other strains. Hexose degradation for homofermentative bacteria is carried out through the Emden-Meyerhoff pathway and yields only lactic acid. The other homofermentative pathway that is commonly used by LAB is the pentose phosphate pathway, degrading pentoses into lactic acid. This pathway requires more energy to produce the lactic acid than the Emden-Meyerhoff pathway though. As La2 did not have much dependency on substrate selection, either of these pathways could be utilized resulting in the steep decrease in pH and high production of lactic acid. The fractions higher in pentoses, the bran, shorts, and whole wheat, had similar acidification curves to the other fraction that have higher levels of hexoses. This shows the ability of La2 to acidify and produce lactic acid regardless of which sugar is available for fermentation.

La1 and La3 had very different acidification compared to La2, which is expected as they are heterofermentative bacteria. They utilize the phosphoketolase pathway to degrade hexoses into lactic acid and ethanol, and pentoses into lactic and acetic acid. This pathway requires a cofactor of thiamin diphosphate. Thiamin diphosphate is the active form of Vitamin B1. Whole wheats have been to have adequate levels of thiamin (Shewry and Hey, 2015). From the mineral analysis done for this study, it is also known that fractions containing higher amount of bran have higher levels of phosphorus. With the availability of the thiamin diphosphate as a cofactor, it can be stated that the heterofermentative strains, La1 and La3, could utilize the phosphoketolase pathway. This is further supported by examining the correlation between

phosphate availability and fermentation characteristics for La1 and La3. For La1, phosphorus was found to be correlated with the minimum pH and the maximum TTA value. For La3, it was correlated with time to pH 4.0, V_{max}, and T_{max}. Similar to how La2 can utilize different pathways depending on the substrate availability, both La1 and La3 have the ability to utilize either of the phosphoketolase pathways depending on the availability of substrate. However, La1 and La3 cannot change as easily seeing as from the results in this study, their acidifications are more dependent on the substrate used to ferment. Those fractions that produced higher amounts of acetic acid, the bran, shorts and whole wheat haver higher amounts of pentosans that can be fermented using the pentose phosphoketolase pathway. The other fractions that have higher amounts of endosperm, and in turn hexoses, produce more ethanol as they use the hexose phosphoketolsase pathway. All of the bacteria were able to utilize the most effective metabolic pathway to produce their metabolites, that then impact the characteristics of each sourdough, reiterating the importance of both substrate and strain selection when fermenting sourdough.

4.7. Overall Conclusions

Overall, it was found that each strain uniquely acidified regardless of the fraction being fermented. As the strains utilize different metabolic pathways this is expected. The fermentations show the various characteristics that can be achieved by formulating with different strains and substrates. The characteristics of the fractions being used, especially the mineral content, impact the acidification and can be used to alter the rate at which acidification occurs. This research provides information on how the strains behave and adapt depending on the metabolite provided. The strain and substrate selection can also be used to vary the metabolite production during fermentation. This can be used to achieve desired compounds and flavor profiles to be applied to industrial baking.

La2 was the most effective of the strains at acidifying. Being the only homofermentative this is expected as it is producing only lactic acid to decrease the pH. La3 had the earliest decrease in pH but could not achieve a low enough pH any faster than the other two strains. La1 lagged in the fermentation characteristics compared to the other strains, but shared some similar attributes to La3, as they are both heterofermentative. Examining the substrates, shorts was the most applicable to be used with any of the strains tested. While not always the most effective, it was the most consistent from strain to strain. Shorts had the ideal blend of available carbohydrate

and minerals to be used in sourdough fermentation. La2 preferred the more endosperm-based samples, especially the middlings, while the other strains preferred those fractions high in bran.

4.8. Future Work

In this study, there were certain process parameters that could be adjusted in an attempt to achieve variation in the results. The absorption in this experiment was adjusted to achieve consistent viscosity between the fractions to ensure sufficient hydration of all dry matter while allowing the mixing paddle to effectively mix the sample. This causes multiple factors being adjusted, which could cause some differences in results. To prevent this, one solution could involve reducing the overall particle size of the shorts and bran to increase absorption and reduce the amount of water required to hydrate the sample. Micronizing the bran particles does change the availability of substrate for fermentation, which would most likely yield dissimilar results from what was previously found in this study. The reduction of bran particle size would also create a need for another processing step to achieve a usable substrate but could provide more insight into how results may differ with constant hydration and inoculation.

This research can easily act as a starting point for many future projects further exploring increasing the efficiency of sourdough acidification. The first area to further explore is the different available strains of LAB that are commonly found in sourdough. *Fructilactobacillus sanfranciscensis* would be an interesting sample to examine as it is abundant in sourdoughs worldwide. While it is a heterofermentative strain like La1 and La3, it shows very different and unique fermentation characteristics. Other homofermentative such as *Lactobacillus amylovorus* and *Lactobacillus delbrueckii* could be explored as strains that utilize the Emden-Meyerhoff or the pentose-phosphate pathway. The next area of study to further explore is how controlling the sourdough process could impact the characteristics of the end-product. A similar process of analysis and characterization could be carried out as was done in this study, with an added analysis of impact on the actual baked goods to better understand impact on consumers. The third area to further explore would be the use of different grains in fermentation. While there are many published articles about this area, there is a lack of studies that examine the individual fractions from the grains. An experiment could be designed in a similar fashion, examining mill streams from a grain cultivar to gain a better understanding of what fraction most impacts the fermentation and acidification. It would be interesting to see how different fractions from rye, a grain synonymous with traditional sourdough, are different from each other and other grains.

There are plenty of opportunities in the future, as the interest in sourdough research continues to rise over the coming years, there will always be a need for novel research on the subject.

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Appendix A - Supplemental Information

Table A1. La1 fermentation characteristic correlations

Variable	*La1 max TTA*		*La1 min pH*		*La1 time to pH 4.0*		*La1 Tmax*		*La1 Vmax*	
	Correlation	**p-value**	**Correlation**	**p-value**	**Correlation**	**p-value**	**Correlation**	**p-value**	**Correlation**	**p-value**
α-amylase	-0.73	0.0266*	-0.40	0.2821	0.21	0.5957	0.23	0.5485	-0.14	0.7291
β-amylase	-0.31	0.4153	-0.03	0.9438	0.27	0.4747	0.23	0.5430	0.66	0.0546
Starch	-0.72	0.0301*	-0.86	0.0027**	-0.06	0.8747	0.62	0.0750	0.08	0.8297
Damaged starch	0.45	0.2191	0.86	0.0030**	0.39	0.2993	-0.32	0.3949	-0.19	0.6218
Ave particle size	0.75	0.0210*	0.54	0.1369	-0.33	0.3836	-0.71	0.0317*	0.04	0.9235
Circularity	-0.40	0.2819	-0.37	0.3309	-0.16	0.6748	0.07	0.8514	0.62	0.0749
Angularity	0.43	0.2522	-0.03	0.9443	-0.18	0.6441	-0.14	0.7232	-0.84	0.0042**
Ash	0.69	0.0416*	0.89	0.0013**	0.12	0.7666	-0.59	0.0955	-0.08	0.8374
Ca	0.63	0.0713	0.10	0.7975	-0.41	0.2751	-0.14	0.7184	0.03	0.9399
Cu	0.75	0.0209*	0.83	0.0052**	0.02	0.9511	-0.62	0.0778	-0.13	0.7320
Fe	0.70	0.0351*	0.88	0.0019**	0.06	0.8851	-0.63	0.0674	-0.04	0.9157
K	0.69	0.0395*	0.89	0.0015**	0.10	0.7908	-0.60	0.0896	-0.08	0.8400
Mg	0.76	0.0171*	0.64	0.0652	-0.04	0.9207	-0.49	0.1815	-0.39	0.2945
Mn	0.73	0.0252*	0.85	0.0038**	0.05	0.9070	-0.61	0.0796	-0.10	0.7893
P	0.69	0.0379*	0.88	0.0016**	0.10	0.7946	-0.59	0.0916	-0.09	0.8185
SO4-S	0.73	0.0244*	0.79	0.0107*	0.00	0.9926	-0.59	0.0960	0.01	0.9850
Zn	0.74	0.0226*	0.83	0.0054**	0.04	0.9090	-0.59	0.0943	-0.10	0.7904
Water absorption	0.73	0.0252*	0.82	0.0064**	0.02	0.9539	-0.62	0.0759	-0.10	0.8075

Note:
*p-value*** indicates *p-value* < 0.01
*p-value** indicates *p-value* < 0.05

Table A.2. La2 fermentation characteristic correlations

Variable	*La2 max TTA*		*La2 min pH*		*La2 time to pH 4.0*		*La2 Tmax*		*La2 Vmax*	
	Correlation	**p-value**	**Correlation**	**p-value**	**Correlation**	**p-value**	**Correlation**	**p-value**	**Correlation**	**p-value**
α-amylase	-0.79	0.0120*	-0.59	0.0942	-0.35	0.3573	-0.53	0.1462	-0.51	0.1570
β-amylase	-0.37	0.3308	-0.05	0.8922	-0.05	0.8980	-0.54	0.1335	-0.26	0.4972
Starch	-0.84	0.0043**	-0.89	0.0014**	-0.86	0.0028**	-0.73	0.0248*	-0.73	0.0264*
Damaged starch	0.63	0.0676	0.72	0.0278*	0.83	0.0056**	0.59	0.0946	0.68	0.0425*
Ave particle size	0.79	0.0105*	0.62	0.0721	0.51	0.1582	0.72	0.0287*	0.58	0.1044
Circularity	-0.56	0.1198	-0.37	0.3287	-0.51	0.1597	-0.65	0.0601	-0.32	0.4037
Angularity	0.46	0.2110	0.10	0.8012	0.17	0.6644	0.63	0.0712	0.25	0.5209
Ash	0.82	0.0067**	0.89	0.0013**	0.88	0.0019**	0.71	0.0321*	0.74	0.0227*
Ca	0.55	0.1231	0.40	0.2895	0.11	0.7872	0.33	0.3811	0.43	0.2430
Cu	0.87	0.0022**	0.88	0.0019**	0.85	0.0041**	0.77	0.0156*	0.73	0.0269*
Fe	0.83	0.0060**	0.90	0.0009**	0.87	0.0021**	0.71	0.0322*	0.71	0.0329*
K	0.83	0.0061**	0.89	0.0015**	0.88	0.0020**	0.72	0.0287*	0.73	0.0242*
Mg	0.87	0.0021**	0.73	0.0252*	0.70	0.0367*	0.82	0.0074**	0.69	0.0410*
Mn	0.86	0.0030**	0.87	0.0022**	0.85	0.0041**	0.76	0.0187*	0.74	0.0232*
P	0.83	0.0056**	0.89	0.0014**	0.88	0.0020**	0.72	0.0273*	0.73	0.0245*
SO4-S	0.82	0.0070**	0.94	0.0002**	0.83	0.0059**	0.62	0.0752	0.64	0.0617
Zn	0.87	0.0023**	0.86	0.0032**	0.83	0.0052**	0.77	0.0162*	0.73	0.0255*
Water absorption	0.86	0.0031**	0.85	0.0036**	0.82	0.0068**	0.75	0.0189*	0.71	0.0310*

Note:
*p-value*** indicates *p-value* < 0.01
*p-value** indicates *p-value* < 0.05

Table A.3. La3 fermentation characteristic correlations

Variable	*La3 max TTA*		*La3 min pH*		*La3 time to pH 4.0*		*La3 Tmax*		*La3 Vmax*	
	Correlation	***p-value***	**Correlation**	***p-value***	**Correlation**	***p-value***	**Correlation**	***p-value***	**Correlation**	***p-value***
α-amylase	-0.79	0.0110*	0.06	0.8719	-0.30	0.4295	-0.50	0.1753	-0.70	0.0366*
β-amylase	-0.31	0.4238	0.05	0.8955	-0.11	0.7703	-0.34	0.3684	-0.49	0.1830
Starch	-0.68	0.0435*	-0.55	0.1250	-0.85	0.0040**	-0.85	0.0039**	-0.78	0.0136*
Damaged starch	0.40	0.2833	0.63	0.0719	0.81	0.0085**	0.60	0.0898	0.71	0.0306*
Ave particle size	0.75	0.0209*	0.23	0.5601	0.58	0.1021	0.86	0.0032**	0.74	0.0219*
Circularity	-0.47	0.1978	-0.05	0.9015	-0.29	0.4445	-0.26	0.5075	-0.48	0.1960
Angularity	0.50	0.1722	-0.28	0.4629	-0.06	0.8772	0.12	0.7507	0.24	0.5283
Ash	0.64	0.0646	0.60	0.0907	0.87	0.0023*	0.83	0.0052**	0.78	0.0125*
Ca	0.50	0.1741	-0.01	0.9829	0.09	0.8083	0.42	0.2619	0.41	0.2727
Cu	0.72	0.0290*	0.50	0.1701	0.81	0.0075**	0.85	0.0041**	0.79	0.0113*
Fe	0.65	0.0567	0.59	0.0933	0.87	0.0022**	0.87	0.0022**	0.77	0.0158*
K	0.64	0.0608	0.59	0.0951	0.87	0.0023**	0.84	0.0042**	0.79	0.0113*
Mg	0.78	0.0125*	0.24	0.5371	0.58	0.0984	0.67	0.0486*	0.73	0.0263*
Mn	0.70	0.0361*	0.52	0.1492	0.83	0.0056**	0.85	0.0039**	0.80	0.0092**
P	0.65	0.0577	0.58	0.1004	0.86	0.0027**	0.84	0.0046**	0.79	0.0117*
SO4-S	0.71	0.0316*	0.47	0.2024	0.75	0.0200*	0.77	0.0158*	0.61	0.0825
Zn	0.72	0.0282*	0.48	0.1909	0.80	0.0091**	0.83	0.0059**	0.81	0.0086**
Water absorption	0.72	0.0286*	0.47	0.1987	0.80	0.0095**	0.83	0.0054**	0.79	0.0109*

Note:
*p-value*** indicates *p-value* < 0.01
*p-value** indicates *p-value* <