

A study on the evaluation of the quality of Bx7^{OE} wheat varieties

by

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Abstract

Improving the end use quality of wheat remains a primary objective for hard winter wheat (HWW) breeders, particularly where bread production is the primary end product. In recent years, one focus has been on the *Glu-B1a1*, which overexpresses the Bx7 glutenin allele, sometimes leading to improvements in dough rheology. While there are numerous studies regarding genetics, molecular characterization, and rheology of wheat lines containing Bx7^{OE}, few have analyzed the baking performance. It has been shown that certain Bx7^{OE} wheats can be significantly stronger than non-overexpressed wheats, while milling extraction, and physical grain characteristics are relatively similar to non-overexpressed varieties. In the existing research regarding the Bx7^{OE} varieties, there is a gap in identifying the appropriate testing methods for the varieties with extreme strength, which surpass standard protocols and current quality targets. The impact of such varieties on end product testing has also been poorly characterized to date. To address these gaps, the following objectives were established for the study. The individual overexpressed varieties were characterized for kernel parameters, experimental milling performance, whole meal and refined flour composition, and dough rheology (GlutoPeak, Farinograph, Alveograph and Mixograph). Blends were created between overexpressed varieties and non-overexpressed base flours in several percentages to assess whether blending could decrease test time while still discriminating quality. Blending was also assessed as a strategy to improve baking quality of base flours, under a standard straight dough method. Finally, a model was developed to determine how the rheological parameters explain the variance of loaf specific volume.

The results indicated that the GlutoPeak instrument was able to discriminate quality for both whole meal and refined flours, though more so with refined flour. For the blend of

overexpressed and base flours, the results demonstrated that at low levels (5-10%) discrimination was diminished as compared to higher levels and to testing individual flours.

Dough rheology testing of refined flours revealed that blending was effective in reducing test time while still allowing discrimination in the quality of rheological parameters, particularly using the Farinograph. Baking revealed that the significant differences seen in rheology did not translate into quality improvements in bread (specific volume range: 3.98-4.57 g/mL). Modeling of rheological parameters for loaf specific volume resulted in only very modest explanatory power ($R^2=0.139$, $p<0.001$).

Based on these results, from a practical perspective, the GlutoPeak testing of both refined and whole meal flour is an appropriate testing method for the Bx7^{OE} lines. The findings presented in this study also suggest that Bx7^{OE} lines can be blended into base flours to improve stability of bread doughs, with no impact on final bread volume. Additionally, both dough rheology and end product quality must be considered together as targets for further wheat quality improvement.

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Preface

Part of my decision to stay at Kansas State University and complete my masters was this project. It was novel and contained industrial significance. When I began this project, the conclusion seemed straightforward; it was only a matter of conducting the tests to get to the conclusion. It was expected the overexpression would improve bread quality. This was exciting as it was useful for many facets of the industry. As I continued to test and conduct data analysis, the straightforward answer became less straightforward. There was no difference in the final bread quality. For a moment, all the time spent milling, conducting rheological tests, and test baking all 200 samples seemed slightly wasted. In reality, the research became more interesting.

The answer was not straightforward, which created fascinating discussions amongst my advisors and friends. It required me to think deeply and stretch my knowledge base to try to explain why results were unexpected. It reminded me that our most educated decisions and calculated responses may not give the intended outcome. That is okay; in fact, it is good. As I am finishing my time here at KSU, I did not provide the next game-changing solution. Nor did I provide a definitive answer of why the expected was not achieved. In fact, I am left with more questions than answers. There is still value in what I have written and concluded, and I did a large amount of testing someone else does not have to do. I believe my personal and professional growth was greater because the answer was not obvious. After all, most of life is mundane, giving your best in the ordinary.

I still do not fully know the industrial relevance of overexpressed wheat. There are still many things that would be interesting to test and try. My time, however, was not wasted. Not scientifically nor personally. As my advisor reminded me, unexpected results are not equal to failure. They allow for good, thought-provoking discussion.

Chapter 1 - A Review on the Current Understanding of Overexpressed versus Non-overexpressed *Glu-B1al* alleles in Hard Wheat Varieties

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Introduction

Wheat breeders seek to find ways to improve hexaploid wheat to increase productivity, disease resistance, and end product quality. Regarding quality, the main focus lies in the protein quantity and quality associated with optimal performance for a specific product. For hard winter wheat (HWW), the end-product of interest is bread, representing an estimated 70% of total HRW utilization.

One of the more recent focuses for HWW wheat breeders has been on the *Glu-B1al* allele. The overexpression of the Bx7 protein subunit was first encountered as a natural phenomenon in bread wheat when the Bx7 subunit was duplicated. This was first located in the wheat variety Red River 68 through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in an article published in 1997 (D'Ovidio et al., 1997). Before the allele entered wheat, however, it has been traced back to a Uruguayan landrace of unknown origin known as Americano 44D from the mid-19th century. The original cultivar of the *Glu-B1al* was an Argentinean landrace known as Klein Universal II (Butow et al., 2004). The allele encodes for high molecular weight (HMW)

glutenin subunit and has been associated with improvements in dough rheology and bread volume. This review covers the current state of the art of Bx7^{OE} wheat varieties, particularly as part of HWW improvement for breeders in the Central Plains region.

Genetics

Wheat has three genes located on the long arms of the Group 1 chromosomes which are the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci (J. Li et al., 2014; Rasheed et al., 2019). Each of these gene loci encode an x-type and y-type subunit leading to numerous combinations of alleles (Rasheed et al., 2019). The HMW subunits are determined by the combination of the six paralogous loci possible; however, usually only three to five subunits are expressed in a given genotype. Some of the subunits are silenced; in fact, Subunit A_y is almost always suppressed in bread wheat. On the other hand, the B_x, D_x, and D_y subunits are usually expressed, and B_y and A_x subunits are expressed in some genotypes (Rasheed et al., 2014). Many allelic variations of the *Glu-B1* and *Glu-D1* loci are known, which contribute to differences in wheat quality. In contrast, the *Glu-A1* locus is known to be less variable (Lee et al., 2023). The x-type subunits have comparatively higher molecular weights than the y-type subunits (Rasheed et al., 2014). Due to the genetic variation of the high molecular weight glutenin subunits, there is significant variation in the quality of wheat across different genotypes (Rasheed et al., 2014). Though the molecular weights are different, the structure of the x- and y-type subunits are quite similar, each containing a signal peptide, an N-terminal domain, a repetitive domain in the center, and a C-terminal domain (Rasheed et al., 2014). The x-type subunit contains a unique tri-peptide motif, and the y-type subunit has a leucine residue instead of a second proline in its repeat sequence (Rasheed et al., 2014). Another difference in structure lies in the x-type subunits having four cysteine residues whereas the y-type subunits have seven cysteine residues (Rasheed et al., 2014, 2019). Different

alleles impact the end use quality of the grain differently, and since quality is the main factor impacted, targeting specific alleles is an important part of producing new wheat varieties (Rasheed et al., 2019). Therefore, understanding the alleles that give an increase in wheat quality is important, as is characterizing the change in quality.

The *Glu-B1a1* allele encodes the overexpression of the Bx7 allele (J. Li et al., 2014; Rasheed et al., 2019). Within the *Glu-B1* locus, there are three homologous x-type subunits which have been characterized as Bx7, Bx7*, and Bx7^{OE} (Lee et al., 2023). Among these, the Bx7^{OE} has become an allele of interest in research, particularly in recent years due to its effects on quality. The overexpression of the Bx7 HMW glutenin subunit likely resulted from a single duplication at the *Glu-B1* locus which was accompanied by the insertion of a retroelement ultimately leading to the Bx7 allele being overexpressed. This mutation event is likely rare as it has only been seen in the *Glu-B1a1* allele and has only occurred in bread wheat (J. Li et al., 2014). The result was a 10.3 Kb segmental duplication and the flanking of a 43 bp long terminal repeat retroelement (Ragupathy et al., 2008). This is understood as a single event since only the varieties with the Bx7^{OE} trait have the retroelement and the duplication genomic structure (Ragupathy et al., 2008). Based on current knowledge, the *Glu-B1a1* allele is the only HMW-GS that has experienced overexpression (J. Li et al., 2014; Ragupathy et al., 2008). The overexpression causes an increase in the amount of Bx7 subunits in the wheat. Usually in bread wheat, the proportion of the Bx7 unit to total HMW-GS is 30%, but over-expression increases the proportion of Bx7 subunit to about 42% (J. Li et al., 2014; Ragupathy et al., 2008). Interestingly enough, wheat containing Bx7^{OE} is fairly limited, but is present in a few bread wheat varieties in the Americas (Lee et al., 2023; J. Li et al., 2014). Canada and Argentina have produced the most wheat varieties with the Bx7^{OE} trait, but it is also present in the United States,

Mexico, and Brazil at lower frequency (J. Li et al., 2014). A few other countries have the trait present in at least one wheat variety, but across the world, the trait is most prevalent in North and South America (J. Li et al., 2014; Ragupathy et al., 2008). Glenlea is one of the most common Canadian wheats with the overexpression that is present in literature. However, since varieties expressing the Bx7^{OE} trait have been seen to improve quality, even though there are few genotypes, breeders are able to capitalize on the trait through traditional breeding methods increasing the quality of bread wheat (Lee et al., 2023; J. Li et al., 2014; Rasheed et al., 2019).

Characterization

Characterizing the Bx7^{OE} trait is essential to allow breeders to identify what crosses and genotypes confer a desired level of gluten strength. Several methods are used to characterize the Bx7^{OE} trait. First, SDS-PAGE can be used to differentiate the target subunit from other variants, but the sensitivity of this method is relatively low (J. Li et al., 2014). The protein is stained and the resulting band intensity of the subunit compared to the Bx7 allele indicates the presence of the Bx7^{OE} allele (Ragupathy et al., 2008). The band of Bx7^{OE} is thicker than that of the Bx7 (S. Li et al., 2020), up to double the size (D'Ovidio et al., 1997). Another possible method for characterization of the Bx7^{OE} allele is Reverse- Phase High Performance Liquid Chromatography (RP-HPLC) which allows the proportion of a specific HMW-GS to total HMW-GS to be determined (J. Li et al., 2014; S. Li et al., 2020; Ragupathy et al., 2008). In the studies conducted, there is some variation in the proportion of Bx7^{OE} compared to Bx7 measured, but the results indicate that when the Bx7^{OE} trait is present, the ratio of that specific HMW-GS is greater than when only the Bx7 allele is present (J. Li et al., 2014; S. Li et al., 2020; Ragupathy et al., 2008). It was found that in hexaploid wheat, the proportion of Bx7^{OE} present was 38.8% to 44.2%, which is significantly higher than when only Bx7 is present (proportion of

26.9% to 32.8% compared to total HMW-GS; (J. Li et al., 2014; Ragupathy et al., 2008). Using RP-HPLC, it was found that in near-isogenic varieties of wheat, which are genetically identical across much of the genome except for expression of the different alleles (Bx7^{OE} versus Bx7) at the *Glu-B1* locus, the proportion of the B_x and B_y subunit were significantly greater for Bx7^{OE} (S. Li et al., 2020). It was also shown that the proportion of A_x and D_x decreased (S. Li et al., 2020). Overall, since the genetic background is the same except for the one allele, the gliadin composition is the same, but the ratio of HMW to LMW and glutenin to gliadin ratio was higher in the Bx7^{OE} line compared to the Bx7 line. This shows an increase in quality is expected to be seen in dough and bread wheat end product quality since there is more HMW-GS overall and more glutenin (S. Li et al., 2020). When the same varieties were characterized with RP-HPLC and SDS-PAGE, the results were identical in characterizing the varieties with Bx7^{OE} compared to Bx7 (J. Li et al., 2014). Polymerase Chain Reaction (PCR) can also be used to determine the presence of *Glu-B1a1* versus *Glu-B1b* using specific markers (Brar et al., 2021; J. Li et al., 2014; Rasheed et al., 2019). The extraction can be made from dry seeds or early leaf tissue, and allele specific markers can be used. There are two current methods. One uses Sequence Tagged Sites markers where two allele specific markers are used, each designed to flank the left and right junctions of the retroelement of the Bx7^{OE} allele (J. Li et al., 2014). The other uses Kompetitive allele specific (KASP) markers to identify the Bx7^{OE} allele (Rasheed et al., 2019). KASP was found to be 95% accurate in the study with 2 false positives among 48 samples (Rasheed et al., 2014).

Microstructure

The microstructure of gluten is made up of three levels: primary, secondary, and tertiary. The secondary structure is made up of primarily α -helices and β -sheets. When wheat varieties

with Bx7 allele and the overexpressed allele were compared, differences in the secondary structure were seen. It was found that the amount of beta-sheets in the Bx7^{OE} varieties was much higher than in the Bx7 varieties (Li et al., 2020). The Bx7^{OE} varieties had a smaller content of α -helices, β turns, and antiparallel β -sheets which are all less desirable than the β -sheets (Li et al., 2020) and overall, contained the most β -sheets compared to any alternative secondary protein structure. The ratio between the α -helices to β -sheets was also much lower in the Bx7^{OE} varieties compared to the Bx7 varieties (Li et al., 2020). The increased proportion of β -sheets contributes to greater strength in dough (Li et al., 2020). Not only are there more β -sheets in the wheat varieties overexpressing the Bx7 allele, but these varieties also have more free sulfhydryl groups which allow more disulfide bonds to form (S. Li et al., 2020). Disulfide bonds are crucial for good gluten quality and dough strength. Overall, the microstructure in Bx7^{OE} varieties is more compact and experiences less change when mixing contributing to overall better rheological and end product quality such as the longer stability times as seen in the Farinograph and Mixolab, discussed below (S. Li et al., 2020).

The microstructure was also compared using scanning electron microscopy. When the microstructure was observed at 2000x after two minutes, the Bx7^{OE} starch apertures on the gluten network were smaller, more dense, and more homogenous than Bx7 at the same magnification (S. Li et al., 2020). After each of the doughs were mixed to development and again observed, there were no significant differences between the apertures and gluten network (S. Li et al., 2020), but after breakdown, both networks were ruptured. However, the network of the Bx7^{OE} was less ruptured than the network of the Bx7 variety (S. Li et al., 2020). Overall, the Bx7^{OE} variety was found to have more gluten network and less apertures disrupting the network

(S. Li et al., 2020). This result is in agreement with higher strength in the varieties with Bx7^{OE}, as measured using recording dough mixers.

Grain Quality

In terms of grain quality, a few differences are found in the varieties containing the Bx7^{OE} trait. The allele causes changes in the percentage of Unextractable Polymeric Protein (% UPP), compared to varieties with the Bx7 allele (Li et al., 2020). The UPP is determined by the amount of protein that is unextractable in sodium dodecyl sulfate (SDS) in comparison to extractable polymeric protein (P. Zhang et al., 2008). UPP is subject to both environmental and genetic effects, but when there is high gluten strength in the genotype, the % UPP is less variable with the environment (P. Zhang et al., 2008). In general, the higher proportion of HMW-GS, the greater the % UPP (P. Zhang et al., 2008). This generally correlates with increased dough quality determining the elasticity and extensibility found in dough (Rasheed et al., 2014). Overall, the varieties with Bx7^{OE} had a higher % UPP during the entire grain filling stage, including at the initial point, indicating the polymeric glutenin protein deposited in the grain earlier in the grain filling stage (S. Li et al., 2020). The most significant increase for varieties with Bx7^{OE}, was between Day 19 and Day 24 after anthesis (S. Li et al., 2020). During this time, Bx7 saw no change. The increase for overexpressed varieties was sustained over the entire grain filling stage and was overall higher (Gao et al., 2013; S. Li et al., 2020). In a study comparing the released Bx7^{OE} varieties Paradox, Breadbox, and Firebox, the grain yield and volume weight was found to be similar to that of the recurrent parent Gallagher, showing the increased quality of the varieties did not deter from grain production (Bock et al., 2025). There is disagreement in literature concerning grain hardness. Some authors saw an increase in kernel hardness for the Bx7^{OE}, but others saw a lower hardness compared to Bx7, although still in target range for hard

wheat (Brar et al., 2021; Cooper et al., 2016; Vancini et al., 2019). The Oklahoma State University wheat quality laboratory analysis saw no shift in hardness compared to other HRW varieties (Bock et al., 2025). Hardness is dependent on more than one factor and is most primarily determined genetically by the puroindoline loci, which may be the reason for disagreement. The grain protein of Paradox, Breadbox, and Firebox were lower than a control of Doublestop CL⁺ (Bock et al., 2025). Not only that, but the grain protein of these three overexpressed varieties was also lower than Gallagher (the recurrent parent) but within a normal average for hard wheats (Bock et al., 2025).

Flour Quality

The milling of the Bx7^{OE} varieties compared to Bx7 varieties seemed to be relatively similar in regard to extraction rate (Bock et al., 2025; Brar et al., 2021). For the Bx7^{OE} varieties, the flour protein was slightly increased, and the flour in one study seemed slightly more yellow (Brar et al., 2021; Lee et al., 2023; S. Li et al., 2020). More differences have been reported in quality tests of the flour than in milling. In the SDS sedimentation test, the values of the genotypes with the Bx7^{OE} trait had a higher sedimentation value than those with the Bx7 trait (Adegbite, 2024; Bock et al., 2025; Gao et al., 2013; Lee et al., 2023; Vancini et al., 2019; L. Zhang et al., 2010). For Firebox, Breadbox, and Paradox, the difference was 1.1-1.3 mL greater than the control of Gallagher taking into account the protein content of each variety (Bock et al., 2025). The value of the Zeleney sedimentation test was also higher with varieties containing the Bx7^{OE} trait compared to those with the Bx7 allele (S. Li et al., 2020). Only one study recorded wet gluten, but in that study varieties with the overexpressed Bx7 allele were found to have a higher wet gluten content than those without the overexpression (S. Li et al., 2020). When the falling

number of overexpressed varieties was determined, there was no significant difference compared to other HRW varieties in a similar environment (Bock et al., 2025).

Dough Quality

In a study looking at three varieties with the Bx7^{OE} trait, it was found the Mixograph peak time was about four minutes greater than that of the recurrent parent - Gallagher, which represents twice the time of Gallagher's peak time (Adegbite, 2024; Bock et al., 2025). The peak time describes the mix time required to achieve maximum resistance (J. Li et al., 2014) and peak times higher than eight minutes are considered an indication of strong doughs (Adegbite, 2024; Bock et al., 2025; J. Li et al., 2014). The target value for the Mixograph peak time for hard wheats according to the Wheat Quality Council is three to six minutes; therefore, these overexpressed varieties far exceeded the recommended target values (Chen et al., 2024). Compared to genotypes with the Bx7 allele, varieties with the Bx7^{OE} allele showed greater strength, having a medium strong to strong gluten quality (Cooper et al., 2016; J. Li et al., 2014). The tolerance of the overexpressed varieties was found to be greater by two scale units on a 0-6 visual scale compared to non-overexpressed varieties. The Mixograph bandwidth 2 minutes past the peak was found to be 3.2-5.9 mm greater than that of Gallagher (Bock et al., 2025). In fact, all Mixograph parameters of genotypes with Bx7^{OE} were found to be higher than those of genotypes expressing only Bx7 (Rasheed et al., 2014). Varieties containing the Bx7^{OE} trait tend to also show greater resistance to overmixing (Gao et al., 2013). However, there are other factors that determine strength. One study indicates that Bx7^{OE} may not overcome the shorter dough mixing time of genotypes with the *Glu-D1a* allele (encoding Dx2+Dy12) which indicates it is important to look at the whole genotype, not just the *Glu-B1* locus (Cooper et al., 2016). Most of

these studies were done on wheat genotypes that had identical genetic backgrounds besides the *Glu-B1* locus.

The Farinograph is another common dough recording mixer used to test wheat quality. Bakers also use it to help establish dough mixing times. Overall, varieties with the Bx7^{OE} showed greater strength with longer peak and stability times and lower MTI than those without Bx7^{OE} (Adegbite, 2024; Bock et al., 2025; Brar et al., 2021; Vancini et al., 2019). Peak time for overexpressed varieties was found to be 7x greater compared to a non-overexpressed control and stability time was found to be 4x greater than the control (Bock et al., 2025). The target Wheat Quality Council Farinograph peak times are 4-8 minutes, and the target stability is 10-16 minutes for hard winter wheats (Chen et al., 2024). The overexpressed varieties had peak times between 21 and 41 minutes and stabilities between 38 and 56 minutes on average (Bock et al., 2025). The excessively long peak times and stability cause warrant from a baker's perspective since the target of 10–16-minute stability allows bakeries to maximize efficiency while delivering desirable quality. The water absorption was also shown to be slightly increased (2.2 to 3%) even though the flour protein levels were similar (Adegbite, 2024; Bock et al., 2025; L. Zhang et al., 2010). More studies have looked at the Mixolab instead of the Farinograph. This assay combines both the dough mixing and starch pasting properties (Rasheed et al., 2019). The results showed that genotypes with the Bx7^{OE} had longer dough development times and longer dough stability than the genotypes with the Bx7 (S. Li et al., 2020; L. Zhang et al., 2010). This indicates that the Bx7^{OE} varieties are stronger than those with the Bx7 allele (S. Li et al., 2020). However, the mixing profiles on the Mixolab were slightly different. At the initial mixing stage, Bx7 had a faster increase in torque compared to Bx7^{OE} and developed earlier likely due to the fact that development time is dependent on the ability for the proteins to hydrate (S. Li et al., 2020). As

the Mixolab test progressed, the genotypes with Bx7^{OE} had a smaller decrease in torque showing a longer dough stability time and greater tolerance to overmixing (S. Li et al., 2020). The second part of the Mixolab test heats the dough and shows the properties of starch. During this period, the torque of Bx7^{OE} was greater than that of genotypes with Bx7, showing that genotypes with Bx7^{OE} swells more with heat (S. Li et al., 2020). At the completion of the test, genotypes with Bx7^{OE} had a greater torque showing that after cooling, they may be more prone to aging (S. Li et al., 2020). When assessing pasting properties using the Rapid Visco Amylograph (RVA), it was found that the final viscosity was higher with the genotypes that contained Bx7^{OE} which is in agreement with the Mixolab results (S. Li et al., 2020; Rasheed et al., 2019). However, one study showed that the parameters in the RVA were significantly lower with genotypes that contain Bx7^{OE} compared to Bx7 which is not in agreement with the Mixolab results (Rasheed et al., 2019).

Only the study by Zhang et al. (2010) mentions alveograph testing, in which Bx7^{OE} was found to increase the P (characterizes elasticity of the dough) and W (characterizes total energy required) parameters. Another study mentions that alveograph parameters would need to be changed to accommodate the overexpressed varieties (increasing mix time), but the data was not presented (Bock et al., 2025).

From the dough mixing parameters, it is apparent that overall, the Bx7^{OE} trait increases dough strength compared to those with the Bx7 allele. As mentioned earlier, the Bx7^{OE} trait gives greater proportion of Bx7 HMW-GS to overall HMW-GS which may be the reason for greater dough strength (Rakszegi et al., 2005). HMW-GS are known to contribute greatly to dough strength and are an important factor in contributing to different dough rheological

properties (S. Li et al., 2020). The dough strength was found to be unrelated to wheat protein concentration (Bock et al., 2025).

When Bx7^{OE} varieties were compared to Bx7 varieties, there were some differences in the measured rheological properties. Overall, Bx7^{OE} had a higher G' (storage modulus) during the whole test, even though the trend was similar during the test (S. Li et al., 2020). The elasticity of the Bx7^{OE} varieties was found to be greater, which correlates with the above-mentioned longer dough development times and longer stabilities shown in Farinograph and Mixolab results (S. Li et al., 2020). All the tested varieties showed increasing G'' (loss modulus), with G'' lower than G' for the entire test (S. Li et al., 2020). While the rheological properties on the rheometer are not well studied, differences in rheology may contribute to the differences in dough quality between the two types of varieties studied.

Baking Quality

Only one assessment of the baking quality of genotypes with Bx7^{OE} has been published so far. The breads from genotypes with the Bx7^{OE} trait were acceptable, but not excellent regarding the bake absorption and final loaf volume (Adegbite, 2024; Bock et al., 2025). All overexpressed varieties across five years had lower absorption (1.4-2.8% lower) compared to the control (average absorption 62.6%), which was Doublestop CL⁺ (Adegbite, 2024; Bock et al., 2025). Doublestop CL⁺ is known for being an exceptional control with high protein. The overexpressed varieties compared were 1.1-1.5% lower in protein than the control, which may explain the decreased absorption and volume seen in the study (Bock et al., 2025). The varieties were baked in one-pound loaves and compared to Doublestop CL⁺ which had a mean volume of 2910 cm³ (Adegbite, 2024). The volumes were on average 77-110 cm³ lower for the Paradox and Breadbox varieties compared to the control. Firebox had an average volume 14 cm³ better than

the control (Adegbite, 2024; Bock et al., 2025). If the strength of the overexpressed varieties causes too much elasticity restricting expansion of the dough, a lower volume could be expected. Although there is no published data to support such claims, authors including Brar et. al (2021) and Cooper et al. (2016) conclude that the overexpressed varieties are effective for improving bread quality. More study needs to be done regarding the baking performance of Bx7^{OE}.

Performance as Improver

Paradox was tested as an improver in a blend percentage with Big Max, with positive effects on performance compared to Big Max alone (Bock et al., 2025). Paradox was added in 20% increments to Big Max, with Farinograph stability increasing linearly at 12.5 min per 20% addition of Paradox, while decreasing the MTI (Bock et al., 2025). A desirable MTI is <10 which with a 20% addition of Paradox, MTI decreased to 12 and decreased below 10 with each increment thereafter. The alveograph optimum balance (P/L=108) was achieved at 40% Paradox inclusion. The study recognized that Paradox added in a blend may improve the baking parameters, but data was not included or discussed in detail since optimum characteristics vary with the end product (Bock et al., 2025). It is expected that 50-75% vital wheat gluten could be replaced with Paradox in pan bread without decreasing bread quality significantly (Bock et al., 2025).

Conclusion

The main discrimination between Bx7^{OE} varieties versus Bx7 varieties is reflected in higher HMW-GS in the grain. Overall, the Bx7^{OE} wheat varieties present greater strength in dough mixing than the Bx7 varieties. However, not enough testing has been completed to allow a conclusive understanding of the differences among varieties. Specifically, there is a knowledge

gap on the comparative end-use quality of Bx7^{OE} and Bx7 varieties. Future work focused on bread quality will shed light on the actual quality gains of the Bx7^{OE} wheat varieties.

Chapter 2 - The Effectiveness of the GlutoPeak™ in Discriminating among Whole Meal and Refined Flour Blend Series of Overexpressed Wheat Varieties and Non-Overexpressed Varieties

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Introduction

In all facets of the industry, understanding the quality of the grain and flour is important. For breeders, selection decisions are based partially on grain and flour quality. For millers, grain quality is crucial for extraction, and flour quality is important to understand the product for a specific customer. For bakers, end product quality is dependent on flour quality (Chen et al., 2024; Delcour & Hoseney, 2010). Therefore, in each sector of the industry, the quality of the grain and flour must be understood. Traditionally, equipment including the Alveograph, Extensograph, Farinograph, and Mixograph have been used, but these tests require extensive time and a large sample size. Breeders, especially in preliminary trials of varieties, seek to differentiate quality of samples to determine what crosses to advance. At these early trials, sample size is minimal and refined flour extraction is not always possible nor time effective. The GlutoPeak is of interest because of its ability to test samples quickly, effectively, with a small sample size, and in a whole meal capacity. The ability for whole meal to be analyzed is important since most rheological tests are not able to be measured using the bran since it interferes with gluten development (Fu et al., 2017). The GlutoPeak analyzes the protein quality by subjecting an 18 gram flour and water slurry to intense mechanical shearing action and

aggregating the protein due to the high speed paddle rotation (Chandi & Seetharaman, 2012; Marti et al., 2014; Marti, Ulrici, et al., 2015; J. Wang et al., 2018; K. Wang et al., 2021; Zawieja et al., 2020). The instrument measures the torque of the paddle against the aggregation of the gluten proteins held in suspension in the slurry which is depicted in a curve (Marti et al., 2014; Marti, Ulrici, et al., 2015; J. Wang et al., 2018; K. Wang et al., 2021; Zawieja et al., 2020). Continued mixing destroys the gluten network, and the torque curve declines with the slurry decreasing consistency (Marti et al., 2014; Marti, Ulrici, et al., 2015). The resulting curve (Figure 1) depicts the following measurements: maximum torque (BEM), which corresponds to the peak at which the max gluten aggregation occurs, peak maximum time (PMT) which is the time to the BEM before the torque falls off and the gluten aggregation breaks down, and the area under the peak which is the curve area depicting energy (Marti et al., 2014).

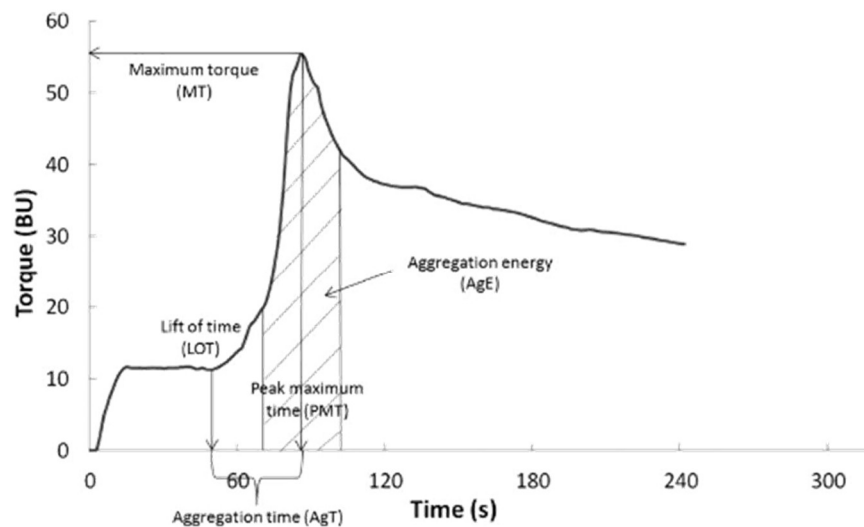


Figure 1: GlutoPeak Curve taken from Rakita et al. (2018) showing parameters measured by the GlutoPeak. It should be noted in this study; maximum torque is abbreviated BEM.

The torque before and after BEM can also be measured (Marti, Ulrici, et al., 2015). Also, multiple classes of wheat with varying protein contents and end use functionality are able to be tested, but this may be protocol dependent (Chandi & Seetharaman, 2012; Fu et al., 2017). The

test is simple to run, so little training is required (Marti et al., 2014; K. Wang et al., 2017).

Research has shown the GlutoPeak is significantly correlated with several traditional rheological test parameters making it intriguing due to small sample size and faster testing (Fu et al., 2017; Marti et al., 2014; Mecitoğlu Güçbilmez et al., 2019; K. Wang et al., 2017; Zawieja et al., 2020). Its high sensitivity to differences in gluten quality merits its desirability for flour evaluation (J. Wang et al., 2018).

While research has shown the test is correlated to the quality of the flour, there are a wide number of methods used, as depicted in Table 1. Both whole meal and refined flour can be used, which is especially important for breeding programs, and the test only requires 8-10 grams of sample. The typical solvents used are distilled water, calcium chloride solution, and sodium chloride solution. Calcium chloride is better suited for flours containing a wide range of protein content (Chandi & Seetharaman, 2012) due to better discrimination. Calcium chloride shortens the time it takes for the gluten proteins to aggregate (Chandi & Seetharaman, 2012). At lower salt concentrations (<0.3 M), all ions have a similar effect on the aggregation of gluten proteins due to the shielding electrostatic forces which allow or prevent protein-protein interaction. At higher salt concentrations (>0.3 M), the protein behavior is dictated by the type of salt and the effect of chaotropic ions (Preston et al., 1982). Calcium is a chaotropic ion so in a solution of CaCl_2 as it dissociates, the ions are effective at solubilizing the glutenin resulting in more extraction occurring with increased concentration. At the same time, the electrostatic interactions of the proteins are negligible, so the hydrophobic interactions increase (Preston et al., 1982). These competing processes allow for increased aggregation of the protein in an efficient time which is especially important for whole meal with the bran interference.

Table 1: Table comparing methods in current literature and results.

Source	Substrate	Method	Results
(K. Wang et al., 2021)	Whole meal (HRS)	Flour: 8 grams flour Solvent: 10 grams distilled water RPM: 2700 rpm Temp: 34 °C	8:10 flour: water ratio best discriminates strength Changing speed did not greatly affect parameters
(Marti et al., 2014)	Semolina (Durum)	Flour: 9 grams flour Solvent: 10 grams distilled water RPM: 2750 rpm Temp: not mentioned	Method appropriate for samples
(Marti, Ulrici, et al., 2015)	Refined flour (common wheat flours)	Flour: 9 grams flour Solvent: 10 grams distilled water/ 0.33 M NaCl RPM: 3000 rpm Temp: 35 °C	Sodium chloride and water, comparable results
(Zawieja et al., 2020)	Whole grain (industrial mill flours)- weak to medium flours	Flour: 8 grams flour Solvent: 10 grams 2% NaCl RPM: 2750 rpm Temp: 36 °C	Method appropriate for samples
(Rakita et al., 2018)	Refined flour (HRW)	Flour: 8.5 grams flour Solvent: 9.5 grams 2% NaCl RPM: 2700 rpm Temp: 36 °C	2% NaCl found to be applicable for weak flour AgE < 1300 GPU considered poor quality and BEM < 50 GPU
(Mecitoğlu Güçbilmez et al., 2019)	Refined flour (HWW)	Flour: 9 grams flour Solvent: 9 grams distilled water RPM: 2750rpm Temp: 36 °C	Weaker gluten peak time (75-201 s) and max peak torque (31-58 GPU)
(Marti, Augst, et al., 2015)	Refined flour (HWW)	Flour: 8.5 grams flour Solvent: 9.5 grams 0.5 M CaCl ₂ RPM: 1900 rpm Temp: 34 °C	Method appropriate for samples
(Fu et al., 2017)	Refined flour (HRS)	Flour: 8 grams flour Solvent: 10 grams distilled water RPM: 2700 rpm Temp: 34 °C	Method appropriate for samples
(J. Wang et al., 2018)	Whole meal (HRS, SW, HW, HRW)	Flour: 8 grams flour Solvent: 10 grams 0.5 M CaCl ₂ RPM: 3000 rpm Temp: 20 °C	Highest efficiency at 3000 rpm More flour, less solvent resulted in higher concentration of gluten Consistent results at this ratio for all classes of wheat tested.
(Marti, Ulrici, et al., 2015)	Intermediate wheat grass	Flour: 8.5 grams flour Solvent: 9.5 grams 0.5 M CaCl ₂ RPM: 1900 rpm	Method appropriate for samples

Temp: 34 °C			
(Gür & Diraman, 2024)	Refined flour (HWW)	Flour: 9 grams flour Solvent: 9 grams distilled water RPM: 2750 rpm Temp: 36 °C	Method appropriate for samples
(K. Wang et al., 2017)	Refined flour (HRS)	Flour: 8 grams flour Solvent: 10 grams water RPM: 2700 rpm Temp: none listed	Increasing mixing speed resulted in reduced PMT and smaller TPA with increase in BEM This flour water ratio seemed most effective in discriminating strength With increased speed, test shortened with little impact in discrimination power
(Chandi & Seetharaman, 2012)	Refined flours and Whole meal (HWW and SW)	Flour: 8.5 grams flour Solvent: 9.5 grams 0.5 M CaCl ₂ RPM: 1900 rpm Temp: 34 °C	Increased concentration of calcium chloride, torque increased Increased rpm and reduced temperature favorable to gluten network Higher temperature and higher flour concentration and calcium chloride shortened the aggregation time for gluten
(Song et al., 2020)	Refined flour	Flour: 9 grams flour Solvent: 10 grams 0.33 M CaCl ₂ RPM: 3000 rpm Temp: 36 °C	Method appropriate for samples
(Melnik et al., 2011)	Refined flour (HRS)	Flour: 8 grams flour Solvent: 10 grams solvent RPM: 2750 rpm Temp: none listed	At low concentration, all salts had similar effect on PMT As ionic concentration increased, differences in PMT evident PMT decreased from most kosmotropic to most chaotropic Most kosmotropic anion produced lowest torque and most chaotropic ion produced greatest torque At low salt conc (<0.3M) differences in PMT much smaller compared to at higher concentration
Flour Type Abbreviations: Hard Red Spring (HRS), Hard Red Winter (HRW), Hard Winter Wheat (HWW), Soft Winter Wheat (SW), Hard White Wheat (HW)			

Table 2 summarizes the significant correlations shown in literature between GlutoPeak parameters and various quality parameters. The results do not all agree in the literature as shown in the table. Ultimately, while correlating the GlutoPeak parameters with other quality test parameters (such as Farinograph and Alveograph) is of some value, of more significant value is correlating the GlutoPeak parameters with the end product testing such as bread. Only one source shows correlations with GlutoPeak parameters and bread specific volume (Rakita et al.,

2018). In general, based on the literature presented, the correlations are positive for strength, so since the values of the overexpressed varieties are larger than KS Mako and the other HRW varieties tested, we expect them to have a higher overall quality. The only exception is the PMT parameter which is found in literature to have a negative correlation to some of the wet gluten parameters, as described in Table 2 (Mecitoğlu Güçbilmez et al., 2019). However, others show strong positive correlations with Farinograph stability and other strength parameters (Song et al., 2020; K. Wang et al., 2017, 2021). Based off of literature, however, it seems that whole meal and refined flour are considered able to determine quality differences and show similar correlations when discussing quality. From what we see, similar trends are seen in the data at a different scale for the whole meal and refined individual varieties.

Despite the evidence of the use of the GlutoPeak for various types of flour, its application in overexpressed varieties is largely undocumented. Therefore, the objectives of this study were to characterize the overexpressed varieties for kernel parameters, milling performance, flour and whole meal composition, and finally the feasibility of the GlutoPeak to assess quality of the flours. A blending series was performed to test the discriminatory ability of the GlutoPeak among flours (whole meal or refined) of a constant genetic background of non-overexpressed (non-Bx7^{OE}) wheats with incremental changes in overexpressed (Bx7^{OE}) wheat flour content.

Materials and Methods

Variety Selection

The overexpressed Bx7 protein subunit varieties used in this study were Breadbox, Firebox, and Paradox. These varieties were developed by the Oklahoma State University breeding program and previously selected for uniquely high dough strength (Bock et al., 2025). These lines were selected from a population of Bx7^{OE} lines due to their strong gluten trait and

performance. Paradox and Breadbox often outperform Firebox regarding gluten strength. Gallagher (not overexpressed) was included in this study since it is the recurrent parent variety for Breadbox and Paradox. Everest, KS Mako, and KS Providence were chosen as base flours in this study since they represent a variety of known qualities. Everest is at the lower end of desirable wheat quality, KS Mako at the higher end, and KS Providence in the middle. Oklahoma Foundation Seed Stocks provided cleaned wheat for Gallagher, Breadbox, Firebox, and Paradox which was grown in Oklahoma. Cleaned wheat for Everest, KS Mako, and KS Providence was obtained from KSU Foundation Seed and grown in Kansas. The differences in growing locations are acknowledged but were not controlled for in this study.

Table 2: Significant correlations between GlutoPeak parameters and quality parameters shown in literature.

Source	PMT	BEM	AM	PM	AgE	TPA	GSI
(K. Wang et al., 2021)	Farino Extenso Rmax, area Negative Correlation with FAB					Farino S Extenso Rmax & area Gluten Index	Farino S Extenso Rmax & area
(Marti et al., 2014)		Protein Content Alveo W			Alveo W		
(Zawieja et al., 2020)	Negatively correlated with FAB	FAB		FAB, Farino S			
(Rakita et al., 2018)	Negatively correlated with Protein Content, Wet Gluten content	Protein Content Wet Gluten content FAB Farino S Alveo W Specific Volume Cohesiveness, Resilience			Protein Wet Gluten content FAB Alveo P, W Farino S Specific Volume Cohesiveness, Resilience		
(Mecitoğlu Güçbilmez et al., 2019)	Negative correlation with Gluten protein content,	FAB Alveo W	FAB Alveo W	FAB Alveo W	Alveo W		

	Glutomatic wet gluten content, FAB					
(Marti, Augst, et al., 2015)		Gliadin content Gluten content				
(Fu et al., 2017)		FAB				
(Gur & Diraman, 2024)	Gluten Index Value Negatively correlated with FAB	Protein Content FAB Alveo W	Protein Content FAB Alveo W	Protein Content FAB Alveo W	Protein Content FAB Alveo W	
(K. Wang et al., 2017)	Extenso Rmax, Area Farino S, MTI	Flour Strength Gluten Strength FAB			Extenso Rmax, Area Farino S, MTI	Extenso Rmax, Area Farino S, MTI
(Song et al., 2020)	Gluten Strength					

Abbreviations: Farinograph Absorption (FAB), Mixing Tolerance Index (MTI), Stability (S), Farinograph (Farino), Alveograph (Alveo), Extensograph (Extenso), Peak Maximum Time (PMT), Maximum Torque (BEM), Torque before BEM (AM), Torque after BEM (PM), Aggregation Energy (AgE), Total Peak Area (TPA), Gluten Strength Index (GSI)

Kernel Testing

The thousand kernel count (TKW) was determined for each wheat variety. The test weight per bushel of each wheat variety was measured using AACC method 55-10.01 (AACC International, 1999). Near infrared (NIR) (Infratec, Foss, Hilleroed, Denmark) was used to measure the protein content of the whole grain wheat. For protein measurement, AACC method 39-25.01 was used (AACC International Method, 1999). To determine the hardness of each variety AACC method 55-32.01 was performed using the Single Kernel Characterization System (SKCS model 4100, Perten Instruments, Springfield IL) (AACC International, 1999). Finally, single kernel imaging was performed using the Cgrain Value Pro machine (Cgrain AB, Uppsala, Sweden). Each kernel test was performed in triplicate for each variety.

Milling

To obtain whole meal, each individual wheat variety was milled using a UDY cyclone mill with a 0.5 mm screen. Samples were stored in glass jars at room temperature until further testing. For refined flour, before milling, wheat was tempered to 15% moisture basis using the NIR moisture value. The calculations were done according to AACC Method 26-95.01 (AACC International, 1999). Tempering and milling were done in 3000 g increments for each variety. Each variety was milled using the Quadrumat Senior experimental mill (Brabender/Anton Paar). The mill was allowed to warm up by running for an hour before milling started. A control sample was run previous to the test samples. The bran, shorts, break flour, and reduction flour were collected and weighed. Total extraction and flour extraction were calculated. The reduction and break flour were then blended to produce refined flour using a V-Blender for ten minutes followed by drum tumbling until homogenous. Samples were stored in hermetic buckets at room temperature until further testing. For both whole meal and refined flour, after milling, the protein, moisture, and ash were measured using the appropriate NIR method on the Foss DS 2500 NIR (Foss, Hilleroed Denmark). Samples were run according to method AACC method 39-10.01 (AACC International Method, 1999) for whole meal and AACC method 39-11.01 (AACC International, 1999) for refined flour.

Blending

The blending scheme is summarized in Table 3. Overexpressed whole meal and refined flours were blended individually into the base whole meal and refined flour respectively, then homogenized. Whole meal blends were tumbled due to a smaller sample size; refined flours were V-blended for 10 minutes to homogenize. Each blend was prepared in triplicate. Whole meal blends were stored in glass jars and refined flour blends were stored in hermetic buckets. All

samples were stored at room temperature. Blends were tested in triplicate using the NIR according to the methods cited above.

Table 3: Summary of blending performed in this experiment.

Base	Bx7^{OE}	Whole Meal Blend Percentages	Refined Flour Blend Percentages
Everest	Breadbox	0	0
	Firebox	5	5
	Paradox	10	10
		25	15
		50	25
		100	50
			100
KS Mako	Breadbox	0	0
	Firebox	5	5
	Paradox	10	10
		25	15
		50	25
		100	50
			100
KS Providence	Breadbox	0	0
	Firebox	5	5
	Paradox	10	10
		25	15
		50	25
		100	50
			100

Note: Bx7OE refers to overexpressed varieties

GlutoPeak

Two different protocols were used to test the whole meal and refined samples on the GlutoPeak. The Extended setting (available on the GlutoPeak software) was used, providing additional parameters from the area under the curve. For whole meal, 8.5 g whole meal was measured and added to 9.5 g 0.5 M calcium chloride solution. The paddle speed was 1900 rpm and analysis temperature was 34 °C (Chandi & Seetharaman, 2012). The test was conducted until 15 seconds after the peak. For refined flour, 9 g refined flour added to 9 g distilled water. The paddle speed was 2700 rpm and analysis temperature was 34 °C (Bouachra et al., 2017). The test was conducted until 15 seconds after the peak. Each test was performed in triplicate. Table 4 summarizes the GlutoPeak parameters analyzed in this study. The Gluten strength index

definition was initially determined by Wang et al. (2017), but further parameters were calculated internally. For whole meal, total peak area (TPA), GSI 3, and GSI4 were not analyzed because the GlutoPeak software does not consistently identify the early parts of the curve.

Table 4: Summary of GlutoPeak parameters collected in this study.

Parameter	Abbreviation	Units	Definition
Peak Maximum time	PMT	Seconds (s)	Time (s) to reach the maximum torque; indicates gluten aggregation time
Maximum Torque	BEM	GlutoPeak Units (GPU)	Highest torque (GPU) reached during aggregation
Torque before maximum	AM	GlutoPeak Units (GPU)	Torque measured 15 s before maximum torque
Torque after maximum	PM	GlutoPeak Units (GPU)	Torque measured 15 s after maximum torque
Aggregation Energy	AgE	GlutoPeak Units (GPU)	Area of the peak between AM and PM
Total Peak Area	TPA	GlutoPeak Units (GPU)	Total area under the torque–time curve.
Gluten Strength Index 1	GSI 1		(AgE*BEM)/1000
Gluten Strength Index 2	GSI 2		(AgE*PMT)/1000
Gluten Strength Index 3	GSI 3		(TPA*BEM)/1000
Gluten Strength Index 4	GSI 4		(TPA*PMT)/1000

Statistical Analysis

Normality was assessed using Shapiro-Wilk ($p < 0.05$) and distribution of residuals. If the normality assumption held true, differences were determined using ANOVA followed by Tukey's HSD test at $p < 0.05$ (JMP® Version 18.0.0., SAS Institute Inc., Cary, NC). If the data was not normal, differences were determined using the Wilcoxon/ Kruskal-Wallis Tests (Rank Sums) followed by Bonferroni at a significance level of $p < 0.05$ (SAS® 9.4, SAS Institute Inc., Cary, NC). Contrasts were also assessed to determine differences between overexpressed varieties compared to non-overexpressed varieties as two distinct groups.

Results and Discussion

Kernel Imaging

Table 5: Kernel characterization obtained from C-grain imaging system of individual varieties.

	Breadbox	Paradox	Firebox	Everest	Gallagher	KS Mako	KS Providence	p-value	Contrast OE vs Base
Avg Area (mm ²)	12.08 ^c ±0.06	12.26 ^{b,c} ±0.09	11.77 ^d ±0.02	12.32 ^b ±0.14	11.55 ^e ±0.09	11.26 ^f ±0.04	12.86 ^a ±0.04	<0.001	0.2864
Avg L (mm)	5.71 ^d ±0.02	5.78 ^c ±0.03	5.43 ^e ±0.01	5.91 ^b ±0.02	5.69 ^d ±0.02	5.40 ^e ±0.01	6.05 ^a ±0.01	<0.001	<0.001
Widest Kernel W (mm)	2.94 ^a ±0.01	2.95 ^a ±0.01	3.06 ^a ±0.01	2.90 ^c ±0.01	2.81 ^d ±0.01	2.96 ^a ±0.01	3.03 ^a ±0.02	<0.001	<0.001
Avg Th (mm)	2.76 ^b ±0.01	2.77 ^b ±0.01	2.87 ^a ±0.01	2.73 ^c ±0.01	2.63 ^d ±0.01	2.76 ^b ±0.01	2.78 ^b ±0.01	<0.001	<0.001
Avg Kernel W (mm)	2.85 ^c ±0.01	2.86 ^c ±0.00	2.97 ^a ±0.01	2.81 ^d ±0.01	2.72 ^e ±0.01	2.86 ^c ±0.01	2.91 ^b ±0.01	<0.001	<0.001
Avg Vol (mm ³)	23.60 ^c ±0.05	24.02 ^b ±0.10	24.04 ^b ±0.04	23.40 ^c ±0.15	21.46 ^e ±0.17	22.47 ^d ±0.12	25.19 ^a ±0.17	<0.001	<0.001
Avg Wt. (g)	0.0315 ^c ±0.0001	0.0321 ^b ±0.0001	0.0321 ^b ±0.0001	0.0313 ^c ±0.0002	0.0286 ^e ±0.0002	0.0300 ^d ±0.0002	0.0337 ^a ±0.0003	<0.001	<0.001
Avg Light	0.5072 ^f ±0.0002	0.5170 ^e ±0.0034	0.5398 ^b ±0.0015	0.5230 ^d ±0.0032	0.4931 ^g ±0.0005	0.5613 ^a ±0.0024	0.5322 ^c ±0.0013	<0.001	<0.001
Avg Hue	25.7 ^d ±0.0	25.7 ^d ±0.1	25.6 ^d ±0.1	27.3 ^a ±0.1	25.5 ^e ±0.0	25.9 ^c ±0.0	26.1 ^b ±0.0	0.0035	<0.001
Avg Sat	0.4846 ^b ±0.0001	0.4823 ^b ±0.0014	0.4825 ^b ±0.0005	0.4361 ^d ±0.0013	0.4979 ^a ±0.0009	0.4625 ^c ±0.0006	0.4606 ^c ±0.0009	0.0037	<0.001
Avg Red	129.3 ^f ±0.1	131.8 ^e ±0.9	137.7 ^b ±0.4	133.4 ^d ±0.8	125.7 ^g ±0.1	143.1 ^a ±0.6	135.7 ^c ±0.3	<0.001	<0.001
Avg Green	94.9 ^e ±0.0	97.0 ^d ±0.8	100.9 ^c ±0.2	102.7 ^b ±0.8	91.1 ^f ±0.1	106.7 ^a ±0.5	101.5 ^{b,c} ±0.3	<0.001	<0.001
Avg Blue	68.7 ^f ±0.1	70.4 ^e ±0.7	73.1 ^d ±0.1	76.7 ^b ±0.6	65.1 ^g ±0.1	78.8 ^a ±0.4	74.9 ^c ±0.2	<0.001	<0.001

Abbreviations: Average (Avg), Length (L), Width (W), (Volume (Vol), Thickness (Th), Weight (Wt.) Saturation (Sat), Overexpressed varieties (OE)

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05). Contrasts of overexpressed versus non-overexpressed varieties are also shown (p<0.05).

For the Cgrain imaging data, differences are seen between all the varieties for the individual parameters as shown in Table 5. There are also differences between the overexpressed varieties

and the non-overexpressed base varieties for all parameters except the average area. There is no information in literature regarding this test for overexpressed varieties. For conventional varieties, there was one study with Cgrain data for hard winter wheat presented and both the overexpressed and non-overexpressed varieties in this study fell within the range given in that reference (Leiva et al., 2022). The overexpressed varieties had kernel size comparable or greater than KS Mako.

Kernel Data

Table 6: Kernel characterization for individual varieties.

Sample	Test Wt. (lb./bu)	TKW (g)	PC (%) (12 MB)	MC (%)	SKCS Avg Weight (mg)	SKCS Avg Diam (mm)	SKCS Avg Hardness
Breadbox	63.82 ^a ±0.08	33.87 ^a ±0.16	10.71 ^d ±0.09	11.84 ^{b,c} ±0.01	34.86 ^{a,b} ±0.37	2.88 ^{b,c} ±0.01	71.46 ^a ±0.92
Paradox	63.15 ^b ±0.22	34.19 ^a ±0.26	10.44 ^e ±0.08	11.87 ^b ±0.01	35.93 ^a ±0.30	2.90 ^b ±0.03	68.32 ^a ±3.10
Firebox	62.58 ^c ±0.06	34.45 ^a ±0.18	11.72 ^a ±0.07	11.81 ^{c,d} ±0.02	35.51 ^a ±0.84	3.00 ^a ±0.04	52.14 ^c ±0.43
Everest	57.87 ^e ±0.29	31.75 ^b ±0.27	11.25 ^b ±0.04	13.68 ^a ±0.03	35.91 ^a ±0.59	2.81 ^{c,d,e} ±0.01	35.93 ^e ±0.92
Gallagher	63.22 ^b ±0.06	30.83 ^c ±0.15	10.98 ^c ±0.07	11.77 ^d ±0.02	32.64 ^c ±1.11	2.73 ^e ±0.05	60.94 ^b ±0.60
KS Mako	59.90 ^d ±0.05	31.63 ^b ±0.15	10.03 ^f ±0.05	11.25 ^e ±0.01	32.91 ^{b,c} ±0.95	2.86 ^{b,c,d} ±0.04	48.35 ^{c,d} ±3.35
KS Providence	58.20 ^e ±0.17	34.38 ^a ±0.47	10.26 ^e ±0.01	11.82 ^{c,d} ±0.02	32.60 ^c ±0.38	2.79 ^{d,e} ±0.01	45.16 ^d ±1.73
p-value	0.0038	0.0083	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast OE vs Base	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: Overexpressed Varieties (OE), thousand kernel count (TKW), Weight (Wt), Protein Content (PC), Moisture Content (MC) Single Kernel Characterization (SKCS)

Note: The different groupings are denoted by the superscript lettering at a significance level of $p < 0.05$. Contrasts of overexpressed versus non-overexpressed varieties are also shown ($p < 0.05$).

Individual varieties differed in test weight, TKW, protein content, moisture content, SKCS hardness and sizing parameters with significant contrasts between the overexpressed varieties and non-overexpressed varieties (Table 6). These are known to be influenced by environmental conditions; however, this was not controlled for this experiment. Test weight for the overexpressed varieties was greater than target (60 lb./bu) (Chen et al., 2024), and similar to Gallagher (Bock et al., 2025). Breadbox (70.00) and Paradox (61.00) had a significantly higher hardness compared to the non-overexpressed varieties (50.00). High hardness was consistent

with the literature for these varieties (Bock et al., 2025). It should be noted that each of the four varieties grown in Oklahoma had greater hardness than those grown in Kansas, indicating weathering or environmental conditions may have contributed a greater difference than genetics. Firebox had the highest protein content at 11.72% followed by Breadbox (10.71%) and Paradox (10.44%). While these differences were found significant in this study, Bock et al. (2025) found non-significant differences. The overexpressed varieties have the highest thousand kernel weight along with KS Providence. It should be noted that on both the Cgrain characterization and kernel characterization the standard deviations are low, so there is strong segregation between every wheat line, not just the contrast between overexpressed and base varieties. This may highlight these tests are excessively sensitive to draw conclusions between kernel analysis and impact on downstream testing and application.

Milling

Table 7: Quadramat senior milling extractability for overexpressed and non-overexpressed varieties.

Sample ID	% Bran	% Shorts	% Bk Flour	% Red Flour	% Total Flour
Breadbox	21.95 ^e ±0.00	11.27 ^a ±0.00	29.88 ^d ±0.00	36.90 ^a ±0.00	66.79 ^{c,d} ±0.01
Paradox	23.36 ^{b,c} ±0.00	11.53 ^a ±0.00	28.55 ^e ±0.00	36.56 ^a ±0.00	65.11 ^f ±0.01
Firebox	22.72 ^d ±0.00	9.43 ^b ±0.00	33.51 ^b ±0.00	34.34 ^{a,c} ±0.00	67.85 ^b ±0.00
Everest	23.59 ^b ±0.00	7.37 ^d ±0.00	36.49 ^a ±0.00	32.55 ^a ±0.00	69.04 ^a ±0.00
Gallagher	22.49 ^d ±0.00	11.05 ^a ±0.00	30.28 ^d ±0.00	36.17 ^c ±0.00	66.45 ^d ±0.01
KS Mako	23.16 ^c ±0.01	9.70 ^b ±0.01	33.13 ^{b,c} ±0.01	34.01 ^b ±0.01	67.14 ^c ±0.01
KS Providence	25.56 ^a ±0.01	8.62 ^c ±0.00	33.07 ^c ±0.01	32.75 ^b ±0.00	65.82 ^e ±0.01
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast OE vs Base	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: Break (Bk), Reduction (Red), Overexpressed Varieties (OE)

Note: The different groupings are denoted by the superscript lettering at a significance level of $p < 0.05$. Contrasts of overexpressed versus non-overexpressed varieties are also shown ($p < 0.05$).

The flour fractions showed differences between varieties and the contrast between the overexpressed and non-overexpressed varieties (Table 7). Again, the small standard deviations should be noted given the ability to discriminate groupings at high sensitivity. Everest had the highest yield at 69.04% and Paradox had the lowest yield at 65.11%. However, in general, the

yield percentages were within the expected range for the Quadrumat senior mill which is 65-70% for HWW (Chen et al., 2024). Overall, the milling performance was comparable in range to the non-overexpressed varieties which is also seen in literature (Bock et al., 2025; Brar et al., 2021). The milling extraction yield was similar or greater than KS Mako which is expected since the kernels were similar or slightly larger than the kernels from KS Mako. The differences in milling may not be fully due to intrinsic differences in the varieties and are likely confounded with the different growing environments of the samples in the study.

Flour Characterization

As expected, correlations between whole grain protein content and whole meal and refined flour protein content are highly positive and significant ($R^2 = 0.94$ and $R^2 = 0.79$ respectively); Figure 2.

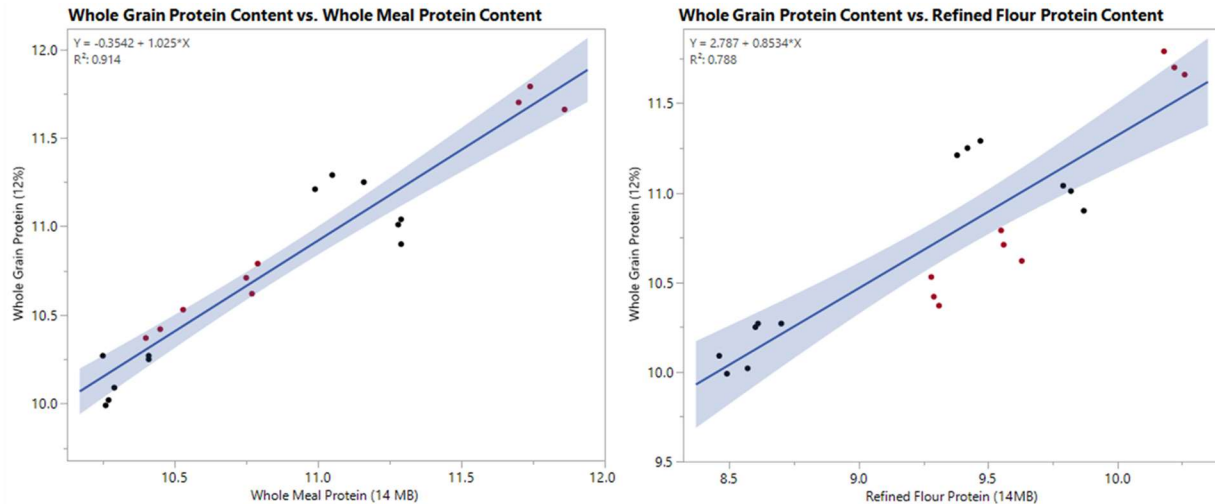


Figure 2: Correlation graphs between whole grain protein content and whole meal and refined protein content. The overexpressed varieties are shown in red.

Some studies showed that the overexpressed varieties had a slightly increased flour protein content (Brar et al., 2021; Lee et al., 2023; S. Li et al., 2020), but the results in this study did not corroborate these findings. In fact, the overexpressed varieties had a slightly lower protein

content than the non-overexpressed varieties for this specific sample set. In comparison to KS Mako, all of the overexpressed varieties had a higher protein content for both whole meal and refined flour. Interestingly enough, KS Mako had the lowest protein content of all the varieties tested in this study. Environmental conditions of the growing location and year may help explain the variation.

Table 8: Characterization of individual varieties using the NIR (14% moisture basis) for both whole meal and refined flour.

Combined Name	Whole Meal			Refined Flour		
	Moisture (%)	Protein (%)	Ash (%)	Moisture (%)	Protein (%)	Ash (%)
Breadbox	9.67 ^{b,c} ± 0.07	10.77 ^d ± 0.02	1.393 ^d ± 0.014	14.83 ^{a,b} ± 0.02	9.58 ^c ± 0.04	0.343 ^{c,d} ± 0.006
Paradox	9.60 ^c ± 0.07	10.46 ^e ± 0.07	1.392 ^d ± 0.025	14.74 ^{c,d} ± 0.02	9.29 ^e ± 0.02	0.347 ^{b,c} ± 0.003
Firebox	9.70 ^{b,c} ± 0.06	11.77 ^a ± 0.08	1.510 ^c ± 0.018	14.86 ^a ± 0.01	10.22 ^a ± 0.04	0.344 ^{c,d} ± 0.003
Everest	10.18 ^{a,b} ± 0.04	11.07 ^c ± 0.09	1.735 ^a ± 0.010	14.33 ^e ± 0.03	9.42 ^d ± 0.05	0.358 ^b ± 0.001
Gallagher	10.34 ^a ± 0.04	11.29 ^b ± 0.01	1.471 ^c ± 0.015	14.68 ^d ± 0.04	9.83 ^b ± 0.04	0.373 ^a ± 0.004
KS Mako	7.78 ^e ± 0.04	10.27 ^e ± 0.02	1.512 ^c ± 0.017	14.77 ^{b,c} ± 0.01	8.51 ^g ± 0.06	0.338 ^{c,d} ± 0.005
KS Providence	8.78 ^d ± 0.43	10.36 ^e ± 0.09	1.641 ^b ± 0.046	14.79 ^{a,b,c} ± 0.04	8.64 ^f ± 0.06	0.349 ^d ± 0.003
p-value	0.0045	0.0042	0.0042	0.0042	<0.0001	<0.0001
Contrast OE vs Base	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Abbreviations: Overexpressed Varieties (OE)

Note: The different groupings are denoted by the superscript lettering at a significance level of $p < 0.05$. Contrasts of overexpressed versus non-overexpressed varieties are also shown ($p < 0.05$).

In general, based on the flour and grain characterization of the individual varieties, the overexpressed varieties perform similarly to the non-overexpressed varieties within reasonable range of HRW and similar to the data presented in the literature (Table 8). From the kernel and flour characterization alone, these varieties do not stand out as unique from other HRW varieties regarding kernel characteristics, milling extraction, or flour characteristics. In comparison to KS Mako the overexpressed varieties either surpass or match KS Mako giving suggestion that the overexpressed varieties may perform in a similar fashion to a high performing HRW variety.

Flour Characteristics of Blends

No study has yet looked at the effect on flour composition of blending the overexpressed varieties with non-overexpressed varieties. In general, protein content for both whole meal and refined flour were different among blend levels (Figure 3). Again, the environmental effects may have a larger effect on the differences than the genetics of the varieties. The two exceptions were for the whole meal protein content for the Paradox-KS Providence series and the Breadbox-Everest series. In both these cases, the proteins of the base flour and the overexpressed variety were very similar so there were no significant differences in the blends at any level. For KS Providence and KS Mako, the overexpressed varieties increased the protein content as blended level increased. This is due to the protein contents of the overexpressed varieties being greater than both KS Mako and KS Providence. Everest, however, has a higher protein content than Breadbox and Paradox whole meal so protein content decreased with increasing levels of Breadbox or Paradox. For refined flour, Everest had a higher protein content than Paradox, so Paradox diluted the protein content. Firebox and Breadbox both had a higher protein content, so their addition led to increase. Blending improved protein content with KS Mako as a base flour since it had such a low protein content in this study.

GlutoPeak

Individual Results for whole meal and refined flour for the GlutoPeak parameters are shown in Table 9. Breadbox and Paradox outperform Firebox, which is expected due to Paradox and Breadbox being pre-selected for extreme gluten performance. From the data collected in this experiment, the overexpressed varieties outperform the base varieties in PMT, AM, and aggregation energy for whole meal and PMT, BEM, AM, Aggregation energy, and TPA for refined flour. The overexpressed varieties also outperform KS Mako for all parameters. There is

no published data on the GlutoPeak for overexpressed varieties. There is literature about base flours and comparisons between the whole meal and refined flours however. Regarding the comparison of whole meal versus refined flour, the present results are similar to the literature. The whole meal had lower peak maximum time (PMT) than the refined flour which is consistent with literature (K. Wang et al., 2021). However, Wang et al., 2021 found that whole meal tends to have a higher maximum torque (BEM), but in general, whole meal had a lower BEM in this data set than refined flour. It must be noted that testing conditions were different. K. Wang et al. (2021) used a solid to water ratio of 8 grams flour to 10 grams distilled water at 2700 rpm for both whole meal and refined flour. The protocols used in this experiment were different for whole meal and refined flour.

For whole meal 8.5 grams whole meal was added to 9 grams 0.5 M calcium chloride solution at 1900 rpm. For refined flour 9 grams refined flour and 9 grams distilled water at 2700 rpm. Therefore, differences in results were partially attributed to differences in protocol. Literature suggests BEM greater than 50-60 GPU and aggregation energy greater than 1300 GPU are appropriate targets for strong refined flour (Mecitoğlu Güçbilmez et al., 2019; Rakita et al., 2018). However, these studies also used different protocols. Rakita et al. (2018) used a different ratio of flour than the refined method used in this study as well as using a 2% NaCl solution instead of distilled water.

While KS Mako is considered a higher performing HRW variety, according to the current data, it does not hit the suggested targets mentioned above. This difference may be explained by the low protein content of this specific sample. KS Mako has a grain protein content of 10.03% for the grain, but for hard winter wheat the target protein is greater than 12% (Chen et al., 2024). This highlights the importance of having a standard method for comparison of data.

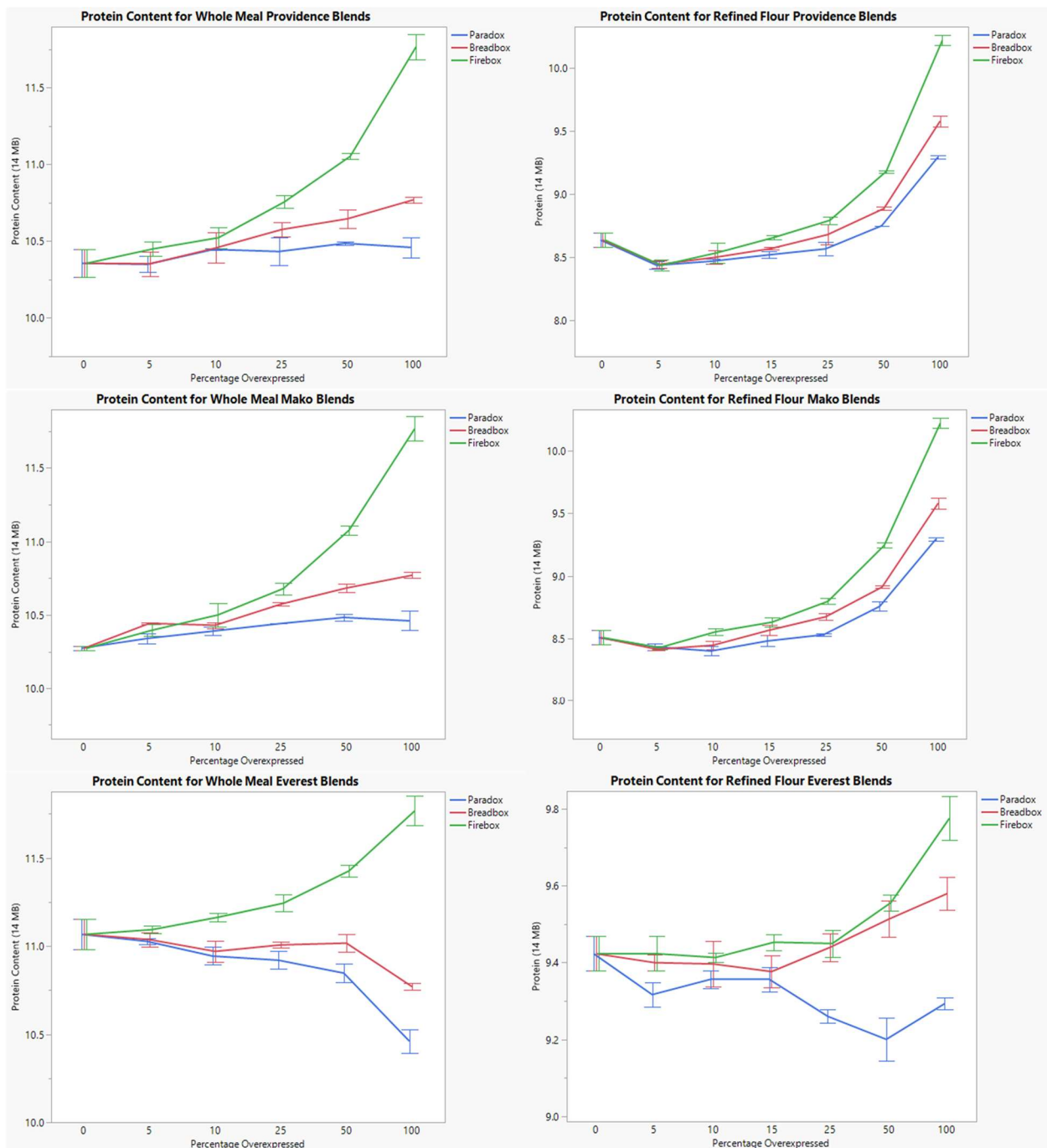


Figure 3: Protein content for each blend segregated by base flour. Both whole meal and refined flour are shown.

GlutoPeak Blend Performance

For the blends, values obtained for most GlutoPeak parameters for both the whole meal and refined flours were significantly different. There were only a few exceptions as follows. In the

whole meal Paradox-KS Providence, Paradox-KS Mako and Breadbox-KS Mako series, the AM data was not significantly different. In the Firebox-KS Providence and Paradox-KS Mako series, the PM data was not significantly different for whole meal. For refined flour, for the Firebox-Everest, Paradox-KS Mako, and Firebox-KS Mako series, the AM data was also not significantly different. The AM and PM parameters denote the torque 15 seconds before and after the BEM respectively. It seems that the steepness before and after the peak is similar. Figures 4 and 5 show graphs of the blends of PMT and aggregation energy which are two parameters commonly looked at to determine quality. As seen in both figures, the standard deviations are much wider in the whole meal graphs likely due to a potential difference in the size of the bran particles leading to variable disruption on the gluten aggregation. In general, the trends are similar to the refined flour, but there is more variance. The variation is more significant in the whole meal data. Literature agrees that the refined flour is more sensitive than whole meal (K. Wang et al., 2021). In general, as the percentage of overexpressed flour increased, both PMT and aggregation energy also increased. For the KS Mako Series, the addition of Firebox did not increase the PMT which is due to the presumed lower level of overexpression. For both whole meal and refined flour, PMT provided better discrimination between varieties than the aggregation energy. In the PMT results, the same trends are present in whole meal and refined flour especially at the higher blend levels. Aggregation energy follows the same trend, but with higher variability. Overall, the results highlight the limitations in whole meal GlutoPeak testing due to the higher heterogeneity of the sample and of the disruption caused by the bran particles. Although this may limit the application of the whole meal GlutoPeak test for definitive results, it might still be applicable. For instance, in a breeding program, it may be valuable for quick differentiation in preliminary breeding stages. In each case, the base blended with an overexpressed variety was able to

surpass KS Mako on its own showing the potential for the overexpressed wheat to correct dough rheology of lower quality wheats. It must be noted that the level of overexpressed wheat required to reach the target KS Mako varies for each overexpressed-base combination.

In order to understand if there are differences between overexpressed varieties at the same level, ANOVA was ran setting the base flour and blend level with only the overexpressed flour as a factor (Table 10). The results indicate the GlutoPeak is better at differentiating the refined flour than the whole meal as indicated by the greater number of parameters with differences. For whole meal, of the overexpressed varieties individually, the GlutoPeak can distinguish between the quality of the overexpressed varieties. However, for the KS Providence and Everest bases, little differentiation is seen at the different levels. For KS Mako, at 25% some discrimination is seen between the overexpressed varieties. A different conclusion is drawn for refined flour, where discrimination is captured at several levels for each base. The GlutoPeak captures differences in quality for the overexpressed varieties individually for all parameters except PM and GSI 1. For the KS Mako base, the greatest discrimination is at 15% with all parameters significantly different except PMT, BEM, and GSI 3. For Everest, the greatest discrimination is seen at 50% with differences for all GlutoPeak parameters except BEM. For KS Providence, the greatest discrimination is seen at 50%, with significant differences between all parameters except aggregation energy. This underscores that the effect of blending is base and level dependent. Also, at low levels, such as 5-10%, the GlutoPeak does not consistently discriminate the quality of the blend. The differences between whole meal and refined flour show the GlutoPeak is not able to differentiate them the same way and there would be no reason to blend the whole meal samples because there is not enough differentiation that occurs.

Conclusions

In this study, the overexpressed varieties were individually characterized regarding kernel characteristics, milling performance, and GlutoPeak testing. While some data exists for the kernel quality, this is the most complete set of data presented characterizing the overexpressed varieties. Kernel quality and milling performance was overall typical of HRW wheat varieties. Differences were seen in PMT and protein aggregation as measured using the GlutoPeak. The blending series confirmed the discriminatory ability of the GlutoPeak when incremental changes were made to the overexpressed (Bx7^{OE}) wheat flour content. The GlutoPeak provided less discrimination of the quality of overexpressed varieties in whole meal blends; thus, testing the original variety is more effective than blending. For refined flour, blending is a potential strategy and does decrease the test time, however dependent on the specific base flour and blend level. It was also seen that blending does improve the quality of weaker varieties underscoring potential to correct dough rheology.

Table 9: GlutoPeak parameters for individual varieties for whole meal and refined flour.

Whole Meal									
Variety	Breadbox	Paradox	Firebox	Everest	Gallagher	KS Mako	KS Providence	p-value	Contrasts: OE vs Base
PMT (s)	105.341 ^a ± 6.425	113.005 ^a ± 3.002	71.342 ^b ± 0.583	46.011 ^d ± 1.001	57.34 ^{c,d} ± 1.533	59.341 ^{b,c} ± 7.098	48.338 ^{c,d} ± 4.166	0.0048	<0.0001
BEM (GPU)	44 ^{a,b} ±2	44 ^{a,b} ±1	48 ^a ±1	41 ^{b,c} ±2	46 ^{a,b} ±1	42 ^{b,c} ±2	37 ^c ±3	0.0001	0.0001
AM (GPU)	39 ^a ± 1	38 ^{a,b} ± 0	30 ^{b,c} ± 1	24 ^c ± 2	27 ^c ± 2	29 ^c ± 7	25 ^c ± 1	0.0109	<0.0001
PM (GPU)	41 ^{b,c} ±2	41 ^{a,b,c} ±2	45 ^a ±1	37 ^c ±1	43 ^{a,b} ±0	37 ^c ±2	33 ^d ±3	<0.0001	<0.0001
AgE (GPU)	1261 ^a ±37	1268 ^a ±42	1302 ^a ±9	1076 ^{b,c} ±34	1221 ^a ±30	1120 ^b ±13	1011 ^c ±58	<0.0001	<0.0001
GSI1	55.954 ^a ± 4.195	56.216 ^a ± 2.617	62.081 ^a ± 1.115	44.169 ^{b,c} ± 3.231	55.751 ^a ± 0.995	46.646 ^b ± 1.538	37.527 ^c ± 5.749	<0.0001	<0.0001
GSI2	132.729 ^a ± 6.350	143.240 ^a ± 5.611	92.911 ^b ± 0.956	49.521 ^d ± 1.81	70.039 ^c ± 3.471	66.470 ^c ± 8.389	48.760 ^d ± 3.369	0.0044	<0.0001
Refined Flour									
Variety	Breadbox	Paradox	Firebox	Everest	Gallagher	KS Mako	KS Providence	p-value	Contrasts: OE vs Base
PMT (s)	396.007 ^b ± 4.581	517.010 ^a ± 8.545	247.338 ^c ± 4.038	148.340 ^d ± 4.510	140.009 ^d ± 1.000	239.339 ^c ± 3.055	136.347 ^d ± 0.577	0.0032	<0.0001
BEM (GPU)	56 ^a ± 1	55 ^{a,b} ± 0	53 ^c ± 1	44 ^d ± 1	54 ^{b,c} ± 1	36 ^e ± 0	37 ^e ± 0	0.0034	<0.0001
AM (GPU)	34 ^a ± 4	35 ^a ± 1	25 ^b ± 1	25 ^b ± 3	21 ^b ±1	24 ^b ± 4	19 ^b ± 1	0.0083	<0.0001
PM (GPU)	48 ^a ± 1	46 ^{a,b} ± 1	44 ^{b,c} ± 1	39 ^d ± 1	43 ^c ± 2	34 ^e ± 1	31 ^e ± 0	0.0035	<0.0001
AgE (GPU)	1518 ^a ± 23	1500 ^a ± 7	1361 ^b ± 9	1206 ^c ± 9	1323 ^b ± 11	1010 ^d ± 36	970 ^d ± 5	0.0039	<0.0001
TPA (GPU)	8267 ^b ± 77	10661 ^a ± 44	4945 ^c ± 66	3235 ^e ± 45	3296 ^e ± 32	3740 ^d ± 124	2598 ^f ± 22	0.0034	<0.0001
GSI1	84.527 ^a ± 2.013	82.500 ^a ± 0.361	72.131 ^b ± 1.291	52.646 ^c ± 0.671	71.023 ^b ± 1.340	36.348 ^d ± 1.300	35.890 ^d ± 0.170	0.0046	<0.0001
GSI2	601.264 ^b ± 10.981	775.478 ^a ± 9.418	336.632 ^c ± 6.348	178.867 ^e ± 6.393	185.286 ^e ± 2.825	241.726 ^d ± 11.671	132.257 ^f ± 0.913	0.0034	<0.0001
GSI3	460.198 ^b ± 8.296	586.373 ^a ± 2.401	262.024 ^c ± 1.643	141.233 ^e ± 1.199	176.897 ^d ± 3.533	134.640 ^e ± 4.448	96.138 ^f ± 0.797	0.0032	<0.0001
GSI4	3273.651 ^b ± 47.561	5512.259 ^a ± 112.978	1223.173 ^c ± 35.721	479.966 ^e ± 21.232	461.492 ^e ± 7.725	895.374 ^d ± 40.588	354.283 ^e ± 4.27	0.0035	<0.0001

Abbreviations: Overexpressed Varieties (OE)

Note: The different groupings are denoted by the superscript lettering at a significance level of $p < 0.05$. Contrasts of overexpressed versus non-overexpressed varieties are also shown ($p < 0.05$).

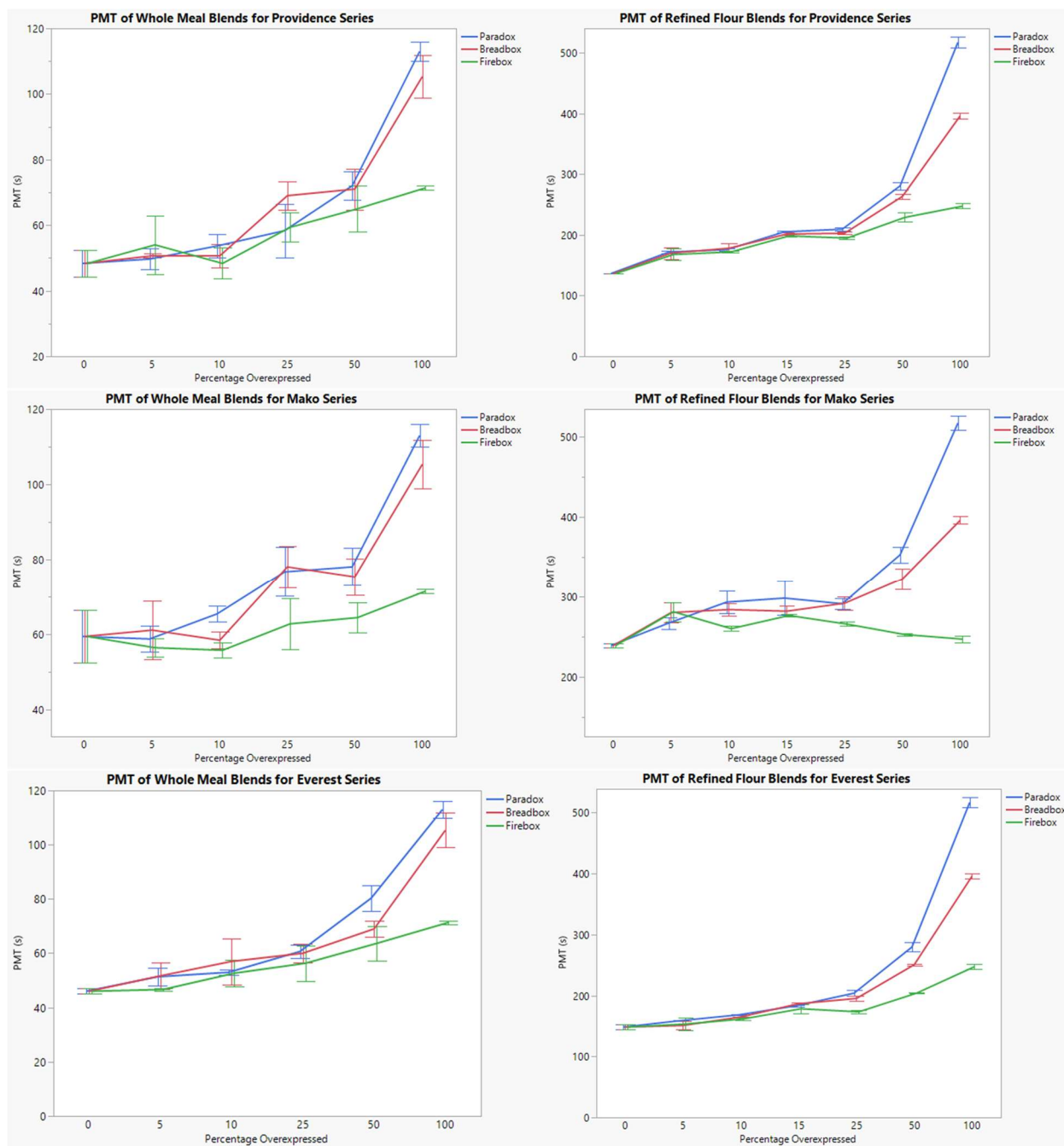


Figure 4: GlutoPeak PMT of whole meal and refined flour blends segregated by base flour.

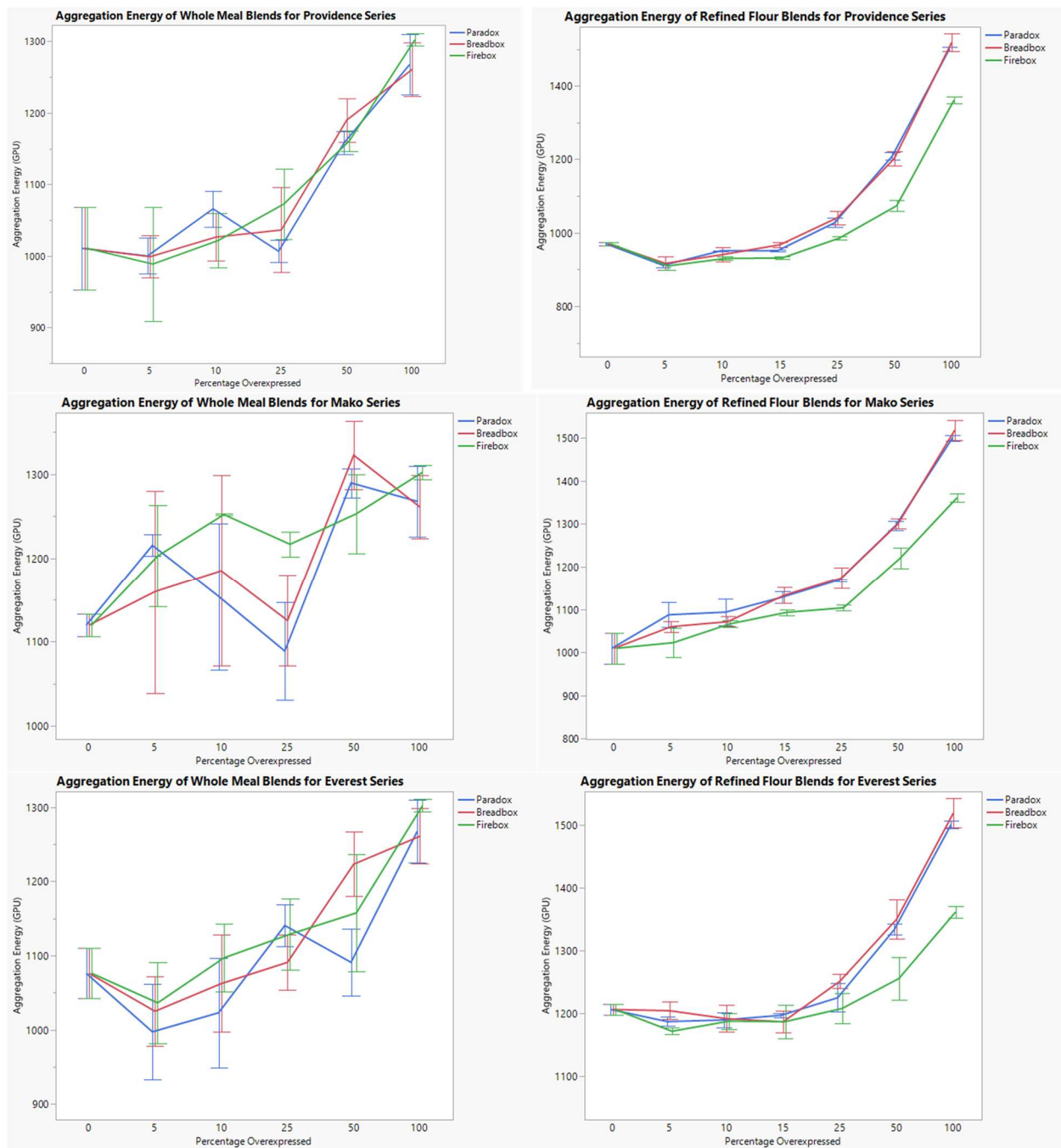


Figure 5: GlutoPeak aggregation energy of whole meal and refined flour blends segregated by base flour.

Table 10: Comparison of whole meal and refined flour GlutoPeak parameters at a fixed percent for each base to determine significance of overexpressed variety (p<0.05).

		Percentage	PMT (s)	BEM (GPU)	AM (GPU)	PM (GPU)	AgE (GPU)	GSI 1	GSI 2			
Whole Meal	KS Mako	5	0.5611	0.9885	0.8167	0.986	0.7326	0.9565	0.3134			
		10	0.0039	0.176	0.0663	0.4065	0.2019	0.2388	0.1931			
		25	0.0452	0.0407	0.6069	0.1151	0.0377	0.0268	0.1651			
		50	0.0255	0.3679	0.0825	0.3554	0.1522	0.2466	0.0103			
	Everest	5	0.2214	0.888	0.8534	0.6564	0.6948	0.7581	0.5219			
		10	0.6145	0.9118	0.3755	0.7046	0.4085	0.713	0.3961			
		25	0.4887	0.7406	0.1542	0.5822	0.3304	0.5747	0.3656			
		50	0.0158	0.1116	0.6191	0.2446	0.0832	0.0947	0.0202			
	KS Providence	5	0.9565	0.9552	0.9508	0.8155	0.9563	0.9795	0.5596			
		10	0.3242	0.1211	0.6787	0.6951	0.2615	0.1846	0.2861			
		25	0.128	0.5529	0.0643	0.0739	0.2777	0.4437	0.0712			
		50	0.3626	0.3396	0.9686	0.3319	0.2166	0.0458	0.4071			
OE on own		0.0156	0.0231	0.0188	0.0107	0.0819	0.0462	<0.0001				
		Percentage	PMT (s)	BEM (GPU)	AM (GPU)	PM (GPU)	AgE (GPU)	TPA (GPU)	GSI 1	GSI 2	GSI 3	GSI 4
Refined Flour	KS Mako	5	0.2772	0.0156	0.1385	0.3041	0.0693	0.0460	0.0309	0.6448	0.0254	0.4150
		10	0.0137	0.7023	0.0306	0.4219	0.2696	0.0007	0.5165	0.0002	0.0360	0.0001
		15	0.1133	0.0608	0.027	0.0469	0.0200	0.0273	0.0130	0.0430	0.0608	0.0062
		25	0.0045	0.0787	0.0098	0.3041	0.0018	0.0594	0.0093	0.0665	0.0011	0.0608
		50	<0.0001	0.0894	0.0576	0.3480	0.0608	0.0273	0.0062	0.0273	0.0273	<0.0001
	Everest	5	0.2019	0.2963	0.5552	0.1787	0.0173	0.2396	0.0700	0.5866	0.0534	0.1184
		10	0.0047	0.3679	0.8424	0.0600	0.9540	0.0007	0.9243	0.0249	0.0071	0.0004
		15	0.1680	0.5647	0.5477	0.0370	0.7475	0.0005	0.6909	0.0191	0.0010	0.0390
		25	0.0002	0.1787	0.2051	0.0787	0.0925	0.0439	0.3105	0.0509	0.0665	0.0390
		50	<0.0001	0.1101	0.0077	0.0319	0.0113	0.0273	0.0278	<0.0001	0.0273	<0.0001
	KS Providence	5	0.8292	1.000	0.4219	0.3679	0.7802	0.1199	0.9406	0.7621	0.2982	0.4992
		10	0.3627	0.5134	0.0600	0.5477	0.1595	0.0025	0.4527	0.0237	0.0304	0.0983
		15	0.0113	0.0025	0.0302	0.0183	0.0005	0.0608	0.0005	0.0608	0.0001	0.0002
		25	0.0012	0.0556	0.1409	0.0211	0.0052	0.0273	0.0058	0.0273	0.0608	<0.0001
		50	0.0001	0.0007	0.0011	0.0080	0.0628	0.0273	0.0002	0.0273	0.0273	<0.0001
OE on Own		<0.0001	0.0066	0.0036	0.007	0.0509	0.0273	0.0509	<0.0001	0.0273	<0.0001	

Abbreviations: Overexpressed Varieties (OE)

Chapter 3 - Dough Rheology and Baking Quality of Bx7^{OE} Wheat

Varieties and their Blends with Non-overexpressed Varieties

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Introduction

Wheat and wheat products are consumed all around the world. In 2023, the global average wheat per annum consumption was 68.82 kg, while in the U.S. it was 88.92 kg (*FAOSTAT*, 2026). This represents 535.7 kcal per day from wheat and wheat products globally, and 599.3 kcal in the U.S. (*FAOSTAT*, 2026). While the United States consumes high amounts of wheat and wheat products, 75 countries consume more, demonstrating that wheat is not only important in the U.S, but also worldwide. The wheat produced in the United States is important for the well-being of people living in this country, but also those all around the world, as 40.6% of the wheat produced in the U.S. is exported (*FAOSTAT*, 2026).

In the Great Plains region, most of the wheat grown is hard winter wheat (HWW), and the end product of most interest is bread, representing about 70% of total HWW utilization. Breeders in the Great Plains regions seek to improve the quality of the wheat by improving productivity, disease resistance, and end product quality. One of the recent focuses of breeders in the Great Plains region has been on the *Glu-B1a1* allele. This allele produces the overexpression of the Bx7 protein subunit and was first encountered as a natural phenomenon in bread wheat

when the Bx7 subunit was duplicated. The wheat variety that was known to first express this mutation was Red River 68 and the overexpressed subunit was first found using sodium dodecyl sulphate polyacrylamide gel electrophoresis (D'Ovidio et al., 1997). The significance of this Bx7 overexpression (Bx7^{OE}) is due to the larger proportion of high molecular weight (HMW) glutenin subunit which has been associated with improvements in dough rheology and bread volume (J. Li et al., 2014). This mutation is rare and has only occurred in bread wheat but produces about 42% HMW glutenin as opposed to the 30% produced in non-overexpressed wheat varieties (J. Li et al., 2014).

Studies on the overexpressed varieties of HWW are limited. They have shown that microstructure of the glutenin is improved with Bx7^{OE} varieties containing more β -sheets with less α -helices, β turns, and antiparallel β -sheets which are all less desirable than the β -sheets (S. Li et al., 2020). Not only that, but the overexpressed varieties have more free sulfhydryl groups allowing more disulfide bonds to form. This results in more crosslinks and a better gluten structure during mixing of dough, which leads to increased dough strength and product quality. The milling extraction rate has been determined to be similar between overexpressed and non-overexpressed wheat varieties (Bock et al., 2025; Brar et al., 2021), but differences are seen in the dough quality. Mixograph peak times are greatly increased and exceed recommended Wheat Quality Council values (Adegbite, 2024; Bock et al., 2025; Chen et al., 2024; J. Li et al., 2014). Farinograph dough development time and stability are much greater, exceeding the Wheat Quality Council values and surpassing the typical 20-minute Farinograph test times (Adegbite, 2024; Bock et al., 2025; Brar et al., 2021; Chen et al., 2024; Vancini et al., 2019). The Alveograph parameters of the overexpressed varieties show an increase in P (characterizing the elasticity of the dough) and W (characterizing total energy required) parameters, but another

study mentions parameters of the test would need to be changed to accommodate the Bx7^{OE} varieties (Bock et al., 2025; L. Zhang et al., 2010). A few studies mentioned bread quality claiming the overexpressed varieties improved volume, but few showed data (Brar et al., 2021; Cooper et al., 2016). In the study that showed data, the overexpressed varieties were deemed acceptable, but not excellent in regard to volume (Adegbite, 2024; Bock et al., 2025).

Due to the novelty of the *Glu-B1a1* allele, there is limited published data. Ultimately, a variety of wheat must have some industrial and processing relevance if it is to be sold to the value-added industry. Due to its excessive strength, it has been suggested as a bread improver. From a testing standpoint, long test times are undesirable for efficiency. Also, few testing protocols are equipped to identify parameters when test times exceed the typical range. The goal of this study is to address these issues and provide a systematic baking approach to address whether the rheological findings are correlated to end product quality. The objectives of this study were to characterize the dough rheology of refined flour milled from overexpressed varieties; use blending as a strategy to test these varieties in a quick and effective manner; blend overexpressed varieties with base non-overexpressed varieties to determine the improvement in end product quality of the base flour; and to develop a model to determine how rheological parameters explain the variance of loaf specific volume.

Materials and Methods

Variety Selection

The overexpressed Bx7 protein subunit varieties used in this study were Breadbox, Firebox, and Paradox which were developed by the Oklahoma State University breeding program. Gallagher was also included in this study which is a variety that does not contain the overexpressed Bx7 subunit but is prominent in the backgrounds of both Breadbox and Paradox.

These varieties were selected from a population of overexpressed lines due to their high gluten performance. Paradox and Breadbox are near isogenic lines and have a higher performance than Firebox. Base flours of variable known qualities were chosen following the quality series of: Everest < KS Providence < KS Mako. Oklahoma Foundation Seed Stocks provided cleaned wheat for Gallagher, Breadbox, Firebox, and Paradox while cleaned wheat for Everest, KS Mako, and KS Providence were obtained from KSU Foundation Seed. The overexpressed lines and Gallagher were grown in Oklahoma whereas KS Mako, KS Providence, and Everest were grown in Kansas.

Milling

Before milling, wheat kernels were tempered to a 15% moisture and milled using a Quadrumat Senior experimental mill. Break flour and reduction flour were combined, and flour composition was determined using the NIR as shown in Table 11.

Table 11: Summary table of milling data.

Sample ID	% Total Flour	Moisture (%)	Protein (%)	Ash (%)
Breadbox	66.79 ^{c,d} ±0.01	14.83 ^{a,b} ±0.02	9.58 ^c ±0.04	0.343 ^{c,d} ±0.006
Paradox	65.11 ^f ±0.01	14.74 ^{c,d} ±0.02	9.29 ^e ±0.02	0.347 ^{b,c} ±0.003
Firebox	67.85 ^b ±0.00	14.86 ^a ±0.01	10.22 ^a ±0.04	0.344 ^{c,d} ±0.003
Everest	69.04 ^a ±0.00	14.33 ^e ±0.03	9.42 ^d ±0.05	0.358 ^b ±0.001
Gallagher	66.45 ^d ±0.01	14.68 ^d ±0.04	9.83 ^b ±0.04	0.373 ^a ±0.004
KS Mako	67.14 ^c ±0.01	14.77 ^{b,c} ±0.01	8.51 ^g ±0.06	0.338 ^{c,d} ±0.005
KS Providence	65.82 ^e ±0.01	14.79 ^{a,b,c} ±0.04	8.64 ^f ±0.06	0.349 ^d ±0.003
p-value	<0.001	0.0042	<0.0001	<0.0001
Contrast OE vs Base	<0.001	<0.0001	<0.0001	<0.0001

Abbreviations: Overexpressed Varieties (OE)

Note: Significant groupings are denoted by subscripts at a significance level of p<0.05.

Blending of Refined flour

Blends of the refined flour were made according to Table 12. The overexpressed flours were individually blended into the base flour and homogenized using a V-blender for 10 minutes.

Samples were then stored in hermetic buckets at room temperature. Blends were tested in triplicate.

Table 12: Summary of blending performed in experiment.

Base	Bx7 ^{OE}	Refined Flour Blend Percentages
Everest	Breadbox	0
	Firebox	5
	Paradox	10
		15
		25
		50
		100
KS Mako	Breadbox	0
	Firebox	5
	Paradox	10
		15
		25
		50
		100
KS Providence	Breadbox	0
	Firebox	5
	Paradox	10
		15
		25
		50
		100

Note: Bx7^{OE} denotes overexpressed varieties

Refined Flour Testing

After milling, 2000 g samples were taken in triplicate for each variety, and the following tests were performed. The protein was measured using the Foss DS

2500 NIR (Foss, Hilleroed Denmark) according to AACC method 39-11.01 (AACC

International, 1999)). Moisture and ash were also measured. GlutoPeak analysis was conducted

on each sample using the Extended method, in which 9 g of refined flour was added to 9 g

distilled water. The paddle speed was 2700 rpm and analysis temperature was 34 °C (Chandi & Seetharaman, 2012). The test was conducted until 15 seconds after the peak.

Mixograph analysis was performed using AACC method 54-40.02 (AACC International Method, 1999). The 10 g bowl was used. Data was collected via the drawn mixogram and using

MixSmart (Version 2.0.609, AEW Consulting, Lincoln NE). Predicted water absorption for the Mixograph was based on an internal regression equation relating GlutoPeak data to known Mixograph water absorption values across several hundred wheat lines. The parameters of interest were chosen to be Mixograph absorption, Mixograph mid-line peak time, and Mixograph mixscore. The mixscore is a calculated value seen below.

$$\text{MixScore} = (3 \times \text{Peak Time}) + \text{Width 2 minutes post peak}$$

This parameter has been internally seen to be related to the Farinograph tolerance score and the historical Mixograph tolerance subjective scoring systems.

Farinograph was performed using the constant dough weight method according to AACC method 54-22.01 for the 50 g bowl (AACC International Method, 2011). The parameters of interest were development time and stability.

Alveograph was performed according to method 54-30.02 using the constant hydration protocol (AACC International Method, 1999). The parameters chosen for analysis were P (characterizes tenacity of dough), L (characterizes extensibility of dough), and W (characterizes total energy required to rupture the dough piece upon inflation).

Baking was performed using a modified AACC optimized straight dough method (AACC International, 1999). The Mixograph absorption and mix time were used for the baking trials, as dough was prepared using a mixer of similar geometry (100 g pin mixer, National Manufacturing, Lincoln NE). Dry inactive yeast was used instead of compressed yeast at 1.7% flour basis according to the conversion given from the manufacturer. Power consumption during mixing and time to maximum power were recorded using a plug-in power logger (HOBOWare Version 3.7.28, Onset Computer Corporation, Buzzards Bay, MA).

Dough properties were labeled based on texture, as judged by an experienced baker (scale of five descriptors, ranging from very extensible to very tight). Laser topography was used to measure loaf volume exactly 1-hour post-baking, using the AACC method 10-16.01 (AACC International Method, 2015). Specific volume was computed.

Baking trials were conducted in triplicates, with a check sample included each day.

Modeling of Specific Volume

Multiple linear regression models were estimated by ordinary least squares (OLS) in a workflow that uses the Statsmodels package/library in Python. The models were developed and iteratively compared to explain the variation of specific volume as a function of the dough rheology predictors. Candidate predictors were specified a priori based on mechanistic plausibility. The categorical variable Dough Property was encoded using $k-1$ indicator (dummy) variables. The workflow started with a theory-based “full” model containing all candidate predictors.

Parameter estimates were reported with associated standard errors, two-sided t-tests, and 95% confidence intervals. Standard regression diagnostics were then applied to assess key regression assumptions and identify potential violations. Heteroscedasticity (unequal variance) was screened using the Breusch–Pagan test, residual autocorrelation was screened using the Durbin–Watson statistic, and residual structure/normality were visually assessed using residual-versus-fitted and normal Q–Q plots. Influential observations were evaluated using Cook’s distance, with points flagged when $D_i > 4/n$. Multicollinearity was assessed using variance inflation factors (VIF).

The workflow evaluated the models using forward selection with an entry criterion of $p \leq 0.05$, and backward elimination with a removal criterion of $p > 0.10$. The resulting candidate models (full, forward-selected, backward-eliminated) were evaluated using adjusted R-squared, Akaike

Information Criterion (AIC), Bayesian Information Criterion (BIC), and predicted residual error sum of squares (PRESS). To limit instability due to collinearity, models were additionally screened against a maximum VIF threshold (default of 10). The workflow pointed to a final explanatory model that prioritized low AIC (with corroboration from PRESS/BIC), acceptable multicollinearity (VIF constraint), and a statistically significant overall F-test.

Statistical Analysis

Normality was assessed using Shapiro-Wilk ($p < 0.05$) and distribution of residuals. If the normality assumption held true, differences were determined using ANOVA followed by Tukey's HSD test at $p < 0.05$ (JMP® Version 18.0.0., SAS Institute Inc., Cary, NC). If the data was not normal, differences were determined using the Wilcoxon/ Kruskal-Wallis Tests (Rank Sums) followed by Bonferroni at a significance level of $p < 0.05$ (SAS® 9.4, SAS Institute Inc., Cary, NC). Contrasts were also assessed to determine differences between overexpressed (OE) varieties compared to non-overexpressed (non-OE) varieties as two distinct groups.

Results and Discussion

Characterization of Individual Bx7^{OE} Varieties

Table 13 summarizes the characterized parameters for the individual varieties. The Mixograph peak time describes the mix time required to achieve maximum resistance with peak times greater than eight minutes an indication of strong doughs (Adegbite, 2024; Bock et al., 2025; J. Li et al., 2014). All overexpressed varieties in this study surpassed the eight-minute mark as well as the targets for the Wheat Quality Council (Chen et al., 2024). The longer peak times in this study are in agreement with literature, but the peak times seen in this study of the overexpressed varieties surpassed the peak times found in literature of these overexpressed varieties. Gallagher in the current study had a peak time of 7.05 ± 0.29 min compared to 4.3 minutes seen previously

(Adegbite, 2024; Bock et al., 2025). In previous studies overexpressed varieties had seen peak times of 3.1 to 9.1 minutes, but for these overexpressed varieties tested peak times are between 8.47 ± 0.41 and 17.07 ± 0.34 minutes. Differences can be attributed to the growing conditions and specific wheat tested. Breadbox and Paradox performed significantly higher than the non-overexpressed varieties. Firebox fell within the range of the stronger HRW lines of Gallagher and KS Mako. Firebox has a lower amount of gluten strength compared to Paradox and Breadbox, which were selected for their unique strength surpassing other overexpressed lines. The Wheat Quality Council HWW water absorption percentage target is 62+, which was achieved for all overexpressed varieties in the current study. The Mixograph absorption was determined using the internal regression equation (unpublished data) from the GlutoPeak. The overexpressed varieties had higher absorptions than the non-overexpressed varieties. There is no data about the overexpressed varieties and the mixscore parameter. In general, a value of 30 or greater typically characterizes a line with good mixing tolerance. In this study, KS Mako, which is known to be a high performing line has a mixscore of 30.87 ± 1.29 . The mixscore values of Breadbox, Paradox, and Firebox are 63.45 ± 1.41 , 76.55 ± 5.48 , 36.79 ± 1.30 , surpassing the mixscore of KS Mako indicating they have a strong mixing tolerance. For the Farinograph, the overexpressed lines show greater strength with longer development and stability times than those without the overexpression which aligns with the data presented here. (Adegbite, 2024; Bock et al., 2025; Brar et al., 2021; Vancini et al., 2019). The target Wheat Quality Council Farinograph development times are 4-8 minutes, and the target stability is 10-16 minutes for hard winter wheat (Chen et al., 2024). This study shows that the overexpressed varieties tested far surpass the targets.

Table 13: Individual lines characterization including rheology and bake data.

Variety	Mixo Abs (%)	Mixo ML Peak Time (min)	Mixo MixScore	Development time (min)	Stability (min)	P (mm H2O)	L (mm)	W (10- ⁴ J)	Specific Volume (cm ³ /g)	Power (W)	Time to Power (min)
Breadbox	63.6 ^a ±0.2	17.07 ^b ±0.34	63.45 ^b ±1.41	51.7 ^b ±3.0	59.2 ^a ±0.2	132 ^a ±4	149 ^a ±13	437 ^a ±54	4.01±0.17	100.21±11.58	17.04 ^a ±1.16
Paradox	63.1 ^{a,b} ±0.3	21.22 ^a ±1.74	76.55 ^a ±5.48	56.2 ^a ±0.6	59.0 ^a ±0.1	114 ^b ±2	169 ^a ±15	465 ^a ±42	4.07±0.04	95.20±6.48	19.28 ^a ±1.52
Firebox	62.3 ^{b,c} ±0.5	8.47 ^c ±0.41	36.79 ^c ±1.30	28.9 ^c ±0.5	48.0 ^b ±3.4	115 ^b ±5	56 ^{b,c} ±6	285 ^b ±23	4.49±0.24	98.44±10.72	8.92 ^b ±0.63
Everest	60.5 ^d ±0.4	6.72 ^c ±0.85	27.74 ^d ±2.05	7.3 ^d ±0.5	13.1 ^d ±0.2	57 ^e ±1	68 ^b ±2	139 ^c ±2	4.22±0.20	103.80±7.36	4.80 ^c ±0.45
Gallagher	62.0 ^c ±0.6	7.05 ^c ±0.29	29.75 ^{c,d} ±0.67	4.8 ^d ±0.3	22.8 ^c ±0.5	97 ^c ±5	44 ^{b,c} ±5	185 ^c ±17	4.23±0.24	104.38±3.65	6.27 ^{b,c} ±0.49
KS Mako	58.4 ^e ±0.2	7.61 ^c ±0.37	30.87 ^{c,d} ±1.29	6.1 ^d ±0.4	12.8 ^d ±0.7	84 ^d ±4	35 ^c ±1	126 ^c ±14	4.26±0.18	98.49±6.15	6.87 ^{b,c} ±0.44
KS Providence	57.3 ^e ±0.1	5.97 ^c ±0.94	24.57 ^d ±2.50	6.6 ^d ±0.3	10.9 ^d ±0.2	44 ^f ±3	67 ^b ±13	110 ^c ±8	4.34±0.02	82.80±3.46	5.80 ^c ±0.89
p-value	0.0036	0.0052	0.0039	0.0035	0.0039	<0.0001	0.0044	0.0042	0.0741	0.0575	0.0048
Contrast OE vs Base	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3683	0.8655	<0.0001

Abbreviations: Mixograph (Mixo), Overexpressed Varieties (OE)

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05). Contrasts of overexpressed versus non-overexpressed varieties are also shown (p<0.05).

According to Bock et al. (2025), the overexpressed varieties had development times between 21 and 41 minutes and stabilities between 38 and 56 minutes on average. The development times and stabilities in this study surpass those seen in literature averaging from 28.9 to 51.7 minutes and 48.0 to 59.2 minutes respectively. Table 13 shows that Bx7^{OE} varieties are stronger than the non-overexpressed varieties with Paradox and Breadbox being the strongest of all for both development time and stability.

Only one study in the literature reports data for the alveograph with an average P of 48 mm H₂O, L of 197.5 mm, and W of 167.0 10⁻⁴ J for the overexpressed varieties. A significant increase was seen in the P and W parameters for the overexpressed varieties (L. Zhang et al., 2010). We see a significant increase in P and W as well for the overexpressed varieties, with values averaging 114 to 132 mm H₂O and 285 to 465 10⁻⁴ J respectively. The increase is significantly greater than the non-overexpressed varieties for both P and W. The value of L ranged from 56 to 169 mm. Breadbox and Paradox had an L significantly greater than the non-overexpressed, but Firebox had a weaker L more resemblant of the non-overexpressed varieties. Due to the strength of these varieties, the large L is not expected. The large L may not accurately reflect the effective extensibility of these varieties but instead indicate the inability of the software to distinguish when the bubble ruptures on the overexpressed lines. During the testing of the overexpressed lines, even after the bubble ruptured, enough elasticity remained, allowing the bubble to maintain shape and resist some air flow prolonging the test time instead of stopping the test at point of rupture. This may be a limitation in the alveograph in testing overexpressed lines leading to some lack of confidence in the data.

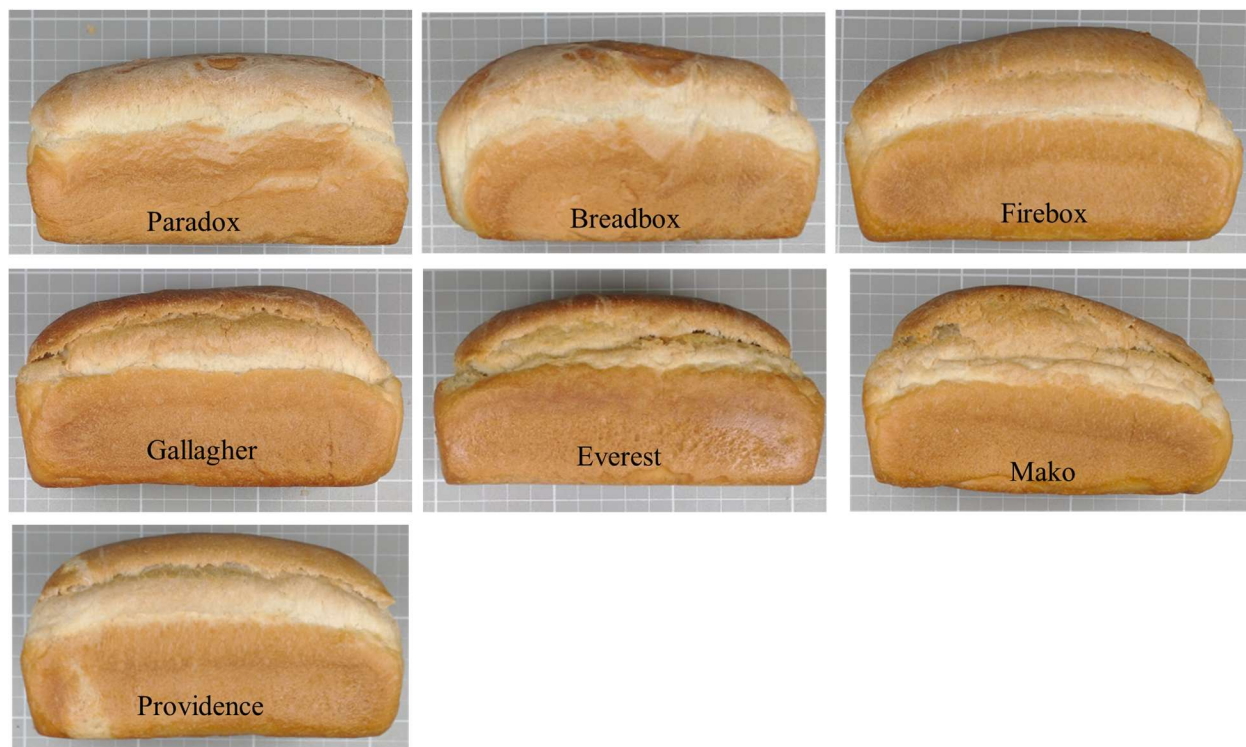


Figure 6: Images of bread produced from individual varieties as labeled.

There are no studies reporting specific volume, mixing power, or time to mixing power for overexpressed wheat varieties. Due to the differences in rheology, it would be expected that there would be differences in the mixing parameters. Interestingly, there were not. To form an elastic system, continuity of a solid polymer phase must be accomplished. In wheat doughs this pertains to high molecular weight glutenin proteins that interact via disulfide bonds, hydrogen bonding, hydrophobic interactions, and low charge repulsion (Delcour & Hoseney, 2010). During dough mixing, the disulfide bonds are constantly broken and reformed via thiol/disulfide exchange reactions to the point to maximum elasticity and dough development (Delcour & Hoseney, 2010). In the baking of these varieties, the AACC straight dough method was used. This method increases the amount of oxidation that occurs due to prolonged fermentation and allows for increased alignment of the gluten network during the punch and fold steps. This builds strength and tolerance into the dough after the mechanical mixing has been completed (Delcour &

Hoseney, 2010). This may be detrimental when the dough itself may be undermixed. When mixing and processing these doughs, the initial observation of the dough presented drastic differences. For the overexpressed doughs, the doughs were very tight and elastic, indicating that optimum mixing was not achieved. For the non-overexpressed doughs, particularly those of known lower quality such as Everest and KS Providence, the doughs tended to be stickier and more extensible. Gallagher and KS Mako tended to feel optimum from a mixing standpoint where they had the correct balance of elasticity and extensibility. During the punching and folding steps, the extent of the differences minimized. This may be one reason why the differences in specific volume were not seen. Increased elasticity leads to greater volume to a point, but if the dough is too elastic, the expansion of the gas cells is resisted leading to overall lower volume (Delcour & Hoseney, 2010). It may be necessary to use a different method for processing these varieties. Secondly, in the overexpressed varieties, long dough development times were seen in the Farinograph. For mixing, the Mixograph peak time was used to determine how long mixing would occur since the pin mixer was used, which is similar to the Mixograph mixing action. The dough development times were significantly longer than the Mixograph peak times for the overexpressed doughs but were more similar for the non-overexpressed doughs. This leads to the question of whether or not proper hydration of the gluten network is achieved in the time to mix according to the Mixograph. If not, this may explain some of the lack of functionality in the non-overexpressed varieties since it is vital for the gluten network to be fully hydrated for optimum performance. In fact, protein or starch that is not hydrated cannot interact in the dough in any beneficial way; dough development is the consequence of complete flour hydration (Delcour & Hoseney, 2010). From this, it can be concluded that mixing should be done to optimum conditions instead of a rheological instrument's parameters to reevaluate

functionality. This leads to the conclusion that the standard methods used to test these varieties were not made for the novelty of these varieties. Therefore, they may not be optimized for the uniqueness of the overexpression. The network could be further quantified by analyzing entanglements of the gluten network using confocal laser microscopy (Lucas et al., 2018; Vidal et al., 2023).

Blending as a Testing Strategy

Due to the strength of the overexpressed lines, the typical rheology equipment used may or may not be able to complete the test or if completion occurs, the test may be longer than desired for efficiency. A potential strategy to evade this issue is to blend the overexpressed varieties with non-overexpressed base values and test the rheology of the composite flour. In each case, differences were able to be determined among the blends of a specific series for all rheological parameters analyzed. The test was also able to be completed and blending effectively decreased the time of the test when possible. As shown in Figure 7, the Mixograph peak time decreased greatly from 100% to 50% especially for Breadbox and Paradox. There is some decrease for Firebox, but it is not as significant. This may be due to a lower gluten strength in Firebox compared to Paradox and Breadbox and performs more similarly to non-overexpressed wheat. In this case, blending may not be necessary for testing. The Mixograph peak time and mixscore are almost always different between 100% and 50%, but after that there is less differentiation in the segregation. Figure 8 shows the Farinograph parameters and again test time is decreased from the test time at 100%. This is especially true for the dough development times. For the KS Mako series with Firebox, the decrease does not come until 15%. In general, blending is also effective for decreasing the stability times especially at the lower blend percentages. It should be noted that the dough development time increases at a slower rate than the stability which may be beneficial

for processing tolerance in a high-speed baking system. In a production facility, maintaining dough development times is important since increasing mix time would lead to a decrease in overall production. However, due to the speed of production, the equipment is aggressive on the dough, requiring tolerance to maintain product quality throughout the process. An increase in stability would lead to an increase in tolerance while maintaining a similar mixing time. The higher percentages of the blends show more discrimination in the Farinograph parameters. The Alveograph is a set time test, so blending does not change test time, but the Alveograph has been known to not be able to test the overexpressed varieties at 100%, so for the Alveograph the important thing is to be able to complete the test and obtain the parameters. The parameters are shown in Figures 9 and 10. As previously noted, the L for the 100% overexpressed varieties was high and may not reflect reality since the software may have not read the value correctly. This can be seen in Figure 9 with the drastic increase in slope from 50% to 100% blend for Paradox and Breadbox especially. Comparing P and L, in general, as P increased, L decreased. At 100% however, P increased greatly as well as L indicating likely the software did not correctly conclude the test giving a higher value of L than reality. If the test did not correctly conclude, there should be some hesitation in relying on the data at 100%; blending does seem to be effective for the Alveograph to give realistic results. In order to understand if there are differences between overexpressed varieties only, ANOVA was ran setting the base flour and blend level with only the overexpressed flour as a factor (Table 14). The results indicate rheological parameters are base dependent. For KS Mako and KS Providence, 15% and 50% are able to discriminate the overexpressed flour performance in most of the total parameters. For Everest, 50% is best able to discriminate the Bx7^{OE} varieties with eight rheological parameters showing significant differentiation. The overexpressed variety on its own also discriminates all

eight rheological parameters, but the test time for the Mixograph and Farinograph is substantially longer than when blended.

It is known that Everest and KS Providence are weaker hard red winter varieties compared to KS Mako, which is known to consistently be a strong performing variety. Therefore, blending can be viewed as a method to raise the rheological quality of a weaker variety to the performance of a stronger variety; in this study KS Mako. The Mixograph peak time of KS Mako was 7.61 ± 0.37 minutes, which is only achieved or surpassed by Providence when blended with Paradox and Breadbox at 15%. Firebox is ineffective up to 50% to correct the peak time of Providence (Appendix A Table 24). The Mixograph mixscore of Mako was 30.87 ± 1.29 , which is equivalent to Providence blended with 15% of Paradox or 25% of Breadbox. Blending with Firebox again does not surpass Mako. To raise Everest to the Mixograph peak time and mixscore of KS Mako, it should be blended with 15% of Paradox, 25% of Breadbox, or 50% of Firebox. For the Farinograph, Providence and Everest both surpass the dough development times of KS Mako on their own, likely influenced by the low protein content of KS Mako in the sample used in this study. The stability of KS Mako is also low, requiring 5% of any of the overexpressed lines to surpass the stability of KS Mako. This is likely due to the differences in environmental conditions of KS Mako more than inherent variety differences. Nonetheless, it can be seen that blending can be an effective method to improve rheological parameters to a stronger line, dependent on the overexpressed line.

Blending as a Strategy to Improve Bread Quality

Just as in the individual varieties, when blends were baked, there was no significant difference in specific volume ($p < 0.05$) for any of the blend combinations. Time to power was

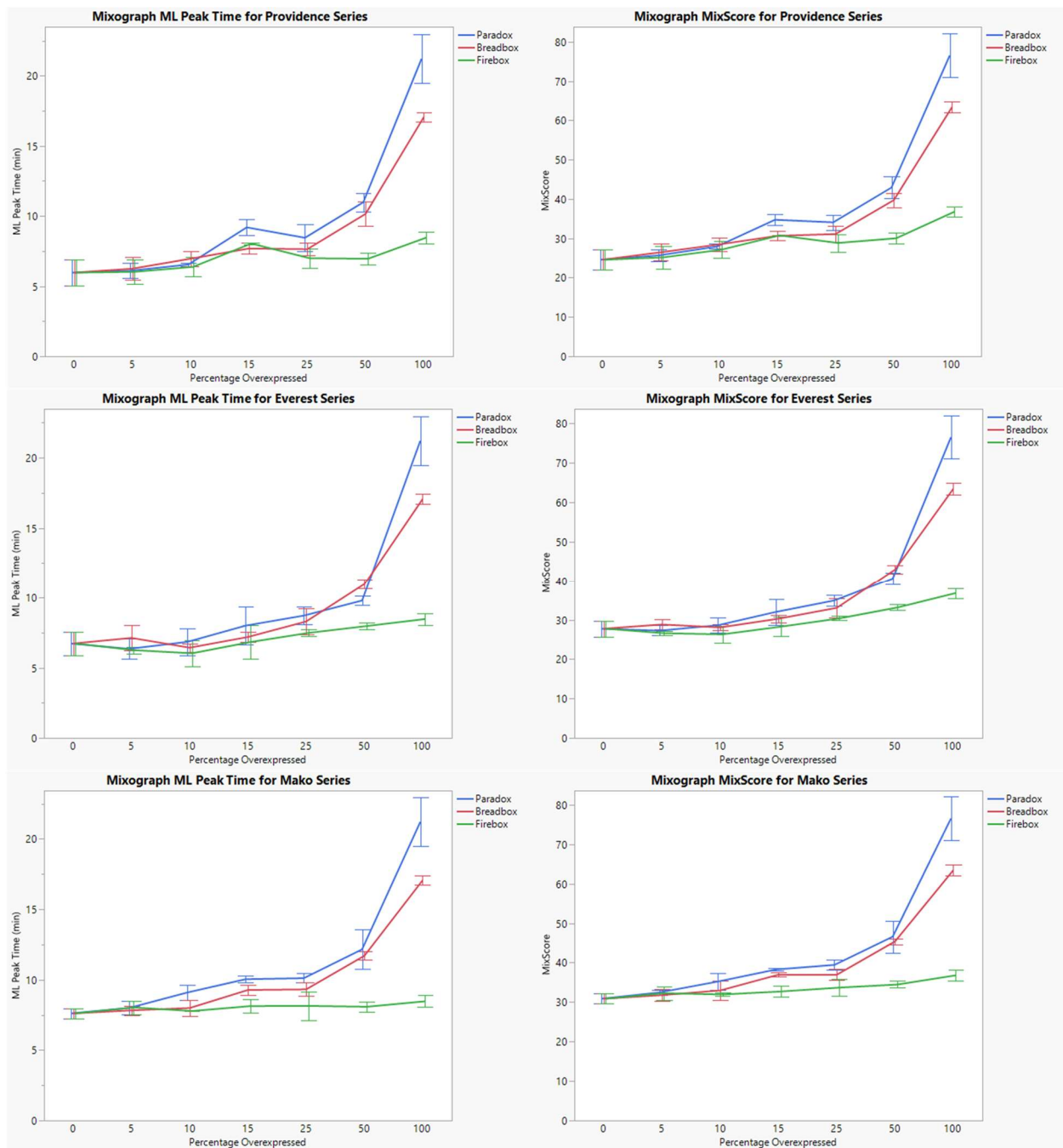


Figure 7: Mixograph parameters for each blend segregated by base flour.

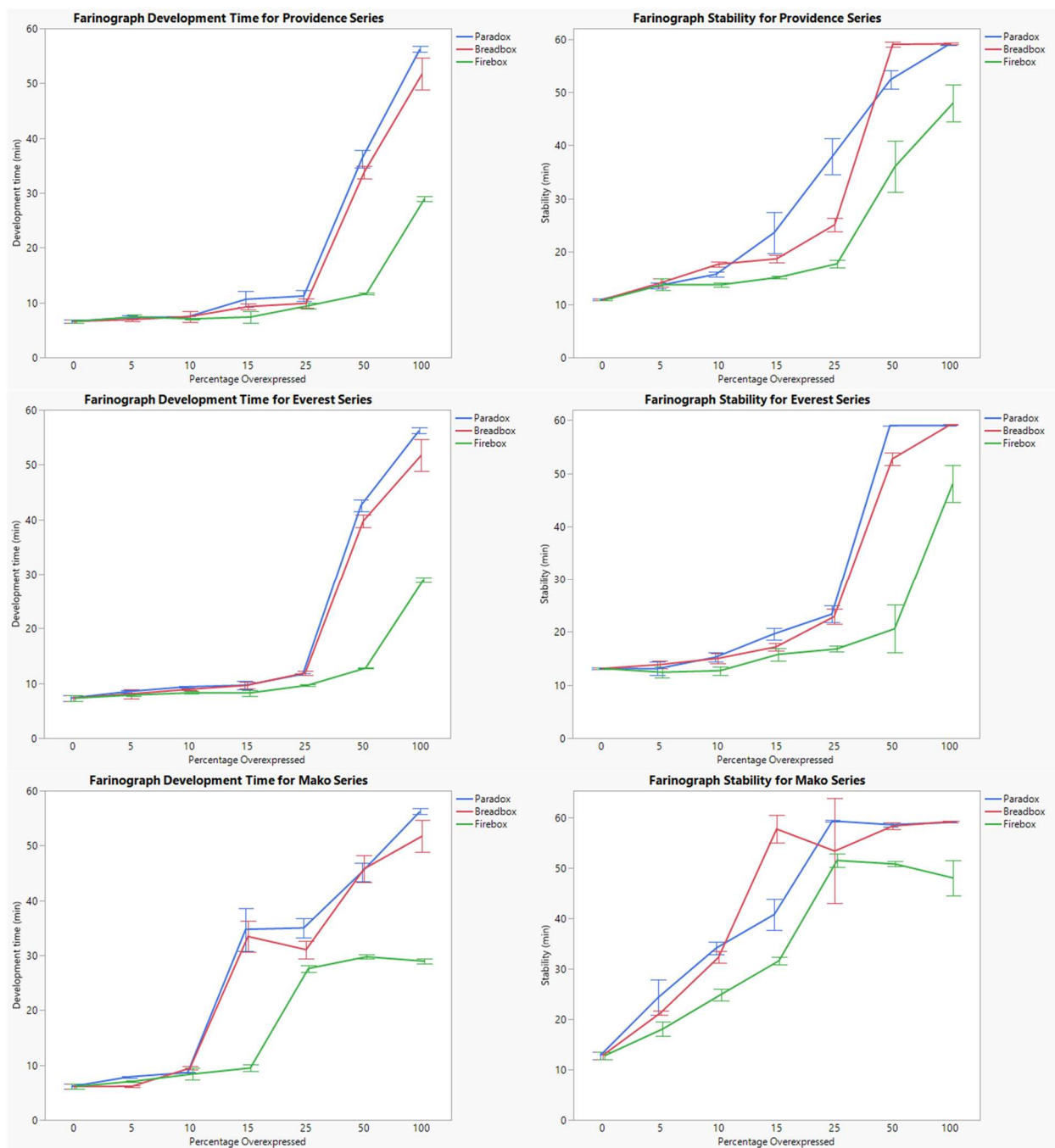


Figure 8: Farinograph parameters for each blend segregated by base flour.

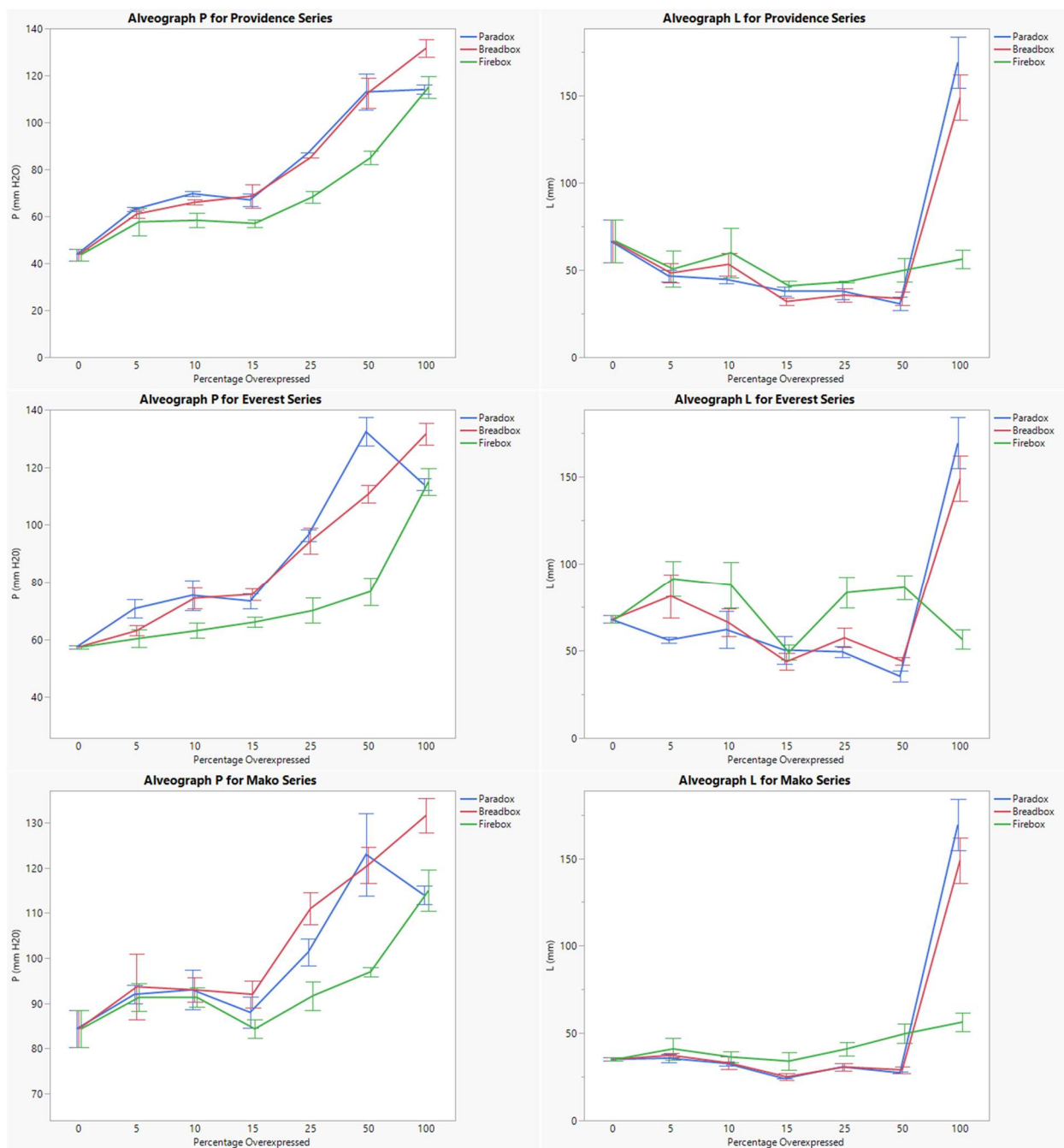


Figure 9: Alveograph P and L parameters for blends segregated by base flours.

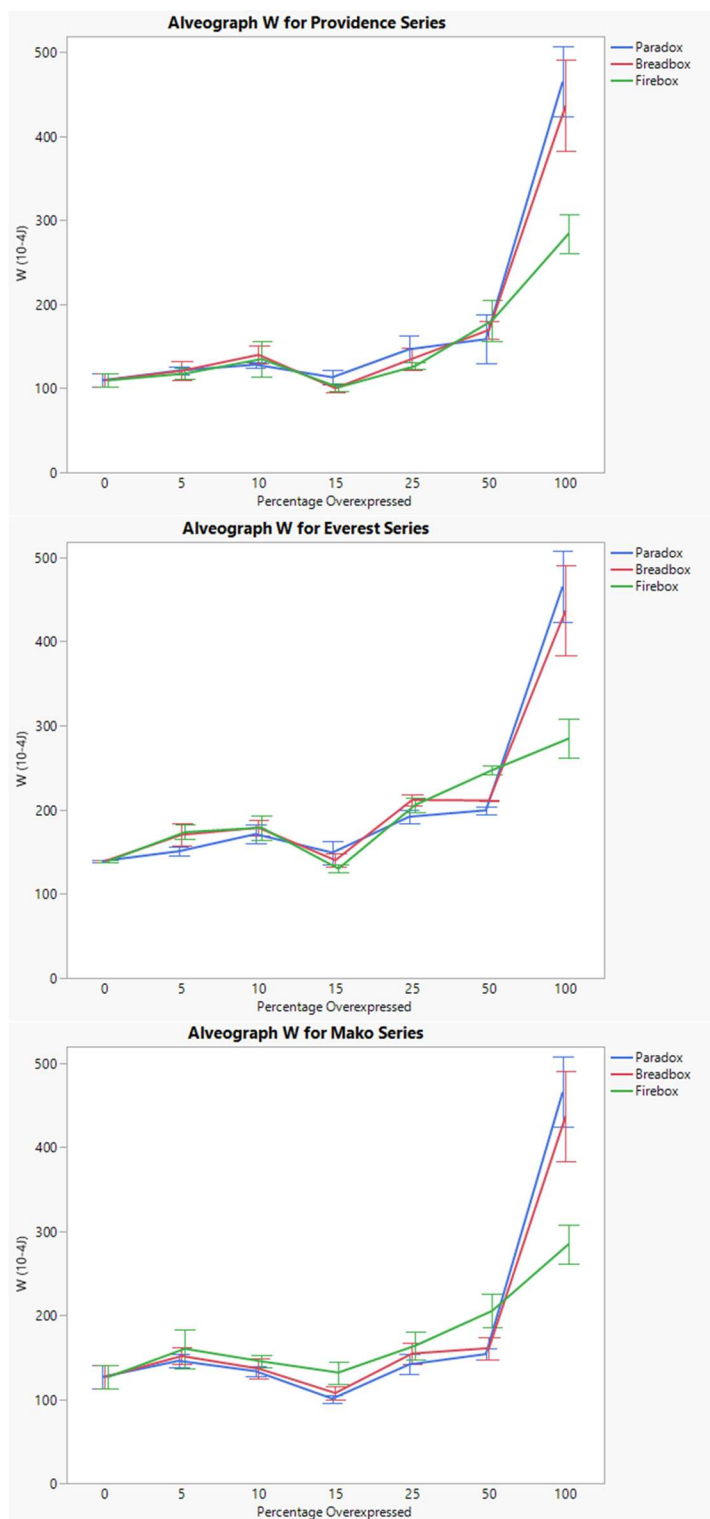


Figure 10: Alveograph W parameter for blends segregated by base flour.

Table 14: Comparison of refined flour rheological parameters at fixed percent for each base to determine significance of overexpressed variety (p<0.05).

	Percentage	Mixograph Absorption (%)	Mixograph Peak Time (min)	Mixograph MixScore	P (mm H ₂ O)	L (mm)	W (10 ⁻⁴ J)	Development time (min)	Stability (min)	Specific Volume (cm ³ /g)	Mixing Power (W)	Time to Power (min)
KS Mako	5	0.4750	0.8303	0.8259	0.8255	0.3041	0.5426	<0.0001	0.0372	0.7379	0.3056	0.5124
	10	0.5787	0.0243	0.1787	0.7688	0.2841	0.3140	0.1880	0.0002	0.3076	0.6703	0.8466
	15	0.0482	0.0024	0.0007	0.0485	0.0538	0.0142	0.0628	<0.0001	0.9175	0.5411	0.1133
	25	0.3041	0.0313	0.0141	0.0011	0.0066	0.2322	0.0021	0.2463	0.3444	0.3134	0.0409
	50	0.4485	0.0020	0.0016	0.0028	0.0459	0.0095	0.0608	<0.0001	0.4349	0.4610	0.0003
Everest	5	0.2713	0.2941	0.1136	0.0090	0.0094	0.0557	0.3802	0.2915	0.9065	0.0145	0.0336
	10	0.0417	0.4952	0.3143	0.0147	0.0545	0.6731	0.0014	0.0179	0.2136	0.0080	0.6422
	15	0.0685	0.4383	0.2512	0.0035	0.4094	0.1319	0.0608	0.0107	0.7279	0.1451	0.5866
	25	0.0854	0.1456	0.0410	0.0628	0.0015	0.0468	0.0536	0.0012	0.8439	0.3772	0.0584
	50	0.0129	<0.0001	0.0001	<0.0001	0.0273	<0.0001	0.0265	0.0241	0.4480	0.8258	0.0029
KS Providence	5	0.2636	0.9352	0.7835	0.2738	0.7898	0.8354	0.2822	0.7552	0.6589	0.3956	0.4740
	10	0.4912	0.4102	0.6120	0.0011	0.1969	0.6034	0.6546	0.0002	0.1143	0.0105	0.5464
	15	<0.0001	0.0084	0.0046	0.0119	0.0140	0.0965	0.0305	0.0110	0.9132	0.4179	0.6628
	25	0.0485	0.1268	0.0525	0.0211	0.0973	0.2035	0.0843	<0.0001	0.5945	0.0264	0.0708
	50	0.0216	0.0008	0.0007	0.0020	0.0063	0.5298	0.0390	0.0002	0.5866	0.6221	0.0068
	OE on own	0.0084	<0.0001	<0.0001	0.0016	0.0390	0.0037	0.0273	0.0439	0.0257	0.8244	<0.0001

Abbreviations: Overexpressed Varieties (OE)

different among the blends as in the case for the individual varieties. Mixing power was only significant for the Paradox-KS Providence, Firebox-KS Providence, and Firebox-Everest series'. The Mixograph absorption which was used for the bake absorption and the specific volumes of the overexpressed blends segregated by base are shown in Figure 11. As seen, the specific volume has no definite trend and there are not differences between the specific volumes of the loaves. The mixing power and time to power are shown in Figure 12. The time to power is different among the blends, but the mixing power is not always significant, which can be shown by the large variation in the curves.

Based on the results shown, blending with overexpressed varieties does not seem to be a potential strategy for improving volume. The volumes presented are not different even though there is significant difference throughout the rheological testing. That begs the question, is the increase in HMW glutenin subunits from the overexpression of the Bx7 subunit not correlated to increase in bread quality? To fully understand this, the quantification of the HMW glutenin subunit must be completed, which is currently in progress. This would allow an understanding of how much actual subunit is being produced and how much is changing in each specific blend increment. Is the amount of Bx7 subunit overexpressed different in each blend increment? Potentially the increment increase is minor with a 5% addition of overexpressed wheat. It is known that the percentage of unextractable polymeric protein is one of the most important factors in dough mixing and quality (Rasheed et al., 2014). Another important consideration is hydration. Even though the dough is mixed to the Mixograph peak time, it is possible that the gluten is not properly developed which may explain some lack of functionality. It could be interesting to see these varieties and blends using rapid hydration systems. Another important consideration is that HMW glutenin subunits are not the sole determinant of bread quality.

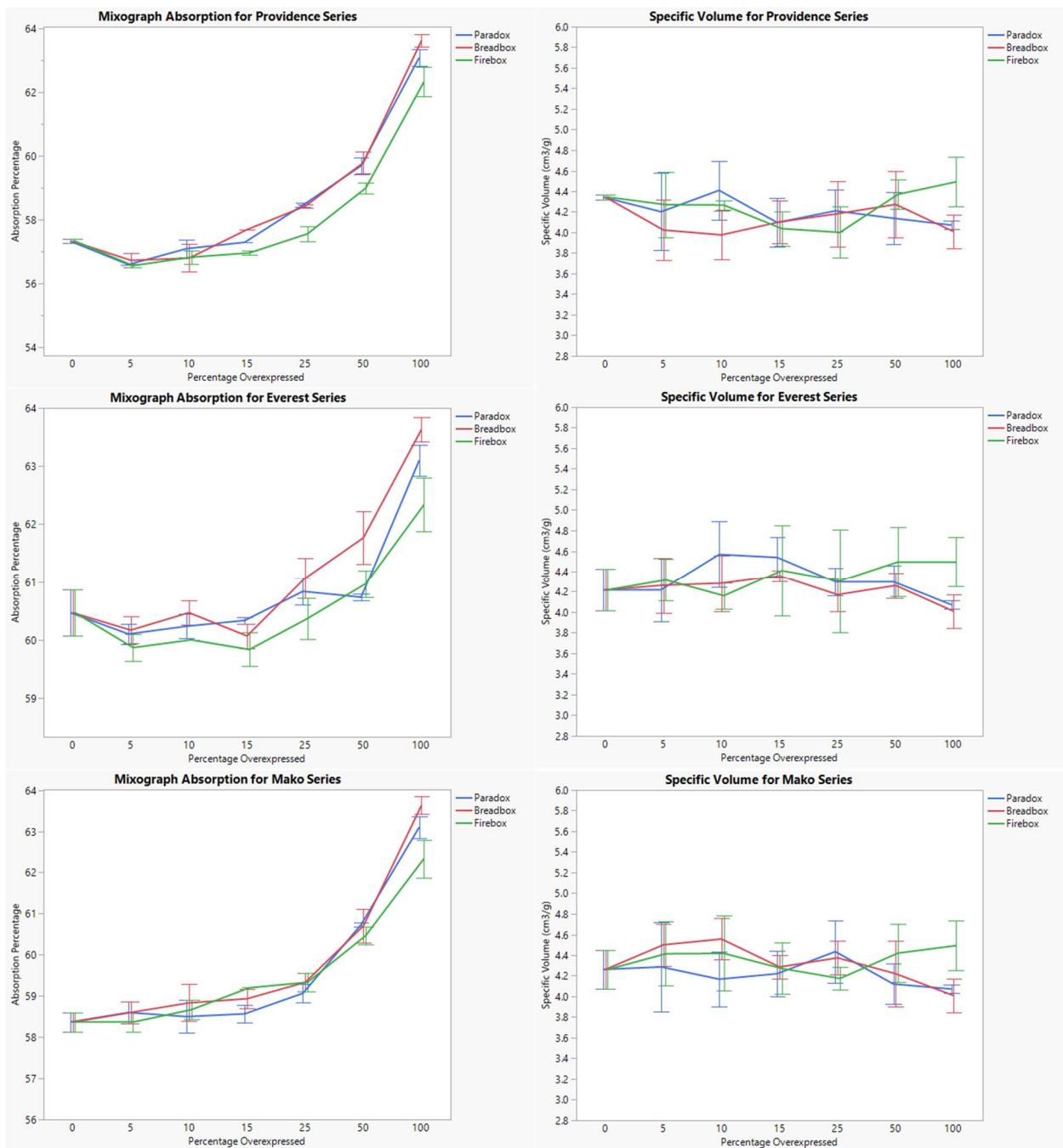


Figure 11: Absorption used for baking and baking specific volume for blends segregated by base flour.

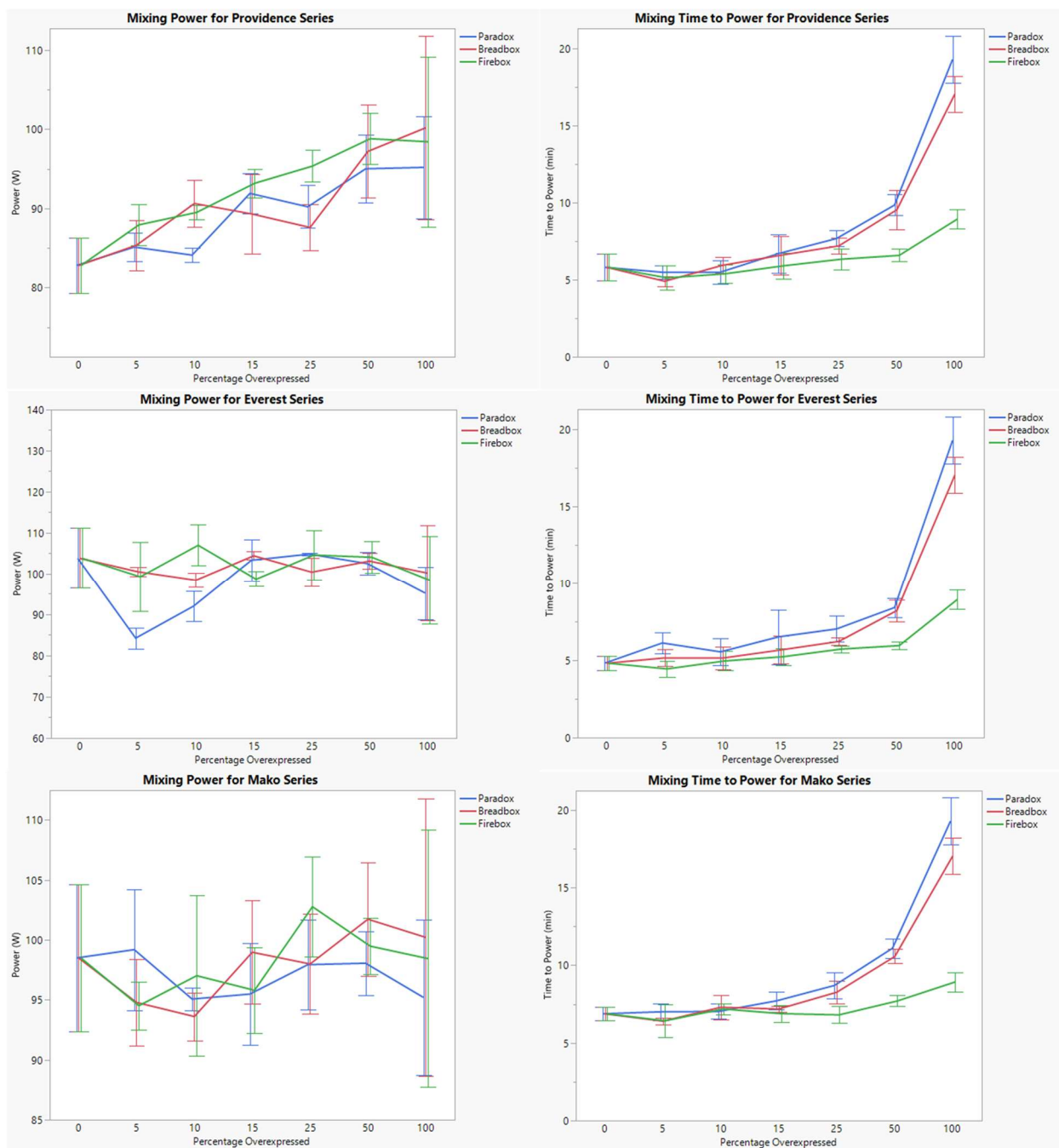


Figure 12: Mixing power and time to max power for blends segregated by base flour. Note the use of different scales.

Part of volume is determined by starch characteristics as well as by the arabinoxylan viscosity in the aqueous phase (Baudouin et al., 2020). Neither the overexpressed varieties nor the base flours are isogenic and do not have the exact same genetic background. Therefore, there may be differences in starch characteristics and arabinoxylan content that may add to some of the confusion. This was not tested in this experiment and is therefore not controlled. It should be emphasized that the protein contents of these particular samples were lower than the wheat quality council target of 12% (Chen et al., 2024) for hard red winter wheat due to the specific environmental conditions of the growing year which may explain some of the lack of variance in specific volume. More study understanding these things may need to be conducted to fully understand the picture.

Modeling of Specific Volume

From the regression workflow, a six-predictor model with a statistically significant overall fit and modest explanatory power was determined ($R^2=0.139$; adj. $R^2=0.111$), which shows that the flour, rheological, and mixing response variables explain only a portion of the total variability in the specific volume result. Backward-elimination rendered the best model, with the final predictors being protein, GlutoPeak peak maximum time, Mixograph peak height, Mixograph mixscore, mixing power, and dough properties (“very tight” descriptor). The model fit parameters are given in Table 15 and residuals analysis is shown in Figure 13. The overall F-test probability ($p = 9.2 \times 10^{-5}$) shows the the model does explain significant variance. Table 16 shows the estimated coefficients from the selected model including the 95% confidence interval. From this, it is seen that higher protein and GlutoPeak PMT were positively associated with higher specific volume, conditional on other variables. This is consistent with the idea that higher protein contents and favorable development characteristics support a stronger gas-holding

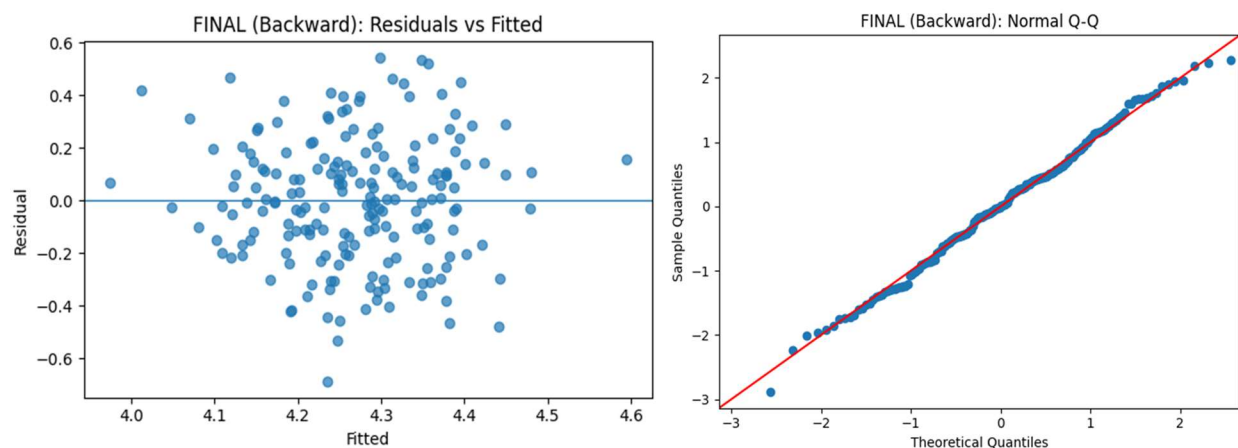
network allowing for a better final loaf volume. In contrast, higher Mixograph mixscore, Mixograph peak height, and mixing power were associated with lower specific volume. A potential explanation for this is that under the process conditions represented here, the higher Mixograph “strength” or resistance values may reflect a tighter, more under-developed, or less extensible dough. This may limit the expansion of the loaf during proofing and baking which may ultimately reduce specific volume. The negative coefficient for mixing power aligns with this explanation: higher mechanical load during mixing can correlate with dough stiffness or lower effective extensibility thereby hindering the expansion of the loaf even if the overall dough characteristics are “strong.” The categorical dough descriptor “very tight” was also negatively associated with specific volume, reinforcing that overly tight dough behavior is detrimental to loaf expansion. It should be noted that the categorical descriptor “very tight” along with the mixing power and time to mixing power were the only parameters analyzed off of a complete bread dough with all ingredients. The other parameters were using flour and water at various proportions with the exception of the Alveograph, which used a sodium chloride solution. This may indicate that the affects of other ingredients on the flour are an important part in the understanding of bread dough and loaf quality.

Table 15: OLS regression results and fit summary.

Model	OLS
Method	Least Squares
R ²	0.139
Adjusted R ²	0.1111
RMSE (Residual standard error)	0.243 cm ³ /g
F-statistic	4.989
Overall F-test probability	p = 9.2×10^{-5}
Log-Likelihood	2.6993
AIC	8.601
BIC	31.4
No. Observations	192
Df Residuals	185
Df Model	6

Table 16: Estimated coefficients (β) from selected model.

	Coefficient	Std. Err.	t	P> t	95% Confidence Interval	
					0.025	0.975
Constant	2.5604	0.479	5.343	0.000	1.615	3.506
Protein %	0.4756	0.119	3.981	0.000	0.24	0.711
GlutoPeak PMT (min)	0.0035	0.001	4.369	0.000	0.002	0.005
Mixograph ML Peak Height	-0.0444	0.021	-2.158	0.032	-0.085	0.004
Mixograph MixScore	-0.0285	0.006	-4.693	0.000	-0.015	-0.003
Power (W)	-0.0093	0.003	-3.026	0.003	-0.015	-0.003
Dough Properties: Very Tight	-0.2741	0.133	-2.054	0.041	-0.537	-0.011

**Figure 13: Final model residuals vs fitted model and final model normal Q-Q graphed.**

Conclusion

In this experiment, three Bx7^{OE} varieties were characterized. The overexpression increased the rheological strength, as assessed using common dough testing equipment. However, increased strength did not lead to differences in specific volume, which is ultimately the pinnacle of bread quality. Blending of Bx7^{OE} varieties with the non-overexpressed varieties was verified to be a strategy to test these varieties in a quick and effective manner. Blends of base flours with 15% or 50% overexpressed flour provided the most discrimination amongst the overexpressed varieties. At both levels, test time was decreased compared to testing at 100% overexpressed variety. Blending was also used and analyzed as a potential to improve end product quality. This did not lead to expected results, as there were no significant differences in the specific volumes of the loaves in any case. Modeling of rheological parameters for loaf specific volume resulted in only

very modest explanatory power. It was found that protein was still the best indicator of dough quality. The manual scoring of dough was also a better predicating parameter than any of the other rheological parameters. This finding reinforces that the performance of the overexpressed varieties may not be able to be correctly analyzed using the current methods, nor do they adequately explain specific volume of the intended end product.

This study provides valuable insights as it was the first systematic test in a controlled baking experiment of the overexpressed lines. There had been some preliminary data, but no prior studies were conducted to this extent. Importantly, it was determined that blending can be used as a method to increase dough stability without increasing dough development time or affecting the product volume.

Chapter 4 - Overall Conclusions

A review of the current literature was conducted to summarize the existing knowledge and historical context of the overexpressed varieties. The *Glu-B1a1* allele has been traced back to the mid19th century where it was first identified in Uruguayan landrace, then reported in the wheat variety Red River 68 in a paper published in 1997. Previous studies have demonstrated the overexpression of the Bx7 protein subunit can have significant impacts on dough rheology; these studies were confirmed in the present study.

Regarding the rheological quality testing methodology, blending proved to be an effective strategy. In most cases, blends containing 15% or 50% overexpressed wheat provided sufficient discrimination while significantly reducing test time. In particular, at 15% overexpressed wheat in a blend, results aligned well with typical test times of conventional methods. These findings indicate blending may be an efficient and effective approach for breeders and quality laboratories to evaluate overexpressed lines for rheological quality.

The GlutoPeak test was shown to be effective in discriminating the overexpressed lines, particularly at 100% overexpressed wheat. Both refined flour and whole meal samples could be adequately discriminated at full inclusion. Refined flour performance was also discriminated at higher blend levels, whereas the test time of whole meal was too short to determine quality differences in blends. Overall, the GlutoPeak may be effective at discriminating strength between overexpressed lines quickly.

It is important to note that the rheological tests examined in this study evaluated flour-water systems, without fully replicating breadmaking conditions, where additional ingredients are added which may influence dough behavior. This suggests that updated methods incorporating complete formulations may be necessary to fully understand the baking potential of

overexpressed wheat. In addition, these protocols were also developed using non-overexpressed wheat and may be inadequate to fully predict the baking quality under the current testing conditions.

A notable gap in the literature was the lack of systematic evaluation of baking quality associated with the overexpressed lines. This was addressed in this work. While significant strength was found in rheology testing, these differences did not translate into the expected measurable improvements in baking performance. Specifically, at lower blend levels in the refined flour, the influence from the overexpression diminished. This may indicate that at lower blend percentages, the actual amount of HMW-GS present may not sufficiently differ from non-overexpressed samples to affect performance. Suggestions for future work include analyzing the amount of Bx7 subunit in each blend to understand whether or not differences occur at low levels and what amount overexpressed subunit is necessary to achieve rheological differences. There may be application of the overexpressed lines regarding stability for processing since it was seen that the dough development time increased at a slower rate than the stability increased. This may benefit high speed bakeries where equipment processing requires dough stability to maintain end product quality where increased dough development time may not be desirable.

One substantial finding in this study was the lack of differences in loaf quality despite the confirmed differences in rheology. Specific volume did not vary significantly between samples even with known differences in subunit content from literature (42% HMW-GS versus 30%). Although the compositional differences appear significant, this was not reflected in the baking outcomes. Several factors may explain this result. First, the Mixograph parameters were used to determine the mixing method of the dough instead of using a trained baker to determine optimum mix. This was done to eliminate any subjectivity and allow for repeatability. The

standard AACC straight dough method was used, which contains several fermentation steps allowing for increased oxidation as well as more forced alignment of proteins fibrils during the sheeting and folding steps. This would reduce the impact of mechanical mixing alone. Regardless, the absence of significant variation in loaf quality remains an intriguing outcome. From a practical perspective, the relevance and application of the Bx7^{OE} lines remain limited to correction of dough rheology. The findings presented in this study suggest that HMW-GS alone may not be sufficient as a target for further wheat quality improvement. Rather than focusing solely on the glutenin content, breeders may need to consider additional factors including protein starch-interactions, flour lipids, or the role of non-starch polysaccharides such as arabinoxylans. In addition, the current rheological and baking evaluation methods may not adequately be designed to capture the functional differences in overexpressed glutenin systems. Further research could explore whether overexpressed lines perform better under alternative processing conditions including extended fermentation, or high hydration systems, where strong, but slow-absorbing flours may be advantageous. Additionally, the type of processing of the overexpressed flours could be explored including air-classification to segregate more functional fractions.

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Appendix A - Supplementary Tables

Chapter 2 Supplementary Tables

Table 17: NIR data for blends in the KS Providence series.

	Percentage	Whole Meal Flour			Refined Flour		
		Moisture (%)	Protein (%) (14 MB)	Ash (%) (14MB)	Moisture (%)	Protein (%) (14MB)	Ash (%) (14MB)
Paradox- KS Providence	0	8.78 ^b ±0.43	10.36 ±0.09	1.641 ^a ±0.046	14.79 ^d ±0.04	8.64 ^c ±0.06	0.349 ^b ±0.003
	5	9.33 ^{a,b} ±0.06	10.35 ±0.05	1.639 ^a ±0.013	15.41 ^a ±0.09	8.44 ^e ±0.03	0.376 ^a ±0.005
	10	9.37 ^{a,b} ±0.16	10.45 ±0.01	1.662 ^a ±0.026	15.31 ^{a,b} ±0.02	8.47 ^{d,e} ±0.02	0.383 ^a ±0.008
	15	ND	ND	ND	15.43 ^a ±0.04	8.52 ^{d,e} ±0.03	0.376 ^a ±0.001
	25	9.42 ^a ±0.12	10.43 ±0.09	1.607 ^{a,b} ±0.01	15.19 ^{b,c} ±0.03	8.57 ^{c,d} ±0.05	0.382 ^a ±0.007
	50	9.53 ^a ±0.08	10.49 ±0.01	1.551 ^b ±0.018	15.12 ^c ±0.02	8.75 ^b ±0.00	0.382 ^a ±0.002
	100	9.6 ^a ±0.07	10.46 ±0.07	1.392 ^c ±0.025	14.74 ^e ±0.02	9.29 ^a ±0.02	0.347 ^b ±0.003
	p-value	0.0300	0.1016	0.0122	0.0039	0.0043	0.0250
Firebox- KS Providence	0	8.78 ^b ±0.43	10.36 ^d ±0.09	1.641±0.046	14.79 ^c ±0.04	8.64 ^d ±0.06	0.349 ^b ±0.003
	5	9.26 ^{a,b} ±0.06	10.45 ^d ±0.05	1.65±0.025	15.43 ^a ±0.03	8.44 ^e ±0.04	0.380 ^a ±0.003
	10	9.27 ^{a,b} ±0.04	10.52 ^d ±0.07	1.679±0.018	15.42 ^a ±0.02	8.54 ^{d,e} ±0.08	0.379 ^a ±0.002
	15	ND	ND	ND	15.38 ^a ±0.05	8.66 ^d ±0.02	0.373 ^a ±0.004
	25	9.4 ^a ±0.06	10.76 ^c ±0.04	1.667±0.015	15.36 ^a ±0.03	8.79 ^c ±0.03	0.378 ^a ±0.004
	50	9.61 ^a ±0.22	11.05 ^b ±0.02	1.63±0.036	15.23 ^b ±0.03	9.18 ^b ±0.01	0.379 ^a ±0.002
	100	9.7 ^a ±0.06	11.77 ^a ±0.08	1.51±0.018	14.86 ^c ±0.01	10.22 ^a ±0.04	0.344 ^b ±0.003
	p-value	0.0077	0.0064	0.0671	0.0056	0.0044	0.0151
Breadbox- KS Providence	0	8.78 ^b ±0.43	10.36 ^c ±0.09	1.641 ^a ±0.046	14.79 ^d ±0.04	8.64 ^{c,d} ±0.06	0.349 ^b ±0.003
	5	9.46 ^{a,b} ±0.16	10.35 ^c ±0.08	1.65 ^a ±0.018	15.52 ^a ±0.01	8.44 ^e ±0.03	0.382 ^a ±0.011
	10	9.34 ^{a,b} ±0.05	10.46 ^{b,c} ±0.1	1.653 ^a ±0.016	15.42 ^a ±0.08	8.50 ^{d,e} ±0.05	0.384 ^a ±0.006
	15	ND	ND	ND	15.28 ^b ±0.01	8.57 ^{c,d,e} ±0.01	0.374 ^a ±0.002
	25	9.34 ^a ±0.1	10.58 ^{a,b} ±0.05	1.627 ^{a,b} ±0.029	15.30 ^b ±0.02	8.68 ^c ±0.08	0.384 ^a ±0.004
	50	9.41 ^a ±0.06	10.65 ^{a,b} ±0.06	1.558 ^b ±0.032	15.11 ^c ±0.02	8.89 ^b ±0.02	0.38 ^a ±0.005
	100	9.67 ^a ±0.07	10.77 ^a ±0.02	1.393 ^c ±0.014	14.83 ^d ±0.02	9.58 ^a ±0.04	0.343 ^b ±0.006
	p-value	0.0205	<0.0001	0.0136	0.0039	0.0041	0.0184

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Table 18: NIR data for blends in the KS Mako series.

	Whole Meal Flour				Refined Flour		
	Percentage	Moisture (%)	Protein (%) (14 MB)	Ash (%) (14MB)	Moisture (%)	Protein (%) (14MB)	Ash (%) (14MB)
Paradox- KS Mako	0	7.78 ^b ±0.04	10.27 ^c ±0.02	1.512 ^a ±0.017	14.77 ^d ±0.01	8.51 ^{c,d} ±0.06	0.338 ^e ±0.005
	5	7.57 ^{a,b} ±0.06	10.34 ^{b,c} ±0.03	1.511 ^a ±0.023	15.04 ^{a,b} ±0.05	8.43 ^{c,d} ±0.03	0.377 ^{a,b,c} ±0.004
	10	7.72 ^{a,b} ±0.07	10.39 ^{a,b} ±0.03	1.508 ^a ±0.03	14.97 ^{b,c} ±0.03	8.40 ^d ±0.04	0.373 ^{b,c} ±0.001
	15	ND	ND	ND	15.08 ^a ±0.03	8.48 ^{c,d} ±0.04	0.369 ^c ±0.002
	25	7.90 ^a ±0.03	10.44 ^a ±0.00	1.513 ^{a,b} ±0.011	14.96 ^c ±0.02	8.53 ^c ±0.01	0.380 ^{a,b} ±0.002
	50	8.10 ^a ±0.06	10.48 ^a ±0.02	1.481 ^b ±0.02	14.93 ^c ±0.02	8.76 ^b ±0.04	0.383 ^a ±0.003
	100	9.60 ^a ±0.07	10.46 ^a ±0.07	1.392 ^c ±0.025	14.74 ^d ±0.02	9.29 ^a ±0.02	0.347 ^d ±0.003
	p-value	0.0059	<0.0001	0.0101	0.0049	0.0069	0.0041
Firebox- KS Mako	0	7.78 ^c ±0.04	10.27 ^e ±0.02	1.512 ^b ±0.017	14.77 ^f ±0.01	8.51 ^{e,f} ±0.06	0.338 ^b ±0.005
	5	7.45 ^d ±0.1	10.40 ^{d,e} ±0.04	1.502 ^b ±0.019	15.07 ^b ±0	8.43 ^f ±0.02	0.377 ^a ±0.003
	10	7.50 ^d ±0.04	10.50 ^d ±0.08	1.525 ^{a,b} ±0.011	15.05 ^{b,c} ±0.01	8.55 ^{d,e} ±0.03	0.377 ^a ±0.005
	15	ND	ND	ND	15.16 ^a ±0.03	8.63 ^d ±0.04	0.363 ^a ±0.01
	25	7.81 ^c ±0.04	10.68 ^c ±0.04	1.557 ^a ±0.014	15.01 ^{c,d} ±0.02	8.80 ^c ±0.03	0.375 ^a ±0.006
	50	8.37 ^b ±0.07	11.08 ^b ±0.03	1.562 ^a ±0.014	14.96 ^d ±0.03	9.24 ^b ±0.02	0.375 ^a ±0.003
	100	9.70 ^a ±0.06	11.77 ^a ±0.08	1.510 ^b ±0.018	14.86 ^e ±0.01	10.22 ^a ±0.04	0.344 ^b ±0.003
	p-value	0.0073	0.0056	0.0015	<0.0001	0.0035	0.0137
Breadbox- KS Mako	0	7.78 ^b ±0.04	10.27 ^e ±0.02	1.512 ±0.017	14.77 ^d ±0.01	8.51 ^{d,e} ±0.06	0.338 ^c ±0.005
	5	7.65 ^b ±0.02	10.44 ^d ±0.01	1.514 ±0.008	15.19 ^a ±0.02	8.41 ^e ±0.01	0.375 ^{a,b} ±0.004
	10	7.65 ^{a,b} ±0.01	10.43 ^d ±0.02	1.507 ±0.023	15.10 ^{a,b} ±0.04	8.45 ^e ±0.03	0.377 ^{a,b} ±0.002
	15	ND	ND	ND	15.07 ^b ±0.05	8.57 ^d ±0.04	0.370 ^b ±0.006
	25	7.75 ^a ±0.04	10.57 ^c ±0.01	1.518 ±0.019	15.03 ^b ±0.04	8.67 ^c ±0.03	0.384 ^a ±0.004
	50	7.95 ^a ±0.02	10.68 ^b ±0.03	1.475 ±0.005	14.86 ^c ±0.01	8.91 ^b ±0.01	0.38 ^{a,b} ±0.001
	100	9.67 ^a ±0.07	10.77 ^a ±0.02	1.393 ±0.014	14.83 ^{c,d} ±0.02	9.58 ^a ±0.04	0.343 ^c ±0.006
	p-value	0.0070	<0.0001	0.0356	0.0044	0.0044	0.0052

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Table 19: NIR data for blends in the Everest series.

	Whole Meal Flour				Refined Flour		
	Percentage	Moisture (%)	Protein (%) (14 MB)	Ash (%) (14MB)	Moisture (%)	Protein (%) (14MB)	Ash (%) (14MB)
Paradox- Everest	0	10.18 ^b ±0.04	11.07 ^a ±0.09	1.735 ^a ±0.01	14.33 ^e ±0.03	9.42 ^a ±0.05	0.358 ^c ±0.001
	5	10.60 ^a ±0.07	11.03 ^a ±0.02	1.722 ^a ±0.028	14.59 ^c ±0.01	9.32 ^{b,c} ±0.03	0.394 ^{a,b} ±0.004
	10	10.5 ^a ±0.05	10.94 ^{a,b} ±0.05	1.701 ^a ±0.011	14.6 ^{b,c} ±0.03	9.36 ^{a,b} ±0.02	0.397 ^a ±0.004
	15	ND	ND	ND	14.51 ^d ±0.02	9.36 ^{a,b} ±0.03	0.383 ^b ±0.008
	25	10.18 ^b ±0.07	10.92 ^{a,b} ±0.05	1.668 ^{a,b} ±0.046	14.66 ^b ±0.03	9.26 ^{c,d} ±0.02	0.390 ^{a,b} ±0.002
	50	9.51 ^c ±0.03	10.85 ^b ±0.05	1.597 ^b ±0.032	14.75 ^a ±0.03	9.2 ^d ±0.06	0.389 ^{a,b} ±0.003
	100	9.60 ^c ±0.07	10.46 ^c ±0.07	1.392 ^c ±0.025	14.74 ^a ±0.02	9.29 ^{b,c,d} ±0.02	0.347 ^c ±0.003
	p-value	0.0075	0.0098	0.0123	<0.0001	<0.0001	0.0092
Firebox- Everest	0	10.18 ^a ±0.04	11.07 ^d ±0.09	1.735 ^{a,b} ±0.01	14.33 ^e ±0.03	9.42 ^d ±0.05	0.358 ^c ±0.001
	5	10.15 ^a ±0.03	11.09 ^{c,d} ±0.02	1.744 ^a ±0.02	14.5 ^d ±0.00	9.41 ^d ±0.01	0.396 ^a ±0.009
	10	10.08 ^{a,b} ±0.02	11.16 ^{c,d} ±0.02	1.749 ^a ±0.013	14.58 ^c ±0.01	9.45 ^{c,d} ±0.03	0.399 ^a ±0.004
	15	ND	ND	ND	14.66 ^b ±0.03	9.45 ^{c,d} ±0.02	0.383 ^b ±0.002
	25	10.02 ^b ±0.03	11.24 ^c ±0.05	1.697 ^{b,c} ±0.005	14.58 ^c ±0.02	9.56 ^c ±0.02	0.393 ^{a,b} ±0.005
	50	9.72 ^c ±0.04	11.43 ^b ±0.03	1.669 ^c ±0.016	14.66 ^b ±0.04	9.78 ^b ±0.06	0.386 ^{a,b} ±0.002
	100	9.70 ^c ±0.06	11.77 ^a ±0.08	1.510 ^d ±0.018	14.86 ^a ±0.01	10.22 ^a ±0.04	0.344 ^d ±0.003
	p-value	0.0075	0.0086	0.0096	<0.0001	0.0064	0.0056
Breadbox- Everest	0	10.18 ^b ±0.04	11.07±0.09	1.735 ^a ±0.01	14.33 ^e ±0.03	9.42 ^{b,c} ±0.05	0.358 ^c ±0.001
	5	10.58 ^a ±0.05	11.04±0.04	1.703 ^{a,b} ±0.014	14.46 ^d ±0.01	9.40 ^{b,c} ±0.02	0.399 ^a ±0.001
	10	10.52 ^a ±0.06	10.97±0.06	1.705 ^{a,b} ±0.027	14.53 ^{c,d} ±0.03	9.40 ^{b,c} ±0.06	0.398 ^a ±0.005
	15	ND	ND	ND	14.6 ^b ±0.03	9.38 ^c ±0.04	0.379 ^b ±0.003
	25	10.06 ^b ±0.05	11.01±0.02	1.661 ^b ±0.018	14.54 ^{b,c} ±0.04	9.44 ^{b,c} ±0.04	0.394 ^a ±0.003
	50	9.34 ^d ±0.06	11.02±0.05	1.604 ^c ±0.021	14.56 ^{b,c} ±0.02	9.51 ^{a,b} ±0.05	0.390 ^a ±0.003
	100	9.67 ^c ±0.07	10.77±0.02	1.393 ^d ±0.014	14.83 ^a ±0.02	9.58 ^a ±0.04	0.343 ^d ±0.006
	p-value	<0.0001	0.0682	0.0082	<0.0001	0.0005	0.0057

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Table 20: Whole meal GlutoPeak data for blends in the KS Providence series.

	Percentage	PMT (s)	BEM (GPU)	AM (GPU)	PM (GPU)	AgE (GPU)	GS1	GS2
Paradox- KS Providence	0	48.338 ^c ±4.166	37 ^c ±3	25 ±1	33 ^b ±3	1011 ^c ±58	37.527 ^c ±5.749	48.760 ^c ±3.369
	5	49.677 ^c ±3.21	36 ^c ±1	26 ±3	32 ^b ±1	1000 ^c ±25	36.341 ^c ±1.431	49.728 ^c ±4.384
	10	53.673 ^c ±3.51	39 ^{b,c} ±0	26 ±2	34 ^b ±1	1066 ^{b,c} ±25	41.574 ^{b,c} ±0.984	57.266 ^c ±4.911
	25	58.342 ^{b,c} ±8.152	37 ^c ±1	26 ±1	34 ^b ±0	1007 ^c ±15	36.913 ^c ±0.945	58.753 ^c ±8.447
	50	72.008 ^b ±4.361	42 ^{a,b} ±1	31 ±6	38 ^a ±2	1158 ^b ±16	48.64 ^b ±0.627	83.457 ^b ±6.256
	100	113.005 ^a ±3.002	44 ^a ±1	38 ±0	41 ^a ±2	1268 ^a ±42	56.216 ^a ±2.617	143.240 ^a ±5.611
	p-value	0.0181	0.0001	0.052	<0.0001	0.0136	<0.0001	0.0165
Breadbox- KS Providence	0	48.338 ^c ±4.166	37 ^c ±3	25 ^c ±1	33 ^c ±3	1011 ^b ±58	37.527 ^b ±5.749	48.760 ^c ±3.369
	5	50.672 ^c ±0.581	36 ^c ±1	26 ^{b,c} ±2	32 ^c ±0	999 ^b ±29	35.642 ^b ±1.604	50.614 ^c ±1.177
	10	50.674 ^c ±3.513	37 ^{b,c} ±1	26 ^{b,c} ±2	34 ^c ±2	1026 ^b ±33	38.336 ^b ±2.262	52.052 ^c ±4.818
	25	69.009 ^b ±4.357	36 ^c ±2	31 ^b ±3	32 ^c ±2	1036 ^b ±60	37.385 ^b ±4.211	71.347 ^b ±1.022
	50	71.011 ^b ±6.245	43 ^{a,b} ±1	31 ^b ±3	40 ^a ±1	1190 ^a ±30	51.169 ^a ±1.733	84.627 ^b ±9.459
	100	105.341 ^a ±6.425	44 ^a ±2	39 ^a ±1	41 ^a ±2	1261 ^a ±37	55.954 ^a ±4.195	132.729 ^a ±6.35
	p-value	0.0121	0.0286	0.012	0.0248	0.0301	0.0342	0.0110
Firebox- KS Providence	0	48.338 ^c ±4.166	37 ^b ±3	25 ±1	33 ±3	1011 ^c ±58	37.527 ^{b,c} ±5.749	48.760 ^c ±3.369
	5	54.008 ^{b,c} ±8.887	36 ^b ±5	26 ±3	32 ±3	989 ^c ±80	35.831 ^c ±7.208	52.925 ^c ±4.204
	10	48.346 ^c ±4.723	37 ^b ±2	25 ±1	33 ±0	1022 ^c ±38	38.178 ^{b,c} ±2.913	49.512 ^c ±6.575
	25	59.341 ^{a,b,c} ±4.511	38 ^b ±2	31 ±3	33 ±1	1073 ^{b,c} ±49	40.473 ^{b,c} ±4.173	63.559 ^{b,c} ±3.657
	50	65.006 ^{a,b} ±7.000	42 ^{a,b} ±1	32 ±5	36 ±4	1161 ^b ±14	48.364 ^b ±0.772	75.537 ^b ±9.024
	100	71.342 ^a ±0.583	48 ^a ±1	30 ±1	45 ±1	1302 ^a ±9	62.081 ^a ±1.115	92.911 ^a ±0.956
	p-value	0.0014	0.0013	0.0430	0.0709	<0.0001	<0.0001	0.0096

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Table 21: Whole meal GlutoPeak data for blends in the Everest series.

	Percentage	PMT (s)	BEM (GPU)	AM (GPU)	PM (GPU)	AgE (GPU)	GSI1	GSI2
Paradox- Everest	0	46.011 ^d ±1.001	41 ^{a,b} ±2	24 ^c ±2	37 ^{a,b} ±1	1076 ^{b,c} ±34	44.169 ^{b,c} ±3.231	49.521 ^d ±1.81
	5	51.339 ^d ±3.215	38 ^b ±2	24 ^c ±1	33 ^b ±4	997 ^c ±64	37.564 ^c ±2.867	51.272 ^d ±5.584
	10	53.004 ^{c,d} ±0.998	38 ^b ±3	24 ^c ±2	35 ^b ±1	1023 ^{b,c} ±74	39.357 ^{b,c} ±5.653	54.182 ^d ±3.128
	25	60.672 ^c ±2.511	41 ^{a,b} ±1	30 ^{b,c} ±2	37 ^{a,b} ±1	1141 ^{a,b} ±28	47.155 ^{a,b} ±1.71	69.213 ^c ±3.603
	50	80.340 ^b ±4.726	38 ^b ±2	32 ^{a,b} ±5	36 ^b ±2	1091 ^{b,c} ±45	41.502 ^{b,c} ±3.43	87.686 ^b ±7.091
	100	113.005 ^a ±3.002	44 ^a ±1	38 ^a ±0	41 ^a ±2	1268 ^a ±42	56.216 ^a ±2.617	143.24 ^a ±5.611
	p-value	0.0064	0.0028	0.013	0.0032	0.0004	0.0003	0.0093
Breadbox- Everest	0	46.011 ^c ±1.001	41 ^{a,b} ±2	24 ^b ±2	37 ^{a,b} ±1	1076 ^b ±34	44.169 ^{a,b} ±3.231	49.521 ^d ±1.81
	5	51.678 ^c ±5.031	38 ^b ±2	25 ^b ±3	34 ^b ±2	1025 ^b ±47	38.652 ^b ±3.622	52.918 ^{c,d} ±4.981
	10	57.012 ^{b,c} ±8.542	38 ^{a,b} ±4	29 ^b ±5	34 ^b ±3	1062 ^b ±66	40.874 ^b ±6.318	60.357 ^{c,d} ±7.397
	25	60.010 ^{b,c} ±3.461	40 ^{a,b} ±2	26 ^b ±1	36 ^{a,b} ±2	1091 ^b ±38	44.056 ^{a,b} ±3.748	65.439 ^c ±3.677
	50	69.003 ^b ±3.005	44 ^{a,b} ±2	31 ^b ±0	40 ^a ±2	1223 ^a ±43	53.883 ^a ±4.319	84.357 ^b ±2.83
	100	105.341 ^a ±6.425	44 ^a ±2	39 ^a ±1	41 ^a ±2	1261 ^a ±37	55.954 ^a ±4.195	132.729 ^a ±6.35
	p-value	0.0112	0.0217	0.036	0.0038	0.0002	0.0017	0.0083
Firebox- Everest	0	46.011 ^c ±1.001	41 ^{a,b} ±2	24±2	37 ^b ±1	1076 ^b ±34	44.169 ^b ±3.231	49.521 ^d ±1.81
	5	46.67 ^c ±0.574	38 ^b ±2	24±3	35 ^b ±0	1036 ^b ±55	39.797 ^b ±4.163	48.383 ^d ±3.052
	10	52.674 ^{b,c} ±5.03	39 ^b ±3	28±5	34 ^b ±1	1097 ^b ±46	43.225 ^b ±4.862	57.665 ^{c,d} ±3.877
	25	56.341 ^{b,c} ±6.503	41 ^{a,b} ±3	33±6	37 ^b ±1	1129 ^b ±48	46.355 ^b ±4.619	63.491 ^{b,c} ±6.1
	50	63.676 ^{a,b} ±6.43	42 ^{a,b} ±4	29±4	37 ^b ±4	1158 ^b ±79	48.851 ^{a,b} ±8.187	73.377 ^b ±2.15
	100	71.342 ^a ±0.583	48 ^a ±1	30±1	45 ^a ±1	1302 ^a ±9	62.081 ^a ±1.115	92.911 ^a ±0.956
	p-value	0.0112	0.0156	0.09	0.0331	0.0005	0.0018	0.0075

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Table 22: Whole meal GlutoPeak data for blends in the KS Mako series.

	Percentage	PMT (s)	BEM (GPU)	AM (GPU)	PM (GPU)	AgE (GPU)	GSI1	GSI2
Paradox- KS Mako	0	59.341 ^c ± 7.098	42 ^{a,b,c} ± 2	29 ± 7	37 ± 2	1120 ^c ± 13	46.646 ^{c,d} ± 1.538	66.470 ^c ± 8.389
	5	58.674 ^c ± 3.518	44 ^{a,b} ± 2	32 ± 4	37 ± 1	1215 ^{a,b,c} ± 13	53.876 ^{a,b,c} ± 1.785	71.295 ^c ± 3.96
	10	65.335 ^{b,c} ± 2.081	41 ^{b,c} ± 2	38 ± 3	34 ± 6	1154 ^{b,c} ± 87	47.061 ^{b,c,d} ± 6.064	75.346 ^c ± 5.179
	25	76.678 ^b ± 6.513	38 ^c ± 2	31 ± 3	36 ± 2	1089 ^c ± 58	41.108 ^d ± 4.63	83.305 ^{b,c} ± 4.61
	50	78.006 ^b ± 5.004	45 ^a ± 1	38 ± 5	40 ± 2	1290 ^a ± 17	58.471 ^a ± 1.52	100.615 ^b ± 6.841
	100	113.005 ^a ± 3.002	44 ^{a,b} ± 1	38 ± 0	41 ± 2	1268 ^{a,b} ± 42	56.216 ^{a,b} ± 2.617	143.240 ^a ± 5.611
	p-value	0.0109	0.0008	0.054	0.055	0.0010	0.0004	0.0097
Breadbox- KS Mako	0	59.341 ^c ± 7.098	42 ± 2	29 ± 7	37 ± 2	1120 ^a ± 13	46.646 ± 1.538	66.47 ^c ± 8.389
	5	61.008 ^c ± 7.809	42 ± 6	30 ± 2	37 ± 5	1160 ^a ± 121	49.195 ± 11.715	70.129 ^c ± 1.746
	10	58.340 ^c ± 2.306	43 ± 5	30 ± 4	39 ± 3	1185 ^a ± 114	51.289 ± 9.971	68.958 ^c ± 4.054
	25	78.009 ^{b,c} ± 5.572	40 ± 2	34 ± 6	35 ± 3	1125 ^a ± 54	44.692 ± 3.829	87.719 ^b ± 6.248
	50	75.341 ^b ± 4.935	47 ± 2	37 ± 4	42 ± 2	1323 ^a ± 41	62.208 ± 4.073	99.536 ^b ± 4.075
	100	105.341 ^a ± 6.425	44 ± 2	39 ± 1	41 ± 2	1261 ^a ± 37	55.954 ± 4.195	132.729 ^a ± 6.35
	p-value	0.0139	0.2265	0.082	0.1283	0.0355	0.0796	0.0110
Firebox- KS Mako	0	59.341 ^{a,b} ± 7.098	42 ^b ± 2	29 ± 7	37 ^c ± 2	1120 ^c ± 13	46.646 ^c ± 1.538	66.47 ^b ± 8.389
	5	56.34 ^b ± 2.516	43 ^{a,b} ± 3	32 ± 6	37 ^c ± 1	1203 ^{b,c} ± 61	52.227 ^{b,c} ± 6.056	67.67 ^b ± 1.739
	10	55.676 ^b ± 2.077	46 ^{a,b} ± 1	32 ± 3	38 ^{b,c} ± 1	1252 ^{a,b} ± 1	57.592 ^{a,b} ± 1.229	69.707 ^b ± 2.614
	25	62.678 ^{a,b} ± 6.804	43 ^{a,b} ± 2	35 ± 7	39 ^{b,c} ± 1	1217 ^{a,b} ± 15	52.728 ^{b,c} ± 3.039	76.227 ^b ± 7.888
	50	64.341 ^{a,b} ± 4.038	46 ^{a,b} ± 1	30 ± 3	41 ^b ± 1	1253 ^{a,b} ± 47	57.663 ^{a,b} ± 3.279	80.653 ^{a,b} ± 6.474
	100	71.342 ^a ± 0.583	48 ^a ± 1	30 ± 1	45 ^a ± 1	1302 ^a ± 9	62.081 ^a ± 1.115	92.911 ^a ± 0.956
	p-value	0.0499	0.0134	0.703	0.0126	0.0004	0.0012	0.0006

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Table 23: Refined GlutoPeak data for blends.

	Percentage	PMT (s)	BEM (GPU)	AM (GPU)	PM (GPU)	AgE (GPU)	Total Peak Area (GPU)	GS1	GS2	GS3	GS4
Paradox- KS Providence	0	136.347 ^e ± 0.577	37 ^c ± 0	19 ^d ± 1	31 ^d ± 0	970 ^d ± 5	2598 ^g ± 22	35.890 ^d ± 0.17	132.257 ^f ± 0.913	96.138 ^f ± 0.797	354.283 ^e ± 4.270
	5	171.671 ^d ± 2.083	34 ^e ± 0	20 ^d ± 1	29 ^e ± 0	911 ^e ± 6	2854 ^f ± 25	30.985 ^f ± 0.187	156.451 ^e ± 2.269	97.036 ^f ± 0.842	489.98 ^{d,e} ± 9.980
	10	175.673 ^d ± 1.532	35 ^d ± 1	20 ^d ± 1	30 ^d ± 1	951 ^d ± 2	3006 ^e ± 22	33.591 ^e ± 0.591	167.006 ^e ± 1.344	106.194 ^e ± 1.237	528.037 ^d ± 8.386
	15	205.007 ^c ± 0.997	35 ^{d,e} ± 0	21 ^{c,d} ± 0	31 ^d ± 0	951 ^d ± 2	3373 ^d ± 22	33.297 ^e ± 0.073	195.031 ^d ± 1.290	118.043 ^d ± 0.780	691.433 ^c ± 7.588
	25	209.340 ^c ± 3.217	38 ^c ± 1	23 ^c ± 1	34 ^c ± 0	1028 ^c ± 13	3644 ^c ± 20	38.713 ^c ± 1.038	215.106 ^c ± 1.335	137.270 ^c ± 2.217	762.899 ^c ± 11.940
	50	280.678 ^b ± 6.026	45 ^b ± 1	30 ^b ± 1	37 ^b ± 1	1208 ^b ± 11	5164 ^b ± 62	53.976 ^b ± 1.147	339.123 ^b ± 5.644	230.655 ^b ± 1.927	1449.755 ^b ± 48.089
	100	517.010 ^a ± 8.545	55 ^a ± 0	35 ^a ± 1	46 ^a ± 1	1500 ^a ± 7	10661 ^a ± 44	82.50 ^a ± 0.361	775.478 ^a ± 9.418	586.373 ^a ± 2.401	5512.259 ^a ± 112.978
	p-value	0.0034	0.0032	0.0052	0.0033	0.0036	0.0032	0.0037	0.0032	0.0035	0.0032
Breadbox- KS Providence	0	136.347 ^e ± 0.577	37 ^{c,d} ± 0	19 ^b ± 1	31 ^{d,e} ± 0	970 ^d ± 5	2598 ^g ± 22	35.890 ^{c,d} ± 0.17	132.257 ^e ± 0.913	96.138 ^e ± 0.797	354.283 ^e ± 4.270
	5	170.009 ^d ± 9.648	34 ^f ± 1	20 ^b ± 1	29 ^e ± 1	917 ^{d,e} ± 18	2827 ^f ± 27	31.179 ^e ± 1.529	155.735 ^d ± 6.063	96.130 ^e ± 3.021	480.728 ^d ± 29.201
	10	178.018 ^d ± 7.94	35 ^{e,f} ± 1	21 ^b ± 0	30 ^e ± 1	941 ^{d,e} ± 19	3016 ^e ± 33	32.623 ^{d,e} ± 1.723	167.357 ^d ± 4.279	104.541 ^e ± 2.31	537.134 ^d ± 29.646
	15	201.338 ^c ± 2.082	36 ^{d,e} ± 0	21 ^b ± 1	32 ^d ± 0	967 ^{d,e} ± 8	3368 ^d ± 13	34.824 ^{d,e} ± 0.280	194.751 ^c ± 0.458	121.260 ^d ± 0.470	678.172 ^c ± 7.093
	25	202.673 ^c ± 2.084	38 ^c ± 0	23 ^b ± 1	34 ^c ± 0	1041 ^c ± 18	3602 ^c ± 27	39.545 ^c ± 0.684	210.891 ^c ± 1.545	136.876 ^c ± 1.041	729.995 ^c ± 3.423
	50	263.342 ^b ± 4.045	43 ^b ± 1	29 ^a ± 2	37 ^b ± 1	1203 ^b ± 19	4889 ^b ± 68	52.120 ^b ± 1.385	316.696 ^b ± 5.785	211.844 ^b ± 4.207	1287.446 ^b ± 30.662
	100	396.007 ^a ± 4.581	56 ^a ± 1	34 ^a ± 4	48 ^a ± 1	1518 ^a ± 23	8267 ^a ± 77	84.527 ^a ± 2.013	601.264 ^a ± 10.981	460.198 ^a ± 8.296	3273.651 ^a ± 47.561
	p-value	0.0039	0.0036	0.0037	0.0036	0.004	0.0032	0.0037	0.0034	0.0036	0.0034

Firebox- KS Providence	0	136.347 ^e ± 0.577	37 ^c ± 0	19 ^d ± 1	31 ^{c,d} ± 0	970 ^c ± 5	2598 ^f ± 22	35.890 ^c ± 0.17	132.257 ^e ± 0.913	96.138 ^{e,f} ± 0.797	354.283 ^e ± 4.27
	5	167.674 ^d ± 9.502	34 ^e ± 0	19 ^d ± 1	29 ^e ± 0	910 ^d ± 11	2761 ^e ± 73	30.929 ^d ± 0.357	152.564 ^d ± 9.669	93.874 ^f ± 2.485	463.210 ^d ± 34.285
	10	172.01 ^d ± 1.729	35 ^{d,e} ± 0	20 ^{c,d} ± 1	30 ^{d,e} ± 1	930 ^d ± 5	2905 ^d ± 13	32.550 ^d ± 0.175	159.964 ^d ± 0.959	101.687 ^e ± 0.457	499.744 ^d ± 5.311
	15	198.341 ^c ± 2.083	34 ^e ± 1	20 ^{c,d} ± 0	30 ^{c,d,e} ± 0	931 ^d ± 4	3208 ^c ± 13	31.977 ^d ± 0.653	184.717 ^c ± 1.258	110.156 ^d ± 2.14	636.338 ^c ± 6.161
	25	194.677 ^c ± 2.08	37 ^{c,d} ± 1	22 ^{b,c} ± 1	32 ^c ± 1	986 ^c ± 5	3288 ^c ± 16	36.143 ^c ± 0.724	191.88 ^c ± 1.204	120.542 ^c ± 1.405	640.049 ^c ± 9.420
	50	228.672 ^b ± 7.574	40 ^b ± 1	22 ^b ± 1	35 ^b ± 1	1074 ^b ± 16	3925 ^b ± 63	42.957 ^b ± 1.694	245.462 ^b ± 6.216	157.006 ^b ± 4.238	897.851 ^b ± 41.545
	100	247.338 ^a ± 4.038	53 ^a ± 1	25 ^a ± 1	44 ^a ± 1	1361 ^a ± 9	4945 ^a ± 66	72.131 ^a ± 1.291	336.632 ^a ± 6.348	262.024 ^a ± 1.643	1223.173 ^a ± 35.721
	p-value	<0.0001	0.0036	0.007	0.0035	0.0037	0.0032	0.0041	0.0036	0.0035	0.0037
Paradox- Everest	0	148.340 ^f ± 4.51	44 ^c ± 1	25 ^b ± 3	39 ^{b,c} ± 1	1206 ^{c,d} ± 9	3235 ^f ± 45	52.646 ^c ± 0.671	178.867 ^f ± 6.393	141.233 ^f ± 1.199	479.966 ^e ± 21.232
	5	159.008 ^{e,f} ± 0.997	44 ^c ± 1	24 ^b ± 1	38 ^c ± 1	1186 ^d ± 7	3286 ^f ± 28	52.202 ^c ± 1.441	188.635 ^{e,f} ± 1.483	144.568 ^{e,f} ± 3.433	522.430 ^e ± 2.105
	10	168.339 ^{d,e} ± 0.581	44 ^c ± 0	24 ^b ± 2	39 ^{b,c} ± 1	1189 ^d ± 12	3455 ^e ± 40	52.316 ^c ± 0.533	200.156 ^e ± 2.243	152.020 ^e ± 1.758	581.609 ^{d,e} ± 6.575
	15	183.340 ^d ± 1.531	44 ^c ± 1	24 ^b ± 1	39 ^{b,c} ± 0	1196 ^{c,d} ± 3	3688 ^d ± 21	53.038 ^c ± 0.763	219.333 ^d ± 1.342	163.487 ^d ± 2.368	676.119 ^d ± 9.440
	25	204.009 ^c ± 5.001	45 ^c ± 1	26 ^b ± 2	40 ^b ± 1	1224 ^c ± 23	4095 ^c ± 40	54.695 ^c ± 1.695	249.720 ^c ± 4.174	182.911 ^c ± 3.033	835.480 ^c ± 25.201
	50	279.671 ^b ± 7.575	47 ^b ± 1	37 ^a ± 2	40 ^{b,c} ± 0	1333 ^b ± 9	5713 ^b ± 61	63.093 ^b ± 0.498	372.800 ^b ± 10.299	270.410 ^b ± 3.816	1597.953 ^b ± 55.395
	100	517.010 ^a ± 8.545	55 ^a ± 0	35 ^a ± 1	46 ^a ± 1	1500 ^a ± 7	10661 ^a ± 44	82.500 ^a ± 0.361	775.478 ^a ± 9.418	586.373 ^a ± 2.401	5512.259 ^a ± 112.978
	p-value	0.0032	0.0153	0.0206	0.0123	0.0079	0.0035	0.0178	0.0032	0.0035	0.0032
Breadbox- Everest	0	148.340 ^e ± 4.51	44 ^d ± 1	25 ^b ± 3	39 ^{c,d} ± 1	1206 ^{c,d} ± 9	3235 ^e ± 45	52.646 ^{c,d} ± 0.671	178.867 ^e ± 6.393	141.233 ^e ± 1.199	479.966 ^e ± 21.232
	5	151.008 ^e ± 6.998	44 ^{c,d} ± 0	25 ^b ± 2	39 ^{c,d} ± 1	1204 ^{c,d} ± 14	3210 ^e ± 136	52.961 ^{c,d} ± 0.618	181.826 ^e ± 10.43	141.240 ^e ± 5.970	485.369 ^e ± 42.463
	10	165.008 ^d ± 0.005	44 ^d ± 1	25 ^b ± 2	39 ^{c,d} ± 1	1191 ^d ± 21	3408 ^e ± 32	52.012 ^d ± 1.468	196.525 ^e ± 3.491	148.810 ^{d,e} ± 3.108	562.293 ^e ± 5.333
	15	186.674 ^c ± 1.526	44 ^{c,d} ± 0	24 ^b ± 1	38 ^d ± 1	1186 ^d ± 18	3689 ^d ± 32	52.184 ^d ± 0.778	221.387 ^d ± 2.959	162.331 ^d ± 1.405	688.683 ^d ± 5.018

Firebox- Everest	25	195.336 ^c ± 3.793	45 ^c ± 1	27 ^{a,b} ± 2	41 ^{b,c} ± 1	1251 ^c ± 11	4035 ^c ± 55	56.698 ^c ± 1.011	244.329 ^c ± 6.898	182.942 ^c ± 3.922	788.386 ^c ± 25.930
	50	250.674 ^b ± 1.525	48 ^b ± 1	31 ^{a,b} ± 5	43 ^b ± 1	1349 ^b ± 31	5254 ^b ± 99	65.230 ^b ± 2.285	338.214 ^b ± 5.912	253.965 ^b ± 7.839	1316.870 ^b ± 18.004
	100	396.007 ^a ± 4.581	56 ^a ± 1	34 ^a ± 4	48 ^a ± 1	1518 ^a ± 23	8267 ^a ± 77	84.527 ^a ± 2.013	601.264 ^a ± 10.981	460.198 ^a ± 8.296	3273.651 ^a ± 47.561
	p-value	0.0036	0.0052	0.0274	0.009	0.0087	0.0037	0.01	0.0036	0.0039	0.0036
	0	148.340 ^d ± 4.510	44 ^d ± 1	25 ± 3	39 ^{b,c} ± 1	1206 ^{b,c} ± 9	3235 ^e ± 45	52.646 ^{c,d} ± 0.671	178.867 ^d ± 6.393	141.233 ^d ± 1.199	479.966 ^e ± 21.232
	5	153.338 ^d ± 10.972	43 ^d ± 1	24 ± 2	38 ^c ± 1	1171 ^c ± 5	3104 ^e ± 148	50.352 ^d ± 1.153	179.555 ^d ± 12.815	133.363 ^e ± 3.543	476.994 ^e ± 56.047
	10	161.676 ^{c,d} ± 2.520	44 ^{c,d} ± 0	25 ± 1	38 ^c ± 0	1187 ^c ± 13	3257 ^{d,e} ± 24	52.228 ^{c,d} ± 0.562	191.894 ^{c,d} ± 2.003	143.308 ^d ± 1.038	526.603 ^{d,e} ± 10.928
	15	178.340 ^c ± 7.766	44 ^{c,d} ± 1	24 ± 1	38 ^c ± 1	1186 ^c ± 26	3482 ^{c,d} ± 48	52.601 ^{c,d} ± 1.727	211.435 ^c ± 4.431	154.352 ^c ± 0.815	621.210 ^c ± 35.156
	25	173.342 ^c ± 2.882	46 ^c ± 1	25 ± 2	39 ^{b,c} ± 1	1207 ^{b,c} ± 23	3501 ^c ± 64	55.144 ^c ± 1.749	209.269 ^c ± 4.574	159.876 ^c ± 4.269	606.875 ^{c,d} ± 18.350
	50	204.013 ^b ± 1.002	48 ^b ± 0	24 ± 2	41 ^b ± 1	1255 ^b ± 34	3971 ^b ± 62	60.24 ^b ± 1.643	256.032 ^b ± 6.893	190.592 ^b ± 2.966	810.067 ^b ± 13.261
Paradox- KS Mako	100	247.338 ^a ± 4.038	53 ^a ± 1	25 ± 1	44 ^a ± 1	1361 ^a ± 9	4945 ^a ± 66	72.131 ^a ± 1.291	336.632 ^a ± 6.348	262.024 ^a ± 1.643	1223.173 ^a ± 35.721
	p-value	0.0044	0.0055	0.9561	0.0103	0.0106	0.0045	0.0078	0.0041	0.0034	0.0046
	0	239.339 ^d ± 3.055	36 ^e ± 0	24 ± 4	34 ^d ± 1	1010 ^e ± 36	3740 ^e ± 124	36.348 ^e ± 1.300	241.726 ^e ± 11.671	134.640 ^f ± 4.448	895.374 ^e ± 40.588
	5	267.337 ^{c,d} ± 7.238	37 ^e ± 1	31 ± 3	34 ^{c,d} ± 1	1087 ^d ± 30	4171 ^e ± 93	40.605 ^{d,e} ± 1.734	290.546 ^d ± 1.772	155.753 ^e ± 5.886	1114.616 ^d ± 6.126
	10	293.342 ^c ± 14.018	38 ^{d,e} ± 2	33 ± 4	34 ^{c,d} ± 1	1093 ^d ± 32	4469 ^d ± 124	41.213 ^d ± 2.831	320.425 ^{c,d} ± 6.106	168.465 ^{d,e} ± 11.271	1310.052 ^{c,d} ± 36.988
	15	298.345 ^c ± 21.225	40 ^{c,d} ± 1	31 ± 1	34 ^{c,d} ± 1	1128 ^{c,d} ± 13	4569 ^d ± 30	44.762 ^{c,d} ± 1.131	336.453 ^c ± 20.084	181.244 ^d ± 3.467	1362.810 ^c ± 90.189
	25	291.344 ^c ± 7.090	41 ^c ± 0	33 ± 2	36 ^c ± 1	1169 ^c ± 3	4963 ^c ± 60	47.915 ^c ± 0.132	340.469 ^c ± 7.363	203.497 ^c ± 2.455	1446.310 ^c ± 52.221
	50	353.008 ^b ± 9.849	48 ^b ± 1	33 ± 1	40 ^b ± 0	1297 ^b ± 11	6300 ^b ± 69	61.812 ^b ± 1.238	457.676 ^b ± 9.802	300.262 ^b ± 2.021	2224.262 ^b ± 85.508
	100	517.010 ^a ± 8.545	55 ^a ± 0	35 ± 1	46 ^a ± 1	1500 ^a ± 7	10661 ^a ± 44	82.500 ^a ± 0.361	775.478 ^a ± 9.418	586.373 ^a ± 2.401	5512.259 ^a ± 112.978
	p-value	0.0056	0.0039	0.0576	0.0094	0.0044	0.0032	0.004	0.0042	0.0034	0.0042

Breadbox- KS Mako	0	239.339 ^d ± 3.055	36 ^d ± 0	24 ^b ± 4	34 ^d ± 1	1010 ^e ± 36	3740 ^f ± 124	36.348 ^d ± 1.300	241.726 ^e ± 11.671	134.640 ^f ± 4.448	895.374 ^e ± 40.588
	5	280.342 ^c ± 12.052	36 ^d ± 1	29 ^{a,b} ± 1	34 ^{c,d} ± 1	1060 ^e ± 12	4164 ^e ± 23	38.505 ^d ± 1.004	296.973 ^d ± 9.368	151.296 ^{e,f} ± 1.626	1167.620 ^d ± 56.523
	10	284.003 ^c ± 8.184	37 ^d ± 0	28 ^{a,b} ± 1	35 ^{c,d} ± 1	1071 ^{d,e} ± 12	4270 ^{d,e} ± 10	39.639 ^d ± 0.434	304.208 ^d ± 6.238	157.978 ^e ± 0.356	1212.627 ^d ± 36.477
	15	282.010 ^c ± 6.551	40 ^c ± 0	30 ^{a,b} ± 3	35 ^{c,d} ± 1	1133 ^{c,d} ± 18	4493 ^d ± 53	45.320 ^c ± 0.711	319.490 ^{c,d} ± 7.402	179.720 ^d ± 2.125	1266.943 ^{c,d} ± 24.625
	25	292.002 ^c ± 8.546	41 ^c ± 1	32 ^{a,b} ± 4	36 ^c ± 1	1174 ^c ± 24	4881 ^c ± 171	48.136 ^c ± 2.157	342.651 ^c ± 9.271	200.247 ^c ± 11.896	1425.113 ^c ± 56.601
	50	322.678 ^b ± 13.205	48 ^b ± 1	32 ^{a,b} ± 3	40 ^b ± 1	1301 ^b ± 12	5796 ^b ± 81	61.998 ^b ± 0.857	419.747 ^b ± 19.274	276.265 ^b ± 4.177	1870.697 ^b ± 95.095
	100	396.007 ^a ± 4.581	56 ^a ± 1	34 ^a ± 4	48 ^a ± 1	1518 ^a ± 23	8267 ^a ± 77	84.527 ^a ± 2.013	601.264 ^a ± 10.981	460.198 ^a ± 8.296	3273.651 ^a ± 47.561
	p-value	0.0101	0.0036	0.0185	0.0062	0.0035	0.0032	0.0034	0.0034	0.0032	0.0035
Firebox- KS Mako	0	239.339 ^e ± 3.055	36 ^{e,f} ± 0	24 ± 4	34 ^a ± 1	1010 ^d ± 36	3740 ^e ± 124	36.348 ^d ± 1.300	241.726 ^d ± 11.671	134.64 ^e ± 4.448	895.374 ^c ± 40.588
	5	281.337 ^a ± 12.056	35 ^f ± 1	26 ± 4	34 ^d ± 1	1023 ^d ± 34	3902 ^{d,e} ± 174	36.143 ^d ± 1.68	287.804 ^{b,c} ± 18.191	137.923 ^e ± 8.379	1098.716 ^b ± 90.716
	10	260.347 ^{c,d} ± 3.058	37 ^{d,e} ± 1	26 ± 2	35 ^{c,d} ± 1	1066 ^{c,d} ± 8	3987 ^{c,d,e} ± 44	39.813 ^{c,d} ± 0.931	277.619 ^c ± 4.146	148.864 ^{d,e} ± 3.897	1037.992 ^b ± 15.682
	15	276.674 ^{a,b} ± 1.531	39 ^{c,d} ± 0	25 ± 2	36 ^c ± 0	1093 ^c ± 7	4102 ^{c,d,e} ± 21	42.627 ^c ± 0.273	302.409 ^{b,c} ± 3.281	159.965 ^{c,d} ± 0.819	1134.837 ^{a,b} ± 10.918
	25	266.006 ^{b,c} ± 2.649	40 ^c ± 1	24 ± 2	36 ^c ± 1	1104 ^c ± 6	4169 ^{c,d,e} ± 36	43.776 ^c ± 0.389	293.588 ^{b,c} ± 4.017	165.363 ^c ± 2.109	1109.043 ^{a,b} ± 20.573
	50	253.009 ^{c,d,e} ± 1.73	46 ^b ± 1	25 ± 1	39 ^b ± 1	1221 ^b ± 25	4448 ^b ± 51	56.165 ^b ± 2.274	308.812 ^b ± 4.074	204.652 ^b ± 6.581	1125.411 ^{a,b} ± 5.115
	100	247.338 ^{d,e} ± 4.038	53 ^a ± 1	25 ± 1	44 ^a ± 1	1361 ^a ± 9	4945 ^a ± 66	72.131 ^a ± 1.291	336.632 ^a ± 6.348	262.024 ^a ± 1.643	1223.173 ^a ± 35.721
	p-value	<0.0001	0.0033	0.8974	0.004	0.0039	<0.0001	0.0037	<0.0001	0.0043	<0.0001

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Chapter 3 Supplementary Tables

Table 24: Refined rheological and bake data for blends.

	Percentage	Mixo Abs (%)	Mixo ML Peak Time (min)	Mixo MixScore	P (mm H ₂ O)	L (mm)	W (10 ⁻⁴ J)	DDT (min)	Stability (min)	Specific Volume (cm ³ /g)	Power (W)	Time to Power (min)
Paradox- KS Providence	0	57.3 ^d ±0.1	5.97 ^d ±0.94	24.57 ^d ±2.49	44 ^d ±3	67 ^b ±13	110 ^b ±8	6.6 ^d ±0.3	10.9 ^e ±0.2	4.34±0.02	82.80 ^c ±3.46	5.80 ^c ±0.89
	5	56.6 ^e ±0.0	6.10 ^d ±0.55	25.67 ^d ±1.47	63 ^c ±1	47 ^{b,c} ±4	121 ^b ±5	7.3 ^d ±0.4	13.5 ^e ±0.6	4.2±0.37	85.13 ^{b,c} ±1.80	5.48 ^c ±0.42
	10	57.1 ^{d,e} ±0.3	6.55 ^{c,d} ±0.12	27.92 ^{c,d} ±0.65	70 ^c ±1	45 ^{b,c} ±2	128 ^b ±3	7.4 ^d ±0.2	15.7 ^e ±0.5	4.41±0.28	84.11 ^c ±0.94	5.49 ^c ±0.74
	15	57.3 ^d ±0.0	9.20 ^{b,c} ±0.57	34.74 ^{b,c} ±1.37	67 ^c ±3	38 ^c ±3	113 ^b ±9	10.6 ^c ±1.5	23.6 ^d ±3.9	4.1±0.24	91.91 ^{a,b,c} ±2.51	6.69 ^c ±1.24
	25	58.5 ^c ±0.1	8.45 ^{b,c,d} ±0.97	34.10 ^c ±1.92	87 ^b ±0	38 ^c ±5	147 ^b ±16	11.2 ^c ±1.0	37.9 ^c ±3.4	4.21±0.21	90.22 ^{a,b,c} ±2.70	7.67 ^{b,c} ±0.52
	50	59.7 ^b ±0.3	10.99 ^b ±0.66	42.95 ^b ±2.83	113 ^a ±8	31 ^c ±4	159 ^b ±29	36.2 ^b ±1.6	52.4 ^b ±1.7	4.14±0.25	95.02 ^{a,b} ±4.33	9.84 ^b ±0.67
	100	63.1 ^a ±0.3	21.22 ^a ±1.74	76.55 ^a ±5.49	114 ^a ±2	169 ^a ±15	465 ^a ±42	56.2 ^a ±0.6	59.0 ^a ±1.0	4.07±0.04	95.20 ^a ±6.48	19.28 ^a ±1.52
	p-value	0.0046	0.0053	0.0042	0.0039	0.0053	0.0061	0.0042	0.0032	0.5440	0.0023	0.0106
Breadbox- KS Providence	0	57.3 ^{d,e} ±0.1	5.97 ^c ±0.94	24.57 ^d ±2.49	44 ^e ±3	67 ^b ±13	110 ^{b,c} ±8	6.6 ^c ±0.3	10.9 ^e ±0.2	4.34±0.02	82.8±3.46	5.8 ^c ±0.89
	5	56.7 ^e ±0.2	6.25 ^c ±0.82	26.47 ^{c,d} ±2.19	61 ^d ±2	48 ^{b,c} ±6	121 ^{b,c} ±12	6.9 ^c ±0.3	14.1 ^d ±0.8	4.02±0.29	85.37±3.17	4.9 ^c ±0.33
	10	56.8 ^e ±0.4	6.96 ^c ±0.55	28.47 ^{c,d} ±1.67	66 ^d ±1	53 ^{b,c} ±6	140 ^{b,c} ±11	7.5 ^c ±1.0	17.6 ^c ±0.5	3.98±0.24	90.64±2.93	5.93 ^c ±0.52
	15	57.7 ^{c,d} ±0.0	7.69 ^c ±0.38	30.65 ^c ±1.16	69 ^d ±5	32 ^c ±2	100 ^c ±6	9.3 ^c ±0.5	18.6 ^c ±0.7	4.10±0.21	89.30±5.02	6.58 ^c ±1.26
	25	58.4 ^c ±0.1	7.64 ^c ±0.45	31.11 ^c ±1.95	85 ^c ±0	36 ^c ±4	135 ^{b,c} ±13	9.9 ^c ±0.8	25.0 ^b ±1.3	4.18±0.32	87.61±2.92	7.19 ^{b,c} ±0.50
	50	59.8 ^b ±0.3	10.16 ^b ±0.86	39.75 ^b ±1.81	113 ^b ±6	34 ^c ±4	170 ^b ±10	33.8 ^b ±1.1	59.1 ^a ±0.4	4.27±0.32	97.22±5.89	9.53 ^b ±1.29
	100	63.6 ^a ±0.2	17.07 ^a ±0.34	63.44 ^a ±1.41	132 ^a ±4	149 ^a ±13	437 ^a ±54	51.7 ^a ±3.0	59.2 ^a ±0.2	4.01±0.17	100.21±11.58	17.04 ^a ±1.16
	p-value	0.0038	0.0069	0.0068	0.0036	0.0055	0.0054	0.0054	0.0039	0.4592	0.0611	0.0094

Firefox- KS Providence	0	57.3 ^{c,d} ±0.1	5.97 ^c ±0.94	24.57 ^c ±2.49	44 ^e ±3	67 ^a ±13	110 ^c ±8	6.6 ^d ±0.3	10.9 ^c ±0.2	4.34±0.02	82.80 ^b ±3.46	5.80 ^b ±0.89
	5	56.6 ^e ±0.1	6.03 ^c ±0.87	25.17 ^c ±2.87	58 ^{c,d} ±6	51 ^{a,b} ±10	118 ^c ±6	7.4 ^d ±0.4	13.7 ^c ±1.1	4.27±0.32	87.90 ^{a,b} ±2.59	5.12 ^b ±0.80
	10	56.8 ^d ±0.2	6.38 ^{b,c} ±0.68	27.14 ^{b,c} ±2.10	58 ^{c,d} ±3	60 ^{a,b} ±14	135 ^{b,c} ±21	7.0 ^d ±0.2	13.7 ^c ±0.4	4.27±0.04	89.50 ^{a,b} ±0.87	5.38 ^b ±0.58
	15	57.0 ^{c,d,e} ±0.1	8.04 ^{a,b} ±0.08	30.77 ^b ±0.11	57 ^d ±2	41 ^b ±3	101 ^c ±5	7.4 ^d ±1.1	15.1 ^c ±0.2	4.04±0.17	93.19 ^{a,b} ±1.84	5.90 ^b ±0.83
	25	57.6 ^c ±0.2	7.00 ^{a,b,c} ±0.69	28.85 ^{b,c} ±2.23	68 ^c ±3	43 ^{a,b} ±1	127 ^c ±4	9.4 ^c ±0.5	17.7 ^c ±0.7	4.00±0.25	95.36 ^{a,b} ±2.00	6.33 ^b ±0.68
	50	59.0 ^b ±0.2	6.96 ^{a,b,c} ±0.44	30.04 ^{b,c} ±1.37	85 ^b ±3	50 ^{a,b} ±7	181 ^b ±24	11.6 ^b ±0.2	36.0 ^b ±4.8	4.37±0.14	98.83 ^a ±3.23	6.57 ^b ±0.41
	100	62.3 ^a ±0.5	8.47 ^a ±0.41	36.79 ^a ±1.30	115 ^a ±5	56 ^{a,b} ±6	285 ^a ±23	28.9 ^a ±0.5	48.0 ^a ±3.4	4.49±0.24	98.44 ^a ±10.72	8.92 ^a ±0.63
	p-value	0.004	0.0017	<0.0001	0.0055	0.0344	0.0071	0.0076	0.004	0.0775	0.0076	0.0003
Paradox- Everest	0	60.5 ^{b,c} ±0.4	6.72 ^{b,c} ±0.85	27.74 ^c ±2.05	57 ^e ±1	68 ^b ±2	139 ^c ±2	7.3 ^e ±0.5	13.1 ^d ±0.2	4.22±0.20	103.80 ^a ±7.36	4.80 ^c ±0.45
	5	60.1 ^c ±0.2	6.34 ^c ±0.71	27.24 ^c ±1.28	71 ^d ±3	56 ^{b,c} ±2	151 ^{b,c} ±5	8.5 ^{d,e} ±0.2	13.1 ^d ±1.2	4.22±0.31	84.25 ^b ±2.54	6.12 ^{b,c} ±0.66
	10	60.2 ^{b,c} ±0.2	6.84 ^{b,c} ±0.95	28.61 ^c ±2.01	75 ^d ±5	62 ^b ±10	171 ^{b,c} ±11	9.4 ^d ±0.2	15.2 ^d ±0.8	4.57±0.32	92.03 ^{a,b} ±3.70	5.54 ^{b,c} ±0.88
	15	60.3 ^{b,c} ±0.1	8.00 ^{b,c} ±1.37	32.01 ^c ±3.33	73 ^d ±3	50 ^{b,c} ±8	149 ^{b,c} ±14	9.6 ^d ±0.8	19.6 ^c ±1.1	4.54±0.19	103.34 ^a ±5.12	6.51 ^{b,c} ±1.75
	25	60.8 ^b ±0.2	8.71 ^{b,c} ±0.62	34.92 ^{b,c} ±1.43	96 ^c ±2	49 ^{b,c} ±3	192 ^{b,c} ±7	11.7 ^c ±0.2	23.3 ^b ±1.6	4.30±0.13	104.84 ^a ±0.25	7.02 ^{b,c} ±0.84
	50	60.7 ^{b,c} ±0.1	9.79 ^b ±0.34	40.60 ^b ±1.42	132 ^a ±5	35 ^c ±3	199 ^b ±5	42.6 ^b ±1.1	59.0 ^a ±0.0	4.30±0.16	102.53 ^a ±2.80	8.41 ^b ±0.62
	100	63.1 ^a ±0.3	21.22 ^a ±1.74	76.55 ^a ±5.49	114 ^b ±2	169 ^a ±15	465 ^a ±42	56.2 ^a ±0.6	59.0 ^a ±0.1	4.07±0.04	95.20 ^{a,b} ±6.48	19.28 ^a ±1.52
	p-value	0.0134	0.0106	0.009	0.005	0.0102	0.0055	0.0036	0.0044	0.1215	0.0005	0.0141
Breadbox- Everest	0	60.5 ^{c,d} ±0.4	6.72 ^c ±0.85	27.74 ^d ±2.05	57 ^e ±1	68 ^{b,c} ±2	139 ^c ±2	7.3 ^d ±0.5	13.1 ^e ±0.2	4.22±0.20	103.80±7.36	4.80 ^c ±0.45
	5	60.2 ^{c,d} ±0.2	7.12 ^c ±0.89	28.80 ^{c,d} ±1.31	63 ^e ±2	81 ^b ±13	170 ^{b,c} ±14	8.1 ^{c,d} ±0.9	13.8 ^e ±0.8	4.26±0.27	100.53±1.12	5.15 ^c ±0.54
	10	60.5 ^{c,d} ±0.2	6.43 ^c ±0.30	28.06 ^d ±0.67	74 ^d ±4	66 ^{b,c,d} ±8	179 ^{b,c} ±9	8.9 ^{c,d} ±0.2	15.0 ^{d,e} ±0.9	4.28±0.27	98.45±1.75	5.14 ^c ±0.72
	15	60.1 ^d ±0.2	7.19 ^c ±0.37	30.27 ^{c,d} ±0.97	76 ^d ±2	44 ^d ±5	140 ^c ±8	9.6 ^{c,d} ±0.7	17.2 ^d ±0.8	4.36±0.05	104.43±1.00	5.66 ^c ±0.89

Firebox- Everest	25	61.1 ^{b,c} ±0.4	8.29 ^c ±0.93	33.03 ^c ±2.46	94 ^c ±5	57 ^{b,c,d} ±6	212 ^b ±6	11.9 ^c ±0.3	22.9 ^c ±1.4	4.17±0.17	100.44±3.54	6.21 ^{b,c} ±0.25
	50	61.8 ^b ±0.5	10.97 ^b ±0.33	42.90 ^b ±1.12	111 ^b ±3	44 ^{c,d} ±2	211 ^b ±1	39.7 ^b ±1.2	52.7 ^b ±1.2	4.26±0.12	103.12±1.95	8.21 ^b ±0.70
	100	63.6 ^a ±0.2	17.07 ^a ±0.34	63.44 ^a ±1.41	132 ^a ±4	149 ^a ±13	437 ^a ±54	51.7 ^a ±3.0	59.2 ^a ±0.2	4.01±0.17	100.21±11.58	17.04 ^a ±1.16
	p-value	0.0076	0.0105	0.0066	0.0036	0.0057	0.0046	0.0037	0.0039	0.4747	0.8039	0.0159
	0	60.5 ^{b,c} ±0.4	6.72 ^{a,b,c} ±0.85	27.74 ^c ±2.05	57 ^d ±1	68 ^{b,c} ±2	139 ^d ±2	7.3 ^d ±0.5	13.1 ^c ±0.2	4.22±0.20	103.80±7.36	4.80 ^{b,c} ±0.45
	5	59.9 ^c ±0.2	6.25 ^{b,c} ±0.24	26.59 ^c ±0.53	60 ^{c,d} ±3	91 ^a ±10	173 ^c ±9	7.9 ^d ±0.3	12.4 ^c ±1.0	4.32±0.20	99.29±8.47	4.44 ^c ±0.53
	10	60.0 ^c ±0.0	6.03 ^c ±0.95	26.31 ^c ±2.17	63 ^{c,d} ±3	88 ^{a,b} ±13	179 ^c ±14	8.3 ^d ±0.2	12.7 ^c ±0.8	4.16±0.13	107.00±5.04	4.96 ^{b,c} ±0.62
	15	59.8 ^c ±0.3	6.83 ^{a,b,c} ±1.17	28.25 ^c ±2.47	66 ^{c,d} ±2	49 ^c ±4	130 ^d ±4	8.2 ^d ±0.5	15.8 ^{b,c} ±1.2	4.41±0.44	98.71±1.87	5.23 ^{b,c} ±0.53
	25	60.4 ^{b,c} ±0.4	7.48 ^{a,b,c} ±0.24	30.34 ^{b,c} ±0.53	70 ^{b,c} ±4	83 ^{a,b} ±9	206 ^c ±9	9.6 ^c ±0.2	16.8 ^{b,c} ±0.6	4.31±0.50	104.61±5.95	5.72 ^{b,c} ±0.22
	50	61.0 ^b ±0.2	7.97 ^{a,b} ±0.22	33.13 ^{a,b} ±0.76	77 ^b ±5	86 ^{a,b} ±7	247 ^b ±5	12.8 ^b ±0.1	20.6 ^b ±4.5	4.49±0.34	104.06±3.95	5.94 ^b ±0.26
Paradox- KS Mako	100	62.3 ^a ±0.5	8.47 ^a ±0.41	36.79 ^a ±1.30	115 ^a ±5	56 ^c ±6	285 ^a ±23	28.9 ^a ±0.5	48.0 ^a ±3.4	4.49±0.24	98.44±10.72	8.92 ^a ±0.63
	p-value	0.0103	0.0057	<0.0001	0.0048	<0.0001	<0.0001	0.0059	0.0062	0.8065	0.6129	0.0148
	0	58.4 ^c ±0.2	7.61 ^c ±0.37	30.87 ^d ±1.29	84 ^d ±4	35 ^b ±1	126 ^b ±14	6.1 ^d ±0.4	12.8 ^c ±0.7	4.26±0.18	98.49±6.15	6.87 ^b ±0.44
	5	58.6 ^c ±0.3	8.00 ^c ±0.48	32.46 ^{c,d} ±0.60	92 ^{c,d} ±2	36 ^b ±3	146 ^b ±8	7.9 ^d ±0.2	24.3 ^c ±3.5	4.29±0.43	99.19±5.05	7.00 ^b ±0.53
	10	58.5 ^c ±0.4	9.09 ^c ±0.54	35.22 ^{c,d} ±2.13	93 ^{c,d} ±4	33 ^b ±1	133 ^b ±6	8.6 ^d ±0.1	34.1 ^c ±1.3	4.17±0.26	95.07±0.92	7.03 ^b ±0.50
	15	58.6 ^c ±0.2	10.04 ^{b,c} ±0.23	38.28 ^{b,c,d} ±0.39	88 ^d ±3	24 ^b ±0	101 ^b ±5	34.7 ^c ±3.9	40.8 ^b ±3.0	4.22±0.22	95.48±4.25	7.71 ^b ±0.58
	25	59.1 ^c ±0.2	10.11 ^{b,c} ±0.32	39.44 ^{b,c} ±1.26	101 ^{b,c} ±3	31 ^b ±2	141 ^b ±12	35.0 ^c ±1.7	59.3 ^a ±0.1	4.44±0.30	97.93±3.75	8.70 ^b ±0.84
	50	60.7 ^b ±0.1	12.16 ^b ±1.40	46.59 ^b ±4.05	123 ^a ±9	27 ^b ±1	154 ^b ±7	45.2 ^b ±1.7	58.6 ^a ±0.5	4.12±0.19	98.05±2.65	11.10 ^b ±0.63
	100	63.1 ^a ±0.3	21.22 ^a ±1.74	76.55 ^a ±5.49	114 ^{a,b} ±2	169 ^a ±15	465 ^a ±42	56.2 ^a ±0.6	59.0 ^a ±0.1	4.07±0.04	95.20±6.48	19.28 ^a ±1.52
	p-value	0.0151	0.004	0.0038	<0.0001	0.0049	0.0108	0.0037	0.0036	0.7048	0.8407	0.0070

Breadbox- KS Mako	0	58.4 ^d ±0.2	7.61 ^d ±0.37	30.87 ^d ±1.29	84 ^c ±4	35 ^b ±1	126 ^b ±14	6.1 ^d ±0.4	12.8 ^c ±0.7	4.26±0.18	98.49±6.15	6.87 ^c ±0.44
	5	58.6 ^{c,d} ±0.3	7.82 ^d ±0.33	31.77 ^d ±1.56	94 ^c ±7	37 ^b ±1	151 ^b ±10	6.1 ^d ±0.1	21.2 ^{b,c} ±0.5	4.50±0.20	94.78±3.63	6.40 ^c ±0.21
	10	58.8 ^{c,d} ±0.5	7.99 ^d ±0.56	32.96 ^{c,d} ±2.48	93 ^c ±3	33 ^b ±4	136 ^b ±12	9.4 ^d ±0.3	32.3 ^b ±1.2	4.56±0.20	93.61±2.01	7.30 ^c ±0.78
	15	58.9 ^{c,d} ±0.2	9.27 ^c ±0.36	36.96 ^c ±0.60	92 ^c ±3	25 ^b ±2	107 ^b ±8	33.4 ^c ±2.8	57.7 ^a ±2.8	4.29±0.11	98.97±4.30	7.18 ^c ±0.17
	25	59.3 ^c ±0.2	9.32 ^c ±0.50	36.94 ^c ±1.39	111 ^b ±4	31 ^b ±2	154 ^b ±13	31.0 ^c ±1.6	53.3 ^a ±10.4	4.37±0.16	97.99±4.16	8.26 ^c ±0.75
	50	60.7 ^b ±0.4	11.70 ^b ±0.30	45.39 ^b ±0.76	121 ^{a,b} ±4	29 ^b ±2	161 ^b ±13	45.8 ^b ±2.4	58.3 ^a ±0.7	4.22±0.32	101.73±4.75	10.57 ^b ±0.46
	100	63.6 ^a ±0.2	17.07 ^a ±0.34	63.44 ^a ±1.41	132 ^a ±4	149 ^a ±13	437 ^a ±54	51.7 ^a ±3.0	59.2 ^a ±0.2	4.01±0.17	100.21±11.58	17.04 ^a ±1.16
	p-value	0.0105	0.0056	0.0055	0.006	0.0065	0.0098	0.0038	0.0099	0.0749	0.6533	0.0073
Firebox- KS Mako	0	58.4 ^d ±0.2	7.61±0.37	30.87 ^b ±1.29	84 ^c ±4	35 ^c ±1	126 ^c ±14	6.1 ^e ±0.4	12.8 ^e ±0.7	4.26±0.18	98.49±6.15	6.87 ^b ±0.44
	5	58.4 ^d ±0.2	8.03±0.49	32.27 ^b ±1.73	91 ^{b,c} ±3	41 ^{b,c} ±6	160 ^{b,c} ±23	7.1 ^{d,e} ±0.1	18.1 ^d ±1.5	4.41±0.31	94.51±1.99	6.42 ^b ±1.04
	10	58.7 ^{c,d} ±0.2	7.78±0.03	31.95 ^b ±0.47	91 ^{b,c} ±2	36 ^c ±3	145 ^c ±7	8.4 ^{c,d} ±1.1	24.9 ^c ±1.2	4.42±0.36	97.01±6.67	7.19 ^{a,b} ±0.34
	15	59.2 ^c ±0.0	8.13±0.50	32.70 ^b ±1.42	84 ^c ±2	34 ^c ±5	132 ^c ±13	9.5 ^c ±0.7	31.6 ^b ±0.8	4.27±0.25	95.79±3.56	6.88 ^b ±0.55
	25	59.3 ^c ±0.2	8.14±1.00	33.68 ^{a,b} ±2.11	92 ^{b,c} ±3	41 ^{b,c} ±4	164 ^{b,c} ±17	27.6 ^b ±0.6	51.5 ^a ±1.3	4.17±0.11	102.77±4.19	6.81 ^b ±0.54
	50	60.5 ^b ±0.2	8.08±0.35	34.47 ^{a,b} ±0.87	97 ^b ±1	50 ^{a,b} ±6	205 ^b ±20	29.7 ^a ±0.4	50.8 ^a ±0.5	4.42±0.28	99.48±2.32	7.70 ^{a,b} ±0.35
	100	62.3 ^a ±0.5	8.47±0.41	36.79 ^a ±1.30	115 ^a ±5	56 ^a ±6	285 ^a ±23	28.9 ^{a,b} ±0.5	48.0 ^a ±3.4	4.49±0.24	98.44±10.72	8.92 ^a ±0.63
	p-value	0.0048	0.5604	0.0032	0.0091	0.0003	0.0115	0.0037	0.0046	0.7372	0.6968	0.0343

Abbreviations: Mixograph (Mixo), Development Time (DDT)

Note: The different groupings are denoted by the superscript lettering at a significance level of p<0.05.

Table 25: Dough parameters used in modeling.

Parameters Considered in Modeling
NIR Flour Parameters
Moisture (%)
Protein (14 MB) (%)
Ash (14 MB) (%)
GlutoPeak Parameters
PMT (seconds)
BEM (GPU)
AM (GPU)
PM (GPU)
Aggregation Energy (GPU)
Total Peak Area (GPU)
Mixograph ML Parameters
Absorption (%)
Peak Time (min)
Peak Height
Peak Width
Peak Integral
MixScore
Alveograph Parameters
P (mm H ₂ O)
L (mm)
W (10 ⁻⁴ J)
Farinograph Parameters
Absorption (%)
Development Time (min)
Stability (min)
Mixing/ Loaf Parameters
Mixing Power (W)
Mixing Time to Power (min)
Dough Properties (very extensible, extensible, good, tight, very tight)
Specific Volume (cm ³ /g)