# MALTING CONDITIONS FOR EVALUATION OF RYE CULTIVARS

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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## ABSTRACT

Malting of rye and use of rye malts presents several challenges to maltsters and brewers, like the lack of a hull and dense packing in steep. While, empirical evidence shows that rye genotypes differ in malting and brewing performance and flavor, there is little published information on the malting of rye or the malt quality attributes of rye genotypes. The objective was to evaluate laboratory micro-malting conditions that could be used in quality screening. Parameters included germination time, moisture and kernel size. Wort arabinoxylan and phenolic acid content were determined in addition to standard malt quality parameters. In general, high extract and lower viscosity were achieved by malting for at least 4 days at 45-48% moisture. However, some commercial maltsters indicated the difficulty of handling of germinating rye at high moisture levels. As such, 5 days of germination at 45% moisture is recommend for future evaluation of rye cultivars.

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"The whole purpose of education is to turn mirrors into windows." -Sydney J. Harris

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# 1. INTRODUCTION

Rye (*Secale cereale* L.) is a cool season cereal grass like wheat (*Triticum aestivum* L.). It is a high yielding crop that can grow even in no-till, less productive fields or in low fertility desert regions (Chmielewski et al. 2000). It has very few insect problems, and it is a strong competitor with weeds (Helm and Schmeiter, 1991). While rye is traditionally used in the production of bread, livestock feed, and spirits (Helm and Schmeiter, 1991), rye malt is gaining increased consideration for its ability to add unique characteristics to beer. These include a "spicy" flavor and "stinging" mouth feel (Wolfe, 2014).

In contrast to ryes' agronomic advantages, the challenge of making rye malt and rye malt beer has been noted by maltsters and brewers. The viscous rye wort makes filtration extremely slow. The high level of soluble arabinoxylan (AX) in rye cell walls contributes to the viscosity of rye wort (Hubner et al. 2010). On the other hand, due to the naked kernel, rye grain presents a dense packing in steep, extreme shrinkage during kilning, and also extends the lautering process. Research on rye malts and use for specialty beers is limited. It is therefore, advantageous to define and qualify the effect of malting methods on rye malt quality.

This research will evaluate process factors on the rye malting process. Particular emphasis will be placed on extract, malt loss, and wort viscosity. The result of this research may give brewers and maltsters, or other researchers a better insight into how to efficiently handle rye grain in malting and brewing.

# 2. LITERATURE REVIEW

## 2.1. Botany and Genetics of Rye

Rye belongs to the grass family, *Gramineae*, and the genus *Secale*. *Secale cereale* L. is the most common cultivated species. There are both winter rye and spring ryes. Generally, winter crops are fall-sown annual crops. Winter rye has better winter hardiness than winter wheat and winter barley. Spring rye, however, can be grown in places where the climate is too severe for winter rye. However, it has poorer yield and end-use quality than winter rye cultivars (Bushuk, 2001). Because rye is a cross-pollinated crop, it is difficult to maintain the genetic purity of cultivars. Hybrid rye varieties, that involve the crossing of inbred lines, were first developed in the 1980's in Germany. Hybrid rye varieties have improved yield, test weight, and intrinsic quality when compared to conventional types (Geiger, 1986; Scoles et al. 2001).

## 2.2. World Production

The winter hardiness of rye and its ability to grow under low fertilization provide a way to confront a global food shortage. However, when compared with the worldwide production of wheat and other cereals, rye is only of minor importance (Bushuk, 2001). Rye is historically indispensable in the farming and eating habits in Northern Europe and several former Soviet Union countries. Europe is the main production region in the world, with as much as 90% of total production from 1993-2013. The top 5 producers in the world are the Russian federation, Poland, Germany, Belarus, and Ukraine (FAOSTAT, 2016). In the USA, the area of rye cultivation has decreased in recent decades (USDA-FAS, 1997), but yields have increased due to improvements in agronomic practices such as the use of fertilizers, crop rotation, and high-yielding cultivars. The increase in rye yield has offset the decrease in cultivated area.

## 2.3. Uses

### *2.3.1. Use in Baking*

The history of rye bread likely goes back to the beginning of rye cultivation. For both historical and health reasons, rye is important in the diets of northern and Eastern Europe. The baking properties of rye have been studied by multiple groups, and were reviewed by Bushuk (2001). The composition of rye presents bakers with several challenges, that include poor gluten strength and a high content of water soluble arabinoxylans. The arabinoxylans make rye dough sticky and difficult to handle. Rye is also often infected with ergot. Ergot scerlotia are produced by a group of fungi in the genus *Claviceps,* can contain alkaloids that cause ergotism in humans and other mammals (Schardl, 2006). The infected grain must be removed before milling or feeding to livestock.

### *2.3.2. Forage and Feed*

Rye is often a feed grain or is used for grazing in countries outside of Europe. Winter rye can be used for extending the grazing season for livestock farmers in some areas. Rye provides forage in late fall and early winter that reduce the cost of stored feed (hay) (Oelke, Oplinger, Bahri, Durgan, Putnam, Doll, and Kelling, 1990).

### *2.3.3. Cover Crop*

Cover crops are used to provide ground cover, protect against soil erosion over the winter time, and to prevent compaction of annually tilled fields. It is common to use ryegrasses or winter rye as cover crops, as the rye root system can grow into late fall. Because rye is the most

winter-hardy crop, once it established well it will provide ground cover protecting against soil erosion (Sullivan, 2002).

### *2.3.4. Rye in Brewing and Distilling*

Rye whiskeys were historically popular in North America because rye was once more widely cultivated, and because of the characteristic spicy or fruity flavor in the final product. America rye whiskeys require use of at least 51 percent of rye grain as well as "rye malt whiskeys", which are produced from a fermented mash of not less than 51 percent of rye malt. For historical reasons the term Canadian whiskey is often used synomously with rye whiskey, even though they may contain no rye grain at all [U.S. Government Publishing Office (GPO), 2008].

Rye ales have thousands of years of history in Europe. Stika (2011) reported that barley, oats, and rye were all used in brewing by the Celts in the early Iron Age through late Medieval times. However, rye had largely fallen out of favor as a brewing grain, with the notable exception being a limited production of roggenbier in Bavaria, Germany. Recently, North American craft brewers have sparked somewhat of a renaissance in rye beers. Draft Magazine reported *just as almost every brewery produces one wheat beer, one pale ale and one stout, now, there's seemingly a rye beer on every beer maker's menu*. (Stambor, 2010). The use of rye grain or malt is associated with spicy and sour-like rye characteristics in the beer flavor profile.

## 2.4. Malting of Rye

Barley is the most common raw material in brewing, and its properties in malting have been extensively studied. On the other hand, there has been limited research on the malting of

rye. Based upon the lack of a hull (husk) it would appear that the malting of rye might be similar to the malting of wheat. Briggs (1998), in fact, mentions in Malts and Malting that the malting of rye follows the same precautions as for wheat. It packs tightly and is easily damaged in malting. Older references cited by Briggs state that although rye steeps rapidly, it is slow to modify, and requires up to 7 days of germination. Pomeranz (et al. 1973) reported than when compared to barley malt, rye malts had higher levels of extract, soluble protein, and alpha-amylase. Taylor (2000) and Maule (1998) reported that compared to barley malt, rye malts resulted in higher viscosity in beers.

Hübner, et al. (2010) evaluated optimal malting conditions for rye in terms of viscosity and other parameters. Variables included germination and steep temperatures, steep moisture and germination time. They found that arabinoxylans (AX) accumulated during the germination process and their extractability increased. The results suggest that longer germination periods resulted in an increased number of AX molecules with lower molecular mass. High wort viscosity is caused by water extractable AX. They indicated the optimal rye malt qualities within the limits of their study were found for a germination time of 6 days at 10 °C. These conditions resulted in an acceptable FAN levels, with the lowest measured viscosity. They also found that with long germination periods, amylolytic and proteolytic enzyme activities were increased, while β-glucanase activity wasn't influenced. Total and soluble nitrogen content were also not significantly affected by the variations in germination conditions. Free amino nitrogen (FAN) was found in higher amounts in worts prepared from rye malts with long germination times. Extract contents were higher in rye malt than in the control barley malt and could be increased by a favorable germination regime while no such impact on wort fermentability was found.

## 2.5. Chemical Composition of Rye

Composition and chemistry had been studied to understand the properties of rye. Based on former studies rye grain has been reported to contain 9%-15 % protein, 57-66% starch, 14 to 20% dietary fiber, and 1.6-2.2% ash (Seibel, 2001; Nilsson, 1997, Bushuk, 2001; Hansen et al., 2003, 2004). However, the composition of rye may vary between reports because of environmental and genotypic influences (Hansen et al., 2004; Békés et al., 2016).

### *2.5.1. Protein*

Rye proteins have been described in numerous studies. Bushuk (2001) divided these proteins into three groups including storage proteins which are located in protein bodies, storage nitrogen, and sulfur and carbon skeletons. The storage proteins found in the endosperm are called secalins. The 7S globulins are the main storage protein in the embryo and aleurone cells. Another protein group is hydrolytic enzymes and their inhibitors, like α-amylases and α-amylases inhibitors, β-amylase, proteinases, esterases, β-glucosidase, and β-galactosidase. A final group of proteins are the resistance-related proteins, which are involved in defense against pests and pathogens.

Although rye has a close relationship with wheat, it has low gluten content. Rye prolamins are referred to as “secalin”, which is the major storage protein of rye grain. Unlike gliadins and glutenins which can form gluten in wheat, it is difficult to prepare a cohesive mass from rye doughs. Secalins lack the cohesiveness of gluten (Gellrich 2003, 2004).

### *2.5.2. Starch*

Starch is the main source of energy in rye. Total content and structure is similar to other cereals like wheat. It consists of amylose and amylopectin, where amylose represents 24-26% of the total amounts (Hew and Unrau, 1970; Berry et al, 1971). The viscosity of rye starch is similar to bread wheat and durum wheat. However, sprouting can be an issue in rye, and lower paste viscosities are seen because of the presence of α-amylase (Berry, et al., 1971, Klassen and Hill, 1971).

### *2.5.3. Cell Wall Polysaccharides*

The non-starch polysaccharides found in cell walls are dietary fiber. The whole grain content of rye is about 17% of dietary fiber, including 3-4% soluble fiber. Arabnoxylans are a major component, and were formerly referred to as pentosans (Bushuk, 2001).

### *2.5.4. Beta-glucan*

Whole grain rye, contains 2.5% beta-glucan, compared to 1.7% in the endosperm. This indicates high levels of beta-glucan in the bran fraction (Bushuk, 2001). Overall numbers are lower than those for barley and oats (Ragaee et al. 2008).

### *2.5.5. Arabinoxylans*

Like beta-glucan, AX levels also are higher in the bran than in the endosperm (Ragaee et al. 2008). Rye has considerably larger amounts of AX than barley, especially water extractable arabinoxylans (WEAX), which are mainly responsible for the increased viscosities. Rye AX structure and its properties have been studied by Vinkx and Delcour (1996), and contribute

between 6.5-12.2% of the total dry weight in rye kernels. AX have a backbone of β -1 → 4 linked xylanopyranose units. Alpha –arabinofuranose can be linked to the C (O)-2 and/or C (O)-3 positions of the xylose units. Some of the arabinose substituents are esterified with ferulic acid at C (O)-5 (Bengtsson et al., 1992).

AX represents a large part of the rye's content of dietary fiber and especially soluble dietary fiber, which is known to have health benefits for the consumer. From a technological point of view, soluble dietary fiber, among other substances like proteins, contribute to body and mouthfeel of the beer. Lu et al. (2000, 2004) described in two studies on the positive effects of AX on postprandial insulin response in healthy individuals and improved metabolic control in diabetes patients. AX breakdown products have also been shown to display prebiotic properties (Cloetens et al.,2008; Courtinetal.,2008; Grootaert et al.,2007; VanCraeyveld et al.,2008).

While there are some positive aspects attributed to AX, they can cause problems in beer production. Properties such as molecular mass, the degree of arabinose branching, and the degree of esterification with ferulic acid will influence AX properties dramatically (Li et al. 2005). WEAX increase the viscosity of solutions as they can bind large amounts of water. Gels can be formed by oxidative cross-linking of AX macromolecules via the ferulic acid residues. During germination of rye kernels, AX is degraded by a number of enzymes. The most important are the endo-xylanases, which form a larger number of AX molecules with shorter chain length by cleaving internal linkages in the main xylan chain. β –Xylosidases slightly decrease the chain length by releasing xylose residues from the non-reducing end. Arabinose molecules can be released from the macromolecules by α-L-arabino furanosidase, while the actions of feruloyl esterase can hydrolyse the linkage between ferulic acid and arabinose residues (Grootaert et al, 2007).

### *2.5.6. Phenolic Acids*

Phenolic compounds are usually studied in subgroups, like phenolic acids, flavonoids, isoflavonoids, lignans, stilbenes, and polyphenols (Dewick, 2001). Rye phenolics were reviewed by Bondia-Pons et al. (2009), as their antioxidant activities offer potential health benefits. However, food processing or other biological effects can make the antioxidant action become more complex (Frankel and Meyer, 2000a). Ferulic acid (85%-90%), sinapic acid (9%-10%), and para-coumaric (3%-5%) have been reported to be the most abundant phenolic acids in rye grain (Bondia-Pons, 2009). There is small amount of syringic, caffeic, vanillic and para-hydroxybenzoic acids present in the rye kernel as well. In terms of milling fractions and products, the levels of phenolic acids have been reported to decrease as follows: rye bran> rye grain> rye flour> rye bread. Katina et al. (2007) fermented both native and germinated rye grains. They found that, during fermentation, yeast fermentation plays the major roll for a 10-fold increase of free ferulic acid in both experimental groups. However, there were no reports about phenolic acid bioactivities in rye malt or wort.

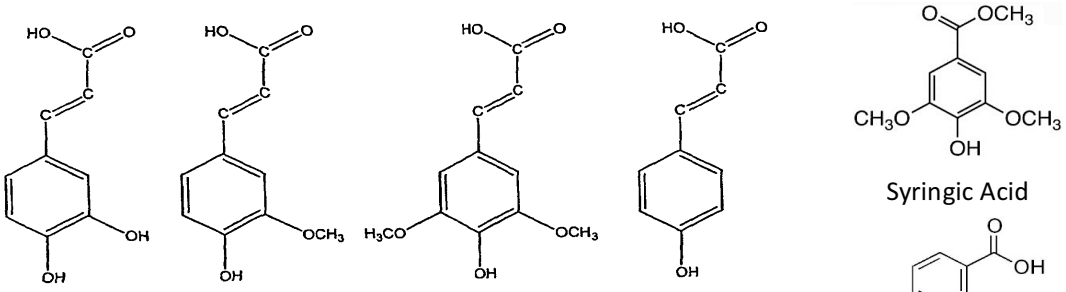


Fig. 1. Chemical Structures of the Main Phenolic Acids in Rye Wort

## 2.6. Beer Flavor

Rye malt contributes a distinctive flavor, body and mouthfeel to the beer. The unique spicy flavor of rye has drawn attention in the craft beer market. The extent of its contribution to the beer flavor profile is affected by the malted grain and several other factors in the brewing process (Pomeranz et al. 1973).

Roggenbier, is the historical beer that was originally brewed in Regensburg, Germany [Beer Judge Certification Program (BJCP), 2015]. Pople often partition wheat and rye together into German Wheat and Rye Beer category as the close relationship between rye and wheat beer BJCP (2008). BJCP (2008, 2015) introduced Roggenbier in the overall impression as *a dunkelweizen made with rye rather than wheat, but with a greater body and light finishing hops*. More than 50% of malted rye (sometimes up to 60-65%) can be used during mashing. Pale malt, Munich malt, wheat malt, crystal malt and/or small amounts of debittered dark malts for color adjustment were been used as the remaining grain. Distinctive banana esters and clove phenols character were provided by Weizen yeast. Usage of Saazer-type hops in bitterness, flavor and aroma is light. Other beverages that use rye as ingredient are traditional Scandinavian or Russian beers such as Kvass, Gotlandsdricka, and Sahti (BJCP, 2015).

Unlike Roggenbier, American rye beer has less yeast flavor and more hop character (BJCP, 2015). The craft brewers and homebrewers in the US make seasonal Rye IPAs, Rye PAs, or RIPAs as their specialty IPA. These rye beers have drier and slightly spicier than an American IPA, and usually have clean or slightly fruity profile. American or New World hops and American or English yeast were also used by craft brewers and homebrewers, with 15-20% rye malt and pale ale or 2-row brewers malt as the base of the grain bills.

People usually change the rye beer flavor by adjusting the percentage of rye malt or exchange the adjunct ingredients. There is not much information about the effect of genotype on rye beer flavor. It would not be a big problem for homebrewed beer, but it is a considerable problem for microbrewers, as the concern of consistency of the final product between batches. Thus, the flavor profile should be involved in the future studies.

## 3. OBJECTIVE

The objective of this research was to evaluate laboratory micro-malting conditions that could be used in the quality screening of rye genotypes. Ideal conditions should achieve high extract with minimal malt loss, and lower wort viscosity/arabinoxylan content.

# 4. EXPERIMENTAL APPROACH

## 4.1. Materials

Upon initiation of the rye malt research project, samples were obtained from Cornell University, the University of Minnesota-Crookston, the NDSU Carrington research extension center, the Oklahoma Seed Foundation (Ardmore, OK), and several commercial or farm sources. All samples (n=48) were screened for grain plumpness, germinative capacity, and a number of other grain quality aprameters (appendix tables A1-A5).

## 4.2. Methods

### *4.2.1. Rye Quality Tests*

#### *4.2.1.1. Test Weight*

Test weight (kg/hL) was determined on cleaned samples, after removal of the dockage [U.S. Department of Agriculture (USDA),2013].

#### *4.2.1.2. 1000 Kernel Weight and Kernel Assortment*

One thousand kernel weight was measured on cleaned samples, by determining the number of kernels in a 10.0g sample. Kernel assortment was performed according to the American Society of Brewing Chemists (ASBC) Method Barley-2C (ASBC, 2009). Kernel size distribution was determined with 2.8, 2.4, and 2.0 x 19mm (7”/64”, 6”/64”, and 5”/64” x 3”/64”) sieves on a Eureka-Niagra Barley Grader (Silver Creek, NY). Rye grain (100g) was spread on the top screen and shaken for 2 min. Kernels remaining on the 2.8 and 2.4 mm sieves were considered plump kernels and kernels retained on 2.0 mm sieve were considered medium kernels. Kernels passing through the 2.0 mm sieve were considered thin. In this experiment,

plump and medium kernel fractions were saved for further analysis, while thin kernels were removed.

#### *4.2.1.3. Grain Moisture*

Grain samples were ground using a Perten LM 3600 disc mill (Perten Instruments. Hägersten, Sweden). Grain moisture was determined with a Brabender Moisture Tester (C. W Brabender Corp. Rochelle Park, NJ) by heating a ground sample for 1 hr at 130$^{o}$C.

#### *4.2.1.4. Protein*

Rye grain protein content was determined using a FOSS 1241 NIR (FOSS in North America, Eden Prairie, MN) using the calibration supplied with the instrument. The accuracy of results was crossed-checked, by determining nitrogen on LECO FP 528 nitrogen analyzer (LECO Corporation, St. Joseph, MI) with the nitrogen factor of 6.5 (LECO FP 528 Application Note, 2017). NIR analysis was on whole grain, while samples for combustion analysis were ground to pass a 0.5 mm screen on a UDY mill (UDY Corp., Boulder, CO).

#### *4.2.1.5. Germinative Capacity*

Germinative capacity was determined by ASBC Barley Method-3B (2009). The percent chitted kernels after 72 hours were recorded as the germinative capacity.

#### *4.2.1.6. Determination of Deoxynivalenol (DON)*

DON was determined based upon the method introduced by Tacke and Casper (1996). Samples were ground using Perten laboratory mill (model 3600, Perten Instruments. Hägersten,

Sweden), and weighed (2.5g) into 50 mL polypropylene tubes. Extraction was with20 ml of 84% acetonitrile/water solution for 60 minutes on a horizontal shaker. After settling, a 2 mL aliquot of the supernatant was transferred to a column containing 1g of 50/50% C18/alumina. The supernatant (2 ml) was gravity filtered, transferred to a 5 ml disposable borosilicate glass culture tube (47729-570, VWR CO), and dried under nitrogen gas. The dried sample was derivatized using trimethlysilayamidazole (TMSI), trimethylchlorosilane (TMCS) 10:1. The derivatized samples were analyzed by gas chromatography with electron capture detection (GC ECD) (model Agilent 6890 GC ECD, Santa Clara, CA).

The samples (1 μL) were injected in duplicate onto a 5% phenyl methyl siloxane column (30 m × 0.25 mm × 2 μm) (Agilent HP-5). A polarity deactivated column (1-2 m × 0.53 mm) (Restek. Bellefonte, PA) was attached as a guard. The system parameters were as follows: the carrier gas was helium; flow pressure: 1.38 bar; the initial inlet temperature was 90°C, and was then ramped at a rate of 20°C/min to 300°C; initial oven temperature 70°C and increased to 170°C at a rate of 25°C/min, and then at 5°C/min to 300°C. Detector temperature was held at 300°C with $ArCH_4$ at 60mL/min. Mirex (ULTRA Scientific, Kingstown, RI) was the internal standard (0.5 mg/mL). A standard curve from 0.1 to 40 ng/μL was prepared by spiking the standard (Biopure, Romer Lab Inc., Union, MO) into a DON free barley extraction. DON results are shown in appendix table A5.

*4.2.1.7. Preharvest Sprouting*

Preharvest sprouting was determined using the stirring number test on a Rapid Visco-Analyzer (Newport Scientific Pty Ltd, Werriewood, New South Wales, Australia) according to AACC method 22-08.01(2000). Rye was ground to pass a 0.5 mm screen in UDY mill (UDY Corp., Boulder, CO) and 4.0 g of flour were mixed with 25g distilled water. The stirring number

test was conducted under the following conditions: 95°C for 3 minutes stirring at 160 rpm, with the initial high speed for 10 seconds. Stirring number (SN, viscosity cP at 3 minute) iss reported in the appendix tables A3 and A4.

### *4.2.2. Malt Analysis*

#### *4.2.2.1. Pilot Malting*

The steeping time each rye sample required to reach 40%, 45%, and 48% moisture was determined by pilot-steeping a 10 g (dry basis) sample according to the procedures of Banasik et al. (1955). Samples were steeped at 16°C in 50 ml perforated round-bottom centrifuge tubes (3122-0050 (Nalgene, Rochester, NY). The samples were removed from steeping after 24, 48, 72 hr and centrifuged at 1500 rpm for 2 min to remove surface moisture, and then weighed. At each time interval, the moisture of steeped sample was calculated. The time required to reach the moisture level (40%, 45%, and 48%) was calculated by plotting log moisture against log time.

Micro-malting was carried out according to method described by Karababa et al (1993). Samples (80 g, dry basis) were steeped for the time determined by pilot-steeping. Steeping includes aeration every four hours for six minutes, and the water is drained every 12 hr., and the samples air-rested for 1 hr. After steeping, the samples were removed from the steep tank, spread over paper towels to eliminate surface water on the grain. Samples were then weighed, and adjusted to desired weight using distilled water. Weights for 40, 45, and 48% moisture levels were 133, 145, and 154 g, respectively.

Samples were placed into 400 mL beakers, germinated for either 3, 4, 5, or 6 days in the germination cabinet at 16°C, and approximately 95% relative humidity. Samples weight was

adjusted every day by adding distilled water, and samples were had hand-turned to preventing matting.

After the completion of germination, all samples were moved into kiln containers. Kilning was a 24-hr schedule, where the temperature was sequentially ramped from 49 to 85$^{o}$C. After kilning, the samples were removed from the kiln, cooled to room temperature and de-rooted by abrading against each other when rubbed by hands. Cleaned samples were weighed and stored at room temperature prior to analysis.

*4.2.2.2. Malt Moisture*

Malt moisture was determined on a coarsely ground 10 g sample with a semi-automatic Brabender Moisture Tester (Brabender Corp., Rochell Park, NJ), heated according to ASBC Malt-3 (2009).

*4.2.2.3. Malt Loss*

Malt loss represents the loss of solubles and $CO_2$ during germination, and the removal of rootlets following kilning. Malt loss was calculated as:

$$\% \text{ Malting loss} = \frac{[\text{Barley wt (d.b.)} - \text{Clean malt wt (d.b.)}] \times 100\%}{\text{Barley wt (d.b.)}}$$

d.b.: Dry basis

wt: Weight

*4.2.2.4. Fine Grind Malt Extract*

Fine grind malt extract was determined according to a modification of ASBC Malt Method (2009), Malt-4. The major modification is that rye samples were centrifuged at 3000 x g

prior to filtration (20 $^{o}$C, 15 minutes).

*4.2.2.5. Malt Diastatic Power*

Malt diastatic power was determined as described in Technicon Industrial Method No. 424-76A (Bran and Luebbe, Inc. Tarrytown, NY). Three malt flour samples of known diastatic power were analyzed with each set of samples. The standard samples were used to prepare a plot of Technicon Autoanalyzer peak height vs. diastatic power. The diastatic power of the standards malts were determined through collaborative testing conducted by the American Society of Brewing Chemists.

*4.2.2.6. Alpha-Amylase Activity*

The alpha-amylase activity of malt was determined according to a modification of the procedure of Banasik (1971) on a Technicon Autoanalyzer, as described in Technicon Industrial Method NO. 424-76A (Bran and Luebbe, Inc. Tarrytown, NY). Three malt flour samples of known alpha-amylase activity were analyzed with each set of samples. The standard samples were used to prepare a plot of Technicon Autoanalyzer peak height vs. alpha-amylase activity. The alpha-amylase activity of the standards was determined according to ASBC Method (2009), Malt-7.

***4.2.3. Wort Analysis***

*4.2.3.1. Wort Soluble Protein*

Wort soluble protein was determined according to ASBC Method (2009), Wort-17.

*4.2.3.2. Kolbach Index (Soluble /Total Protein)*

Kolbach Index was calculated as:

$$\text{Kolbach Index} = \frac{Wort\ Soluble\ Protein \times 100\%}{Grain\ Total\ Protein}$$

Wort soluble protein was determined according to ASBC Method (2009), Wort-17.

*4.2.3.3. Wort Viscosity*

Wort viscosity was determined at 20$^{o}$C according to ASBC Method (2009), Wort -13A.

*4.2.3.4. Wort Carbohydrates*

Wort carbohydrates were determined by high-performance liquid chromatography (HPLC) method using an Aminex HPX-87 column (Catologh No. 125-0095, Bio-Rad Laboratories, Hercules, CA) according to ASBC Method (2009), Wort-14B.

*4.2.3.5. Free Amino Nitrogen (FAN)*

FAN was determined according to ASBC Method (2009), Wort-12.

*4.2.3.6. Arabinoxylans (AX)*

The arabinoxylan content of wort was determined by gas chromatography according to the modified method of Carpita and Shea (1989). A 100 µl aliquot of each rye wort sample was derivatized to alditol acetates according to the method of Blakeney et al. (1983) with some modifications. The frozen wort samples were thawed at 25°C and 100 µl was added to a screw cap tube (16 x 125mm). The samples were hydrolyzed using 4.17M trifluoroacetic acid (1500 µl)

by heating at 121°C 1 hr. After hydrolysis, the internal standard (inositol) was added to each sample and the samples were dried at 55°C under nitrogen. The samples were then reduced by adding ammonium hydroxide (1M, 100µl) and sodium borohydride in DMSO (20mg/ml, 500µl). After heating at 40°C for 90 minutes, 6 drops of glacial acetic acid were added to each tube. To acetylate the samples, 100 µl 1-methylimidazol and 500 µl acetic anhydride were added to each tube and the reaction was stopped after 10 minutes with the addition of 4ml of water. The samples were then partitioned with 1 ml of methylene chloride two times, and the methylene chloride fractions were combined and dried at 45 °C under nitrogen. The samples were finally redisolved in 1 ml of acetone and placed in2 mL auto-sampler vials (Agilent Technologies) for GC analysis. The samples were analyzed with an Agilent 7890 gas chromatograph (GC) with flame ionization detector (Agilent technologies, Santa Clara, CA). The samples (5 µl) were injected in duplicate onto a Supelco SP-2380 fused silica capillary column (30 m × 0.25 mm × 2 µm) (Supelco Bellefonte, PA, U.S.A.). The system parameters were as follows: flow rate, 0.8 mL/min; flow pressure, 82,737 Pa; oven temperature, 100°C; detector temperature, 250°C; and injector temperature, 230°C. The carrier gas was helium (Mendis et al. 2013). A standard curve was prepared that contained monosaccharide standards in the concentrations of 250, 500, 750 and 1000 ng/ul and inositol was added at 750 ng/ul as an internal standard. Arabinoxylan content was calculated as:

$$\boldsymbol{Arabinoxylan\ (mg/L)} = \frac{[((\mathbf{Arabifnose\ ng/ul + Xylose\ ng/ul}) * \mathbf{0.88})) * \mathbf{1000}]}{\mathbf{100}}$$

*4.2.3.7. Phenolic Acids*

The phenolic acid content of wort was determined according to a modification of method reported by McMurrough et al. (1984). Congress wort samples (5 mL) were adjusted to a pH of

2.0 by adding 2.0*M* HCL. Samples were then extracted twice by shaking vigorously for 60 minutes with hexane (5mL). The mixtures were centrifuged for 5 minutes at 3000 x g after extraction, and the organic phase was discarded. The remaining aqueous phases were extracted three times with ethyl ether/acetate (1:1, v: v) (5mL). After vigorously shaking for 15 minutes, the mixtures were centrifuged (3000 x g) for 5 minutes. The pooled ethyl ether/acetate extracts were dried under nitrogen gas. Acetonitile (0.5 mL) was added and the samples were filtered through a Whatman-40 filter (Whatman, UK) into 2.0 ml amber (Agilent Technologies,). These concentrated samples were then analyzed on an Agilient 1290 series liquid chromatography with a 6540 UHD Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS (Agilent Technologies, Santa Clara, CA). Separation was performed on a ZORBAX SB-C18 column (1.8 μm, 2.1 × 50 mm, Agilent, Santa Clara, CA, USA) at 30℃. The mobile phase consisted of water containing 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). Gradient conditions were as follows: 0-1 min isocratic with 3% B; then a linear increase from 3 to 97% B for 1-10 min; followed by an isocratic washout step for 5 min and shifting back to initial setting for 2 min. Flow rate was 0.4 mL/min, and injection volume was 2.0 μL.

Detection was (diode array detector) was carried out by scanning the absorption between 250 to 400 nm with a step of 2.0 nm. The wavelengths were 260.0 nm, 275.0 nm, 294.0 nm and 324.0nm with the band width of 2.0 nm. The AJS electrospray ionization interface (ESI) interface was used in the positive mode, and the absorbance threshold of the 21 entroid data storage was 200 (Rel. 0.01%). The stop time was 15 min and cycle time 0.5 s. Source parameters were set as follows: drying gas at the temperature of 300℃ with the flow rate of 10 L/min; 30 psig nebulizer gas at 300℃ and 7 L/min; and 125 V fragmentor energy. The mass range (m/z) of TOF Spectra was 100-1000, and the acquisition rate and time were 2 spectra/s and 5

ms/spectrum, respectively. The m/z of reference masses were 121.0509 and 922.0098. Ferulic acid, p-coumaric acid, vanillic acid, sinapinic acid, caffeic acid, catechin, syringic acid and gallic acid were quantitated with their hydrogen adduct of m/z 195.0654, 165.0545, 169.0494, 225.0756, 181.0494, 291.0882, 199.0600 and 171.0287, respectively.

The calibration curves were prepared by spiking phenolic acids standards into the extract, and the response area was calculated by deducting that in control extract. The limit of detection (LOD) and quantification (LOQ) for Ferulic acid, p-Coumaric acid, Sinapinic acid, Caffeic acid and Catechin were 0.02 µg/100 mL (0.02 µg/mL wort) and 0.1 µg/100 mL (0.1 µg/mL wort), respectively. For Vanillic acid, Syringic acid and Gallic acid, LOD and LOQ were 0.05 µg/100 mL (0.05 µg/mL wort) and 0.2 µg/100 mL (0.2 µg/mL wort), respectively.

*4.2.3.8. Wort Beta-Glucan*

Wort beta-glucan was determined according to adjusted ASBC Method (2009), Wort-18B. Wort samples were placed in an autosampler (Waters Corporation, Milford, MA). The flow rate was 3.0 mL/min with Waters 515 HPLC Pump (Waters Corporation, Milford, MA). Waters 474 Scanning Fluorescence Detector (Waters Corporation, Milford, MA) set to 420 nm emission and 365 nm excitation wavelengths.

### *4.2.4. Experimental Design and Statistical Analysis*

This study was designed according to a randomized complete block design (RCBD) with five factors in a factorial arrangement. The factors were 2 levels of sample, three levels of steep moisture (40%, 45% and 48%), four levels of germination days (3, 4, 5, and 6 days), and 2 levels of kernel size (plump and medium). Malting was replicated (n=2). Data was analyzed by

Analysis of Variance (ANOVA), performed with procedures of the Statistical Analysis System (version 9.3, SAS Institute, Cary, NC), and analyzed using interactions. Main effects and interactions were evaluated using the general linear models (GLM) procedure. The sources of variation for germination days and germination moisture were portioned into single degree of freedom polynomial contrasts. Duncan's multiple range test was used to compare treatment means. Stepwise regression was used to evaluate how much variability could be explained by each independent variable (e.g. sample, kernel size, germination moisture, days of germination) for the dependent variable (e.g. malt loss, extract, DP, α-amylase, soluble protein, FAN, wort viscosity, AX, β-glucans, fermentable sugars, and polyphenolics).

## 5. RESULTS AND DISCUSSION

Upon the initiation of this project, the first task was to identify rye grain samples for study. As mentioned in the materials section, samples were obtained from small grains programs at Cornell University, North Dakota State University, the University of Minnesota, and the Oklahoma Seed Foundation. In addition, several samples were obtained from maltsters and growers, in cooperation with the North American Craft Maltsters Guild. A total of 48 samples were obtained the initial quality screening and results are shown in appendix tables A1-A5. Cultivars included forage types, and both conventional and hybrid grain types. The samples exhibited a wide range in kernel plumpness (1.2-90.0%, mean= 48.2), 1000 kernel weight (15.2-37.7 g mean = 26.9g), protein (7.6-19.6%, mean = 11.6%), and germination (72.5-100%, mean=93%). There was also a range in determental characters including deoxynivalenol (0.0-3.0 mg/kg, mean= 0.40 mg/kg), and preharvest sprouting (stirring number 19.4-172.2 sec, mean= 118.5 sec). Examination of appendix tables A1-A5 shows that the forage types generally had higher protein (mean=15.8), lower plumpness (mean= 12.5%), and low 1000 kernel weight (mean=20.8 g). As such, grain form the forage types was considered unacceptable for the production a conventional rye malt. The current study also required several kilograms of sample when treatments and replications were considered. Two rye samples were selected for further study based upon germinative capacity, kernel plumpness, and adequate sample amount. These were the genotype DR02 (ND Dylan) and an unidentified genotype of winter rye. DR02 was grown at the NDSU Carrington research extension center in 2014, and had acceptable plumpness (63.1%), protein (12.7%), and germination (96.5%). The unidentified sample was from the 2014 crop in Iowa, and was obtained from Embden Grain in Embden, North Dakota. The plumpness (42.1%) and protein (10.9%) were acceptable, but germination was slightly less than optimal

(91%). As the design of this study cannot be used to determine variety effects, DR02 and the Iowa sample will subsequently be referred to as samples A and B, respectively.

### 5.1. Statistical Interpretation

The statistical significance of the parameters was determined by ANOVA. Interactions are discussed prior to main effects, as a statistically significant interaction indicates the results for a certain analytical parameter may not have responded uniformly across a combination of factors (e.g. sample, germination moisture level, etc.). However, while statistically significant, some interactions may not be a "true" interaction, as the rank of one factor may have remained the same across all levels of treatments. In these cases, the significance is usually caused by differences in the magnitude of responses to different treatments. For example, while the interaction of germination time × germination for malt loss, was statistically significant, examination of Figure 2 shows that the trends were similar for each moisture level and ranks did not change. However, the magnitude of responses across the germination moisture times was different. Not all interactions are included in the subsequent discussion. Only 2-way interactions are shown in figures 1-28. The factor in the ANOVA table had been participate into separate single degree of freedom comparisons. The number of comparisons is equal to the number of levels of a factor minus one. For example, in table 2, in the malt loss analysis, there were 3 levels of the factor of germination moisture, then there were two comparisons, linear and quadratic. This analysis shown the significance of the linear, quadratic, and/or cubic effects for these treatments.

### 5.2. Malt Loss

Malt loss reflects the amount of material lost in converting grain into malt, and is an important economic consideration to the maltster. When considered on a dry basis, malt loss can be attributed to respiration losses, loss of soluble material in the steep, and the removal of rootlets. In the current study, malt loss was significantly affected ($P \leq 0.05$) by all malting operational parameters except kernel size (Table 1, Table 2). The interaction of germination time × germination moisture was also significant, but as previously discussed this was due to differences in magnitude of malt loss at different moisture levels, when considered across germination times (Figure 2). Although the relative rank was the same across times, the difference in malt loss between days increased as the germination moisture level changed from 40% (3.0%), 45% (6.9%), and to 48% (7.9%). This indicates the losses increase with time, but became more pronounced at a higher germination moisture levels. The response of malt loss to germination moisture was not linear, as indicated by the significant quadratic contrast ($P < 0.0001$) (Table 2). However, response of malt loss to germination time was linear ($P<0.0001$). Sample was significant, but had a relatively small impact on malt loss (Table 1). The average loss of sample A was only 0.67% higher than that for sample B.

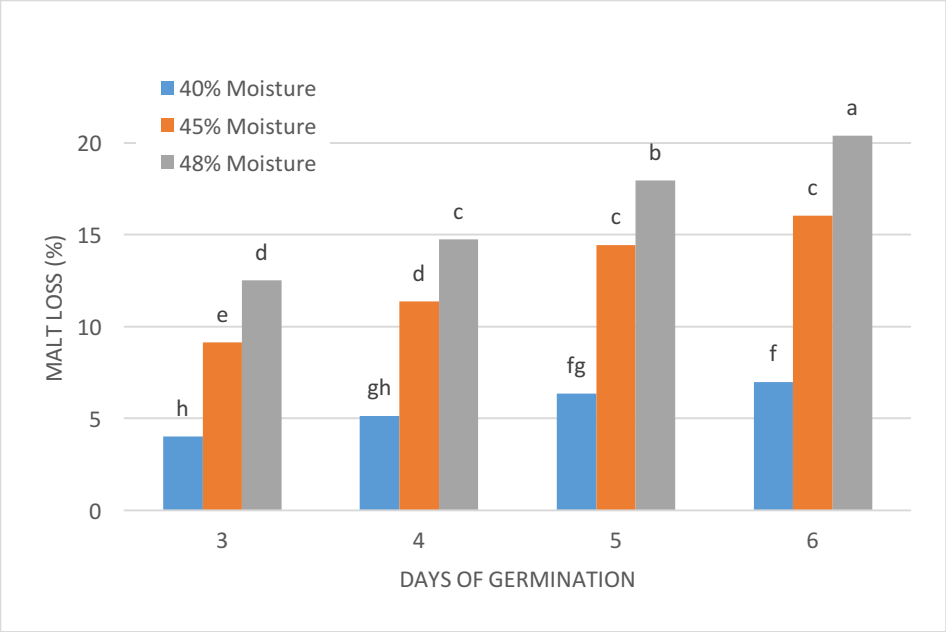


Fig. 2. Interaction of Germination Moisture Level and Germination Time on Malt Loss. Columns within each moisture level denoted by the same letter are not significantly different ($P \leq 0.05$).

**Table 1. Mean of Rye Malt Quality Values as Affected by Malting Operational Parameters [a]**

| | Malt Loss (%) | Extract (% Malt, db) | Diastatic Power (°ASBC) | Alpha-Amylase | Wort Soluble Protein (% Malt, db) | S/T (%) | Viscosity (cP) | FAN (mg/L) | AX (mg/L) | A/X Ratio (%) | β-Glucan (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | | | | | | | | | | | |
| A | 11.92 a | 84.02 a | 131.83 a | 83.20 a | 8.15 a | 64.16 a | 5.23 a | 223.02 a | 3791.50 a | 85.23 a | 68.61 a |
| B | 11.25 b | 87.08 b | 115.19 b | 88.59 b | 7.19 b | 65.93 b | 4.47 b | 221.79 a | 3849.21 a | 88.13 a | 59.43 b |
| **Germination Time** | | | | | | | | | | | |
| Three day | 8.55 a | 84.98 a | 117.75 a | 69.27 a | 7.46 a | 63.41 a | 5.37 a | 217.09 a | 3579.73 a | 88.92 a | 113.18 a |
| Four day | 10.41 b | 85.69 b | 121.46 ab | 80.62 b | 7.68 b | 65.15 b | 4.98 b | 223.41 ab | 4112.21ab | 87.65 a | 66.89 b |
| Five day | 12.91 c | 85.66 b | 129.87 b | 93.45 c | 7.68 b | 65.06 c | 4.59 c | 227.05 b | 3860.13 b | 84.43 a | 43.11 c |
| Six day | 14.47 d | 85.90 b | 124.96 c | 100.23 d | 7.85 b | 66.55 d | 4.46 c | 222.06 ab | 3729.34 b | 85.72 a | 32.89 d |
| **Grain Size** | | | | | | | | | | | |
| Plump | 11.52 a | 85.93 a | 123.41 a | 83.24 a | 7.92 a | 67.17 a | 4.83 a | 230.58 a | 3823.27 a | 84.82 a | 63.66 a |
| Medium | 11.65 a | 85.18 a | 123.61 a | 88.55 b | 7.42 b | 62.91 b | 4.87 a | 214.23 b | 3817.43 a | 88.54 a | 64.38 a |
| **Moisture** | | | | | | | | | | | |
| 40% | 5.61 a | 85.87 a | 108.17 a | 71.30 a | 7.61 a | 64.71 ab | 5.36 a | 215.91 a | 3907.19 a | 85.12 a | 136.13 a |
| 45% | 12.74 b | 84.75 a | 108.97 a | 82.81 b | 7.79 b | 66.10 b | 4.76 b | 211.03 a | 3834.16 a | 90.51 a | 40.19 b |
| 48% | 16.40 c | 86.05 b | 153.39 b | 103.57 c | 7.61 a | 64.31 a | 4.43 c | 240.28 b | 3719.70 a | 84.41 a | 15.74 c |

[a] Means followed by the same letter are not significantly different (P≤0.05).

**Table 2. Analysis of Variance for Malt Loss and Extract**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Malt Loss** | Sample (V) | 1 | 10.65 | 11.28 | 0.0013 |
| | Size (S) | 1 | 0.39 | 0.41 | 0.5219 |
| | Germination Moisture (G_M) | 2 | 963.55 | 1020.53 | <0.0001 |
| | Linear G_M | 1 | 1862.97 | 1973.12 | <0.0001 |
| | Quadratic G_M | 1 | 64.15 | 67.94 | <0.0001 |
| | Germination Days (G_D) | 3 | 165.34 | 175.11 | <0.0001 |
| | Linear G_D | 1 | 492.53 | 521.66 | <0.0001 |
| | Quadratic G_D | 1 | 0.51 | 0.54 | 0.4657 |
| | Cubic G_D | 1 | 2.97 | 3.14 | 0.0806 |
| | V*S | 1 | 0.96 | 1.02 | 0.3166 |
| | V*G_M | 2 | 0.38 | 0.40 | 0.6714 |
| | S*G_M | 2 | 0.28 | 0.30 | 0.7423 |
| | V*G_D | 3 | 0.57 | 0.60 | 0.6176 |
| | S*G_D | 3 | 0.61 | 0.64 | 0.5895 |
| | G_M*G_D | 6 | 10.90 | 11.55 | <0.0001 |
| | Error | 70 | 1.03 | | |
| **Extract** | Sample (V) | 1 | 208.88 | 122.54 | <0.0001 |
| | Size (S) | 1 | 12.62 | 7.41 | 0.0083 |
| | Germination Moisture (G_M) | 2 | 13.75 | 8.07 | 0.0007 |
| | Linear G_M | 1 | 0.51 | 0.30 | 0.5875 |
| | Quadratic G_M | 1 | 27.00 | 15.84 | 0.0002 |
| | Germination Days (G_D) | 3 | 3.18 | 1.86 | 0.1440 |
| | Linear G_D | 1 | 7.88 | 4.63 | 0.0351 |
| | Quadratic G_D | 1 | 1.24 | 0.73 | 0.3969 |
| | Cubic G_D | 1 | 1.22 | 0.72 | 0.4007 |
| | V*S | 1 | 0.19 | 0.11 | 0.7366 |
| | V*G_M | 2 | 22.26 | 13.06 | <0.0001 |
| | S*G_M | 2 | 1.01 | 0.59 | 0.5551 |
| | V*G_D | 3 | 2.16 | 1.27 | 0.2932 |
| | S*G_D | 3 | 3.21 | 1.88 | 0.1411 |
| | G_M*G_D | 6 | 2.67 | 1.56 | 0.1714 |
| | Error | 70 | 1.70 | | |

### 5.3. Malt Extract

Malt extract was significantly affected ($P \leq 0.05$) by cultivar, germination time, and germination moisture level (Table 1). The interactions of sample × germination moisture, sample × germination time, and germination moisture × germination time were also significant (Table 2). The response of extract to germination moisture was non-linear response (Table 2) and appears to be due to the low extract of sample A at 45% moisture. Figure 3 clearly shows the different responses of two samples across germination moisture levels. Compared with sample A, sample B had a relatively high extract at each germination moisture level. However, the extract of sample A was significantly lower at 45% than at either 40% or 48%. These reason for these results is not clear, but experimental error should not be ruled out.

By contrast, the model indicatesthat the overall response of extract to germination was linear (Table 2), and as expected extact increased with germination time (Table 1). However, differences were only significant between days 2 and days 4-6. The samples also did not respond uniformly to germination time (Fig. 4). Increasing germination time helped sample A achieve higher extract from 3 to 4 days, while the differences for sample B across times were minimal. Examination of Figure 5 shows that high levels of extract were achieved at four days of germination with 40 and 48% moisture levels, and did not changed greatly thereafter. Again the behavior at 45% is unusual. There was a more pronounced effect of increasing time at this moisture level, which suggests some problem with modification that did not exsist at the lower and higher moisture levels. While the effect of kernel size on extract was not significant (Table 2), the interaction of kernel size × germinations time was significant at $P \leq 0.05$. Extract levels for the medium kernel fraction continued to increase across six days, while levels for the plump fraction peaked at 4 days, and declined thereafter. The decline in extract is not unexpected in

malts as they become over-modified, but the rate of modification in barley is generally seen to progress faster with smaller kernels.

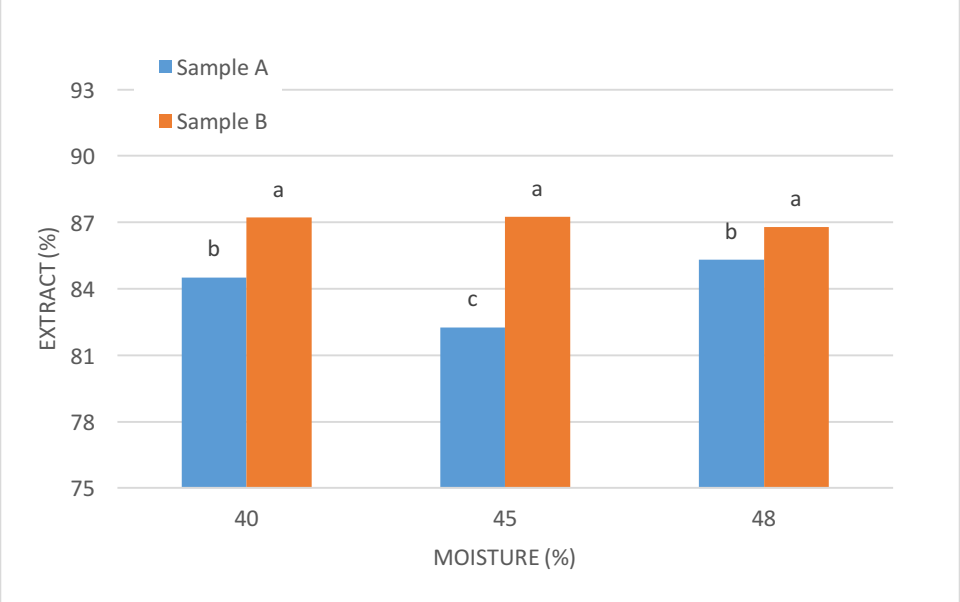


Fig. 3. Interaction of Sample and Germination Moisture Level on Extract.
Columns within each sample level denoted by the same letter are not significantly different ($P \leq 0.05$).

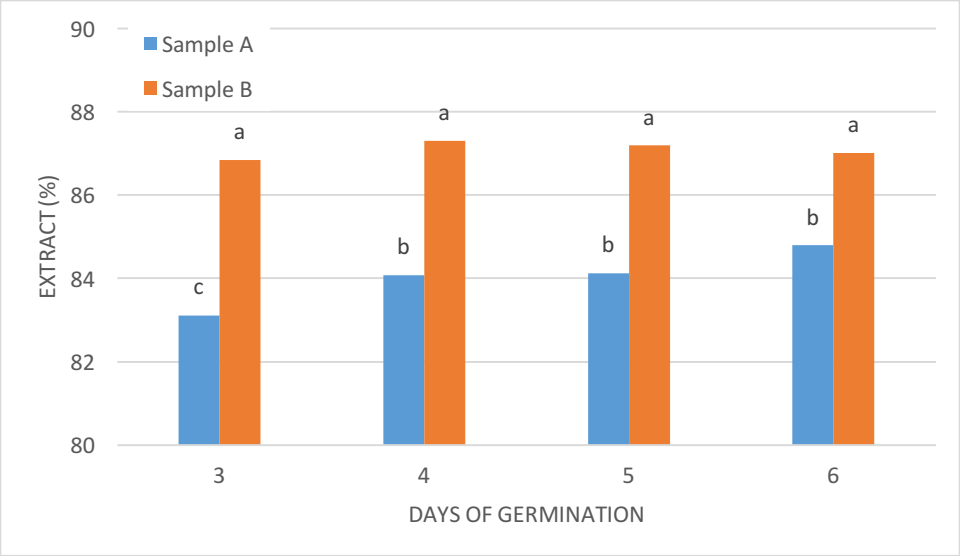


Fig. 4. Interaction of Sample and Germination Time on Extract.
Columns within each sample level denoted by the same letter are not significantly different ($P \leq 0.05$).

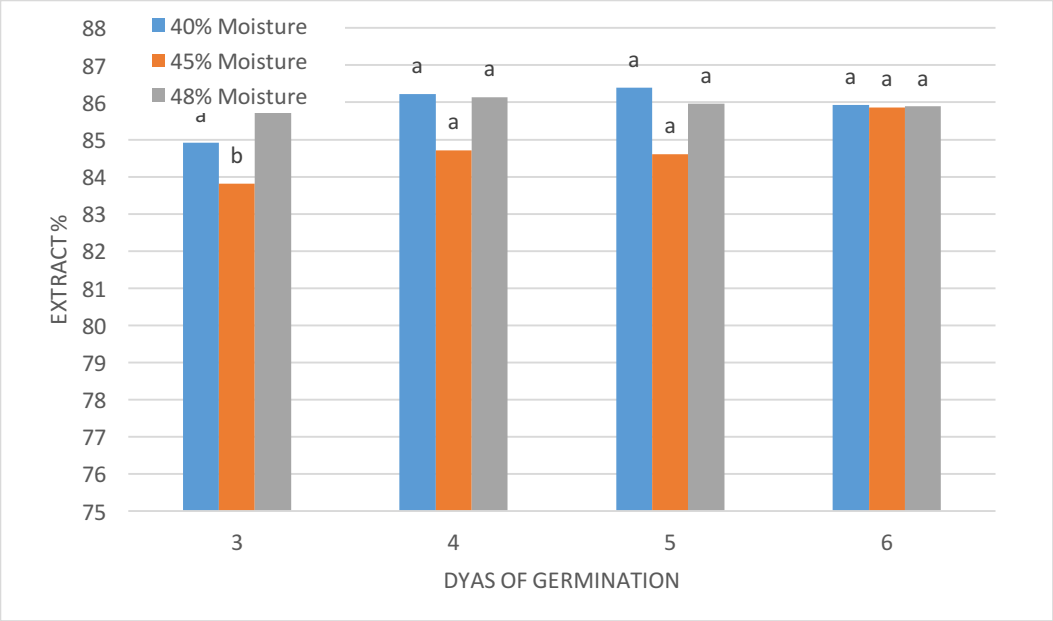


Fig. 5. Interaction on Germination Moisture and Germination Time on Extract.
Columns within each moisture level denoted by the same letter are not significantly different (P≤0.05).

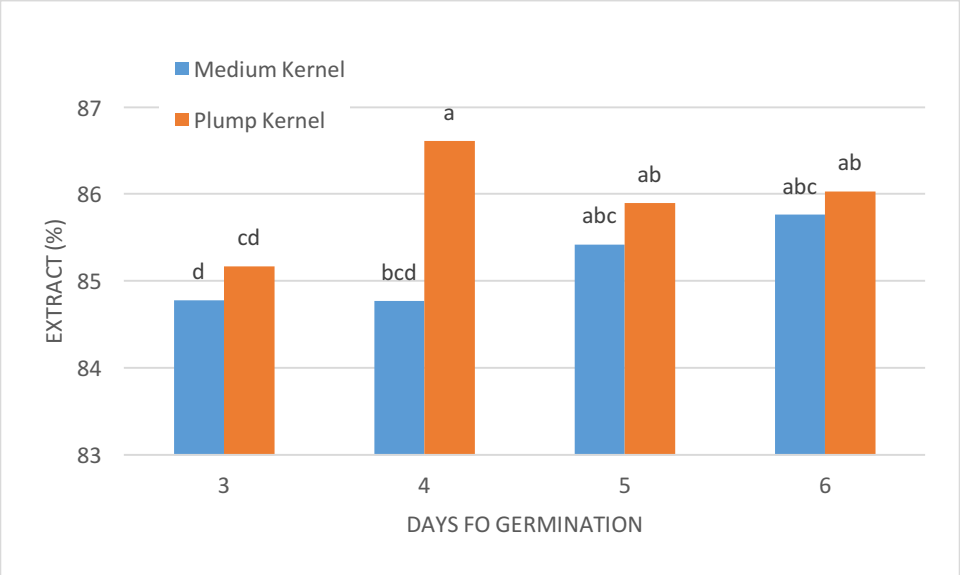


Fig. 6. Interaction of Size and Germination Time on Extract.
Columns withing each kernel size level denoted by the same letter are not significantly different (P≤0.05).

### 5.4. Malt Enzymes

Diastatic power and alpha-amylase levels are routinely determined as part or malt quality analyses, and are measures of starch degrading activity. Alpha-amylase is an endo-enzyme, and is largely responsible for reducing starch molecular weight and viscosity, and providing substrate for the action of beta-amylase (Birggs, 1998). Diastatic power is a measure of all enzymes that hydrolyze starch, but is predominately influenced by beta-amylase levels. Beta-amylase hydrolyzes alpha-1-4 glucosidic linkages in starch and dextrins to yield maltose, and as such is important in wort fermentability. Previous research has shown rye malt to have high levels of amylolytic activity (Briggs, 1998; Pomeranz, 1973) Barley malt also contains alpha-glucosidase and limit-dextrinase, but as these are not routinely measured they were not determined on the malted rye samples.

The level of diastatic power (DP) was significantly affected by sample, germination moisture, and germination time (Tables 1 and 3). However, there were significant interactions between sample × germination moisture, sample ×germination time and grain size x germination moisture (Table 3). Figure 7 shows a true interaction between grain size and moisture as ranks changed at the 48% moisture level. Examination of figure 7 shows that DP levels were in the range of 100 $^{o}$ASBC at 40 and 45% moisture, and differences due to kernel size were really not of practical significance. Levels of DP dramatically increased to the range of 150+ $^{o}$ASBC at 48% moisture. It is generally accepted that there is no *de novo* synthesis of beta-amylase during germination (Birggs, 1998), so this increase in DP at high moisture is surprising. It is likely due to another component of DP, and alpha-amylase levels were also seen to be significantly higher at 48%.

The interaction between sample × moisture (Figure 8) can be similarly explained. There were clearly differences between sample A and B at 40 and 45% moisture, but levels were in the range of 100 °ASBC. The DP of both samples increased to >150 °ASBC at 48% moisture, and overall differences were minimal. Figure 9 shows that samples did not respond uniformly across germination times in terms of DP. Sample A clearly has slightly higher levels, but perhaps of more importance is the observation that DP levels due not change to any practical degree as germination time increases.

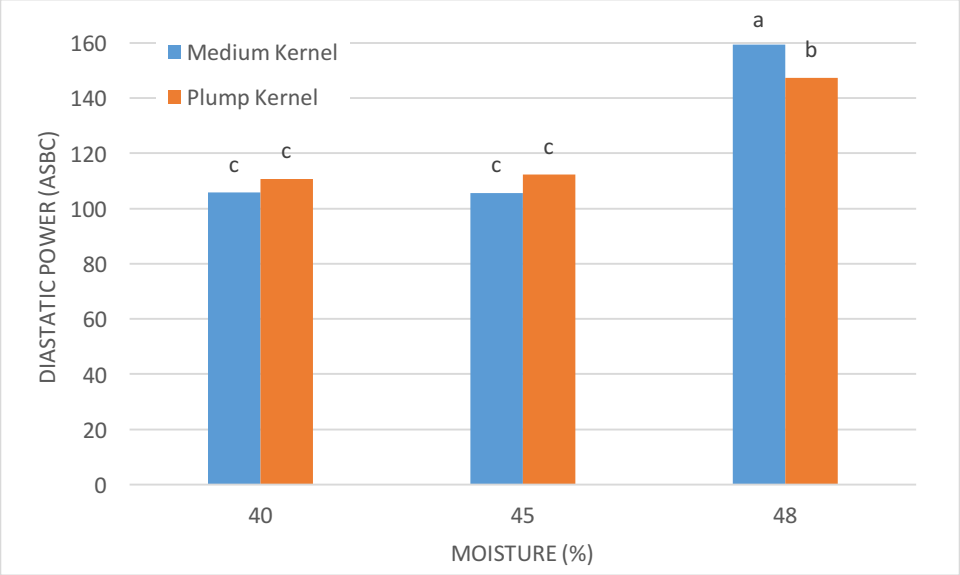


Fig. 7. Interaction of Size and Germination Moisture for Diastatic Power (DP).
Columns within each kernel size level denoted by the same letter are not significantly different (P≤0.05).

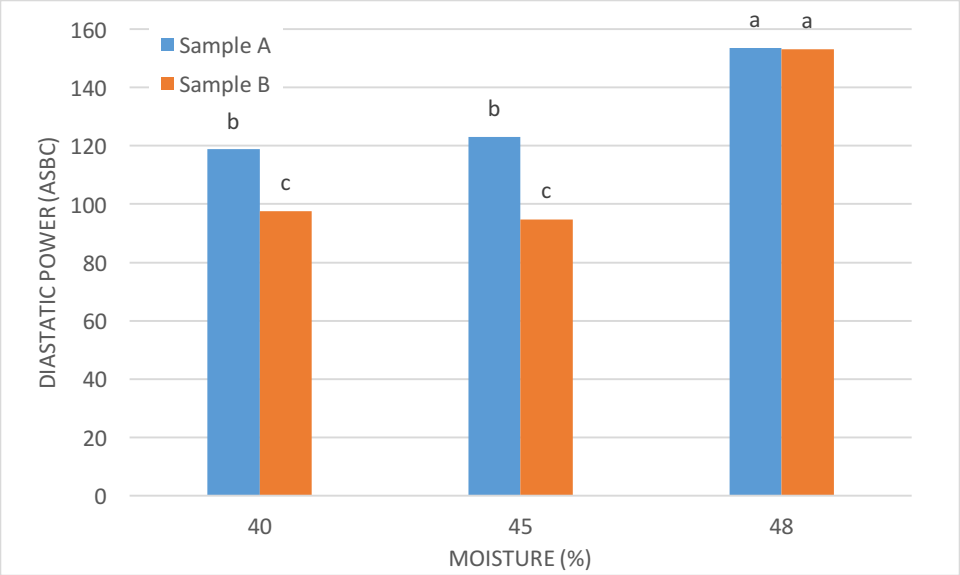


Fig. 8. Interaction of Sample and Germination Moisture for Diastatic Power (DP). Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

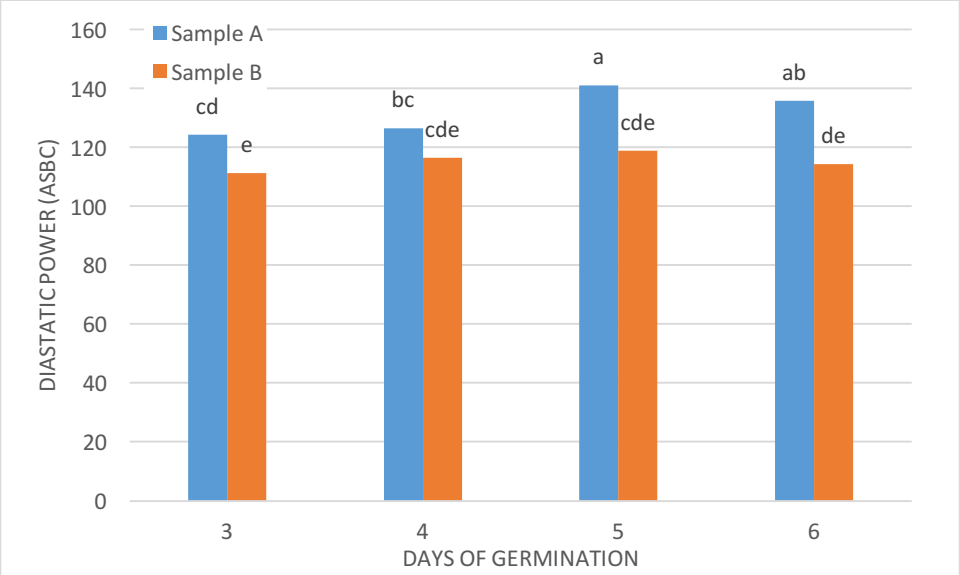


Fig. 9. Interaction of Sample and Germination Time for Diastatic Power (DP). Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

Alpha-amylase was significantly affected (P≤0.05) by all malting parameters (Table 1), but interactions between size × germination moisture and sample x germination were also significant (Table 3). Figure 10 shows that of α-amylase increased with increasing germination

moisture levels, but did not respond uniformly across the two kernel size fractions. In general alpha-amylase levels were between 70 and 80 DU at 40 and 45% moisture but increased to near 100 DU at 48% moisture. The high alpha-amylase levels of malted rye were previously noted by Pomeranz (1973), and levels at 100 DU are normally only observed in distillers' malts prepared from barley. The levels observed at 48% moisture, may in fact, be far in excess of what is required for a brewers' malt. Figure 11 clearly shows the different responses between samples A and B across germination moisture levels. While moisture level had a significant impact, data in Table 1 shows that alpha-amylase activity significantly increased (69 to 100 DU) as the germination time was extended from 3 to 6 days. These results are not surprising as alpha-amylase in barley is synthesized *de novo*, and levels are known to increase in germination (Birggs, 1998).

Table 3 also shown the responses of both α-amylase and diastatic power to germination moisture and germination days were non-linear.

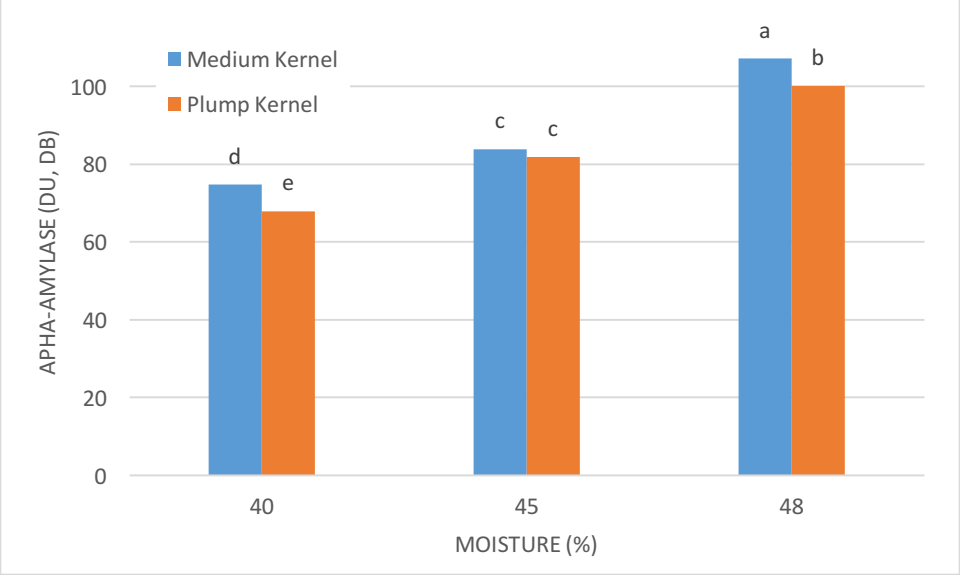


Fig. 10. Interaction of Grain Size and Germination Moisture for α-Amylase.
Columns within each kernel size level denoted by the same letter are not significantly different (P≤0.05).

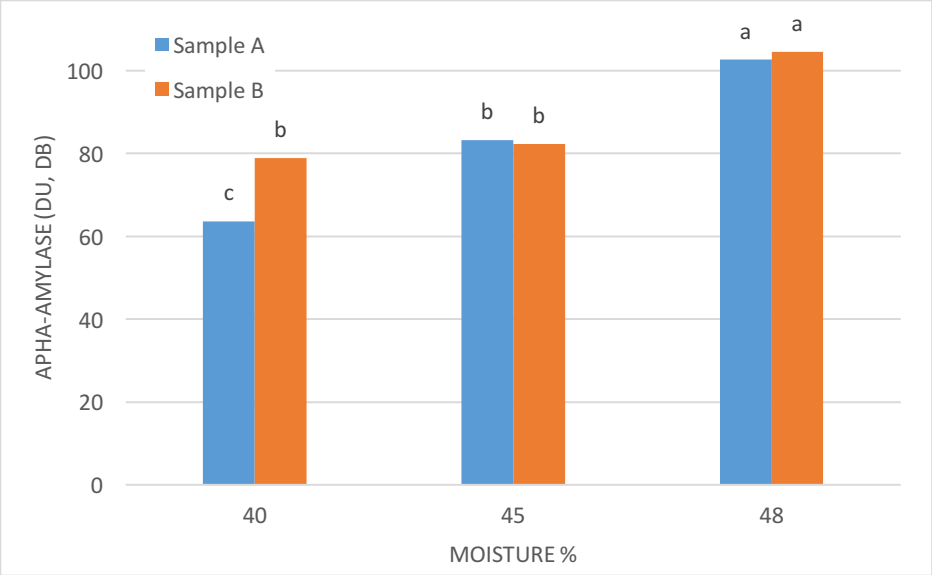

Fig. 11. Interaction of Sample and Germination Moisture for α-Amylase.
Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

### 5.5. Wort Soluble Protein Content and Free Amino Nitrogen

Soluble protein is expressed as a percentage of the malt extract. Some soluble protein is considered necessary for beer foam and proper yeast nutrition. Normal values in barley malt are 5-6% of the malt extract (Birggs, 1998; Clerck, 1957). High values are often considered problematic because of the potential for haze formation in beer, and loss of fermentable extract. The ratio of soluble (wort) to total grain protein is often referred as the Kolbach index or S/T protein, and is considered a measure of protein modification during malting. Values for barley malts are usually around 40-45%, but values for wheat and rye are likely higher due to the lack of husk (hull) and different total protein contents. Free amino nitrogen is considered as a measure of amino acids in the wort, which are necessary to support yeast growth. Levels of 150 mg/mL in barley malt are generally considered adequate.

Soluble protein was significantly (P≤0.05) affected by sample, grain size and germination time (Table 1). Interactions of sample x germination moisture, grain size x germination moisture, sample x germination time, and grain size x germination time also were significant. In former

studies on barley and rye, (Schwarz et al., 2007; Hübner et al., 2010) soluble protein was reported to increase as germination time progressed. There was no comparison of germination moisture levels in either of the cited studies. In the current study, the soluble protein of sample A increased as germination moisture levels increased, while levels in sample B decreased slightly at higher moisture levels (Figure 12). The proteolytic activities of the plump rye fraction were found to increase with higher germination moisture levels until a maximum was reached at 45%, after which, they declined. Soluble protein levels of the medium fraction slightly decreased as the moisture level increased (Figure 13). As shown in Figure 14, sample A had more soluble protein than sample B, across germination times. The trend was for soluble protein to increase in sample A, while it remained stable in B.

The impact of grain size × germination time can be observed in Figure 15. Rye malts germinated for longer times had more soluble protein, while the kernel size effect had been offset. As germination time increased in this study, the difference of medium kernels and plump kernels were 0.63%, 0.81%, 0.31, and 0.24. Main outcomes of this study were that soluble protein did not increase significantly after 4 days of germination, and that samples did not respond uniformly over germination time.

The Kolbach index was significantly affected by sample, germination time, and grain size (Table 4). Interactions between sample × germination moisture, grain size × germination moisture and sample × germination time were also significant (Table 4). However, mean values in this experiment only varied from 62.9 to 67.2%, which represents very little variation. Values only increased from 63.4 to 66.6% from 3 to 6 days of germination. Values in barley malt normally range from 35 to 45% (Birggs, 1998; De Clerk, 1957), and generally increase with germination time/modification The high values for rye malts are due to a higher levels of soluble

**Table 3. Analysis of Variance for DP and α-Amylase**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Diastatic Power** | Sample (V) | 1 | 6648.33 | 64.29 | <0.0001 |
| | Size (S) | 1 | 0.96 | 0.01 | 0.9234 |
| | Germination Moisture (G_M) | 2 | 21427.63 | 207.21 | <0.0001 |
| | Linear G_M | 1 | 32710.67 | 316.32 | <0.0001 |
| | Quadratic G_M | 1 | 10144.58 | 98.10 | <0.0001 |
| | Germination Days (G_D) | 3 | 639.16 | 6.18 | 0.0009 |
| | Linear G_D | 1 | 1081.85 | 10.46 | 0.0019 |
| | Quadratic G_D | 1 | 446.24 | 4.32 | 0.0414 |
| | Cubic G_D | 1 | 389.39 | 3.77 | 0.0563 |
| | V*S | 1 | 111.52 | 1.08 | 0.3026 |
| | V*G_M | 2 | 1701.17 | 16.45 | <0.0001 |
| | S*G_M | 2 | 854.12 | 8.26 | 0.0006 |
| | V*G_D | 3 | 221.05 | 2.14 | 0.1032 |
| | S*G_D | 3 | 35.87 | 0.35 | 0.7915 |
| | G_M*G_D | 6 | 93.10 | 0.90 | 0.4998 |
| | Error | 70 | 103.41 | | |
| **Alpha-Amylase** | Sample (V) | 1 | 696.45 | 29.37 | <0.0001 |
| | Size (S) | 1 | 676.62 | 28.53 | <0.0001 |
| | Germination Moisture (G_M) | 2 | 8557.64 | 360.88 | <0.0001 |
| | Linear G_M | 1 | 16658.33 | 702.49 | <0.0001 |
| | Quadratic G_M | 1 | 456.94 | 19.27 | <0.0001 |
| | Germination Days (G_D) | 3 | 4534.57 | 191.23 | <0.0001 |
| | Linear G_D | 1 | 13410.23 | 565.52 | <0.0001 |
| | Quadratic G_D | 1 | 125.11 | 5.28 | 0.0246 |
| | Cubic G_D | 1 | 68.37 | 2.88 | 0.0939 |
| | V*S | 1 | 0.64 | 0.03 | 0.8703 |
| | V*G_M | 2 | 604.50 | 25.49 | <0.0001 |
| | S*G_M | 2 | 67.91 | 2.86 | 0.0638 |
| | V*G_D | 3 | 36.40 | 1.53 | 0.2131 |
| | S*G_D | 3 | 10.23 | 0.43 | 0.7311 |
| | G_M*G_D | 6 | 38.65 | 1.63 | 0.1517 |
| | Error | 70 | 23.71 | | |

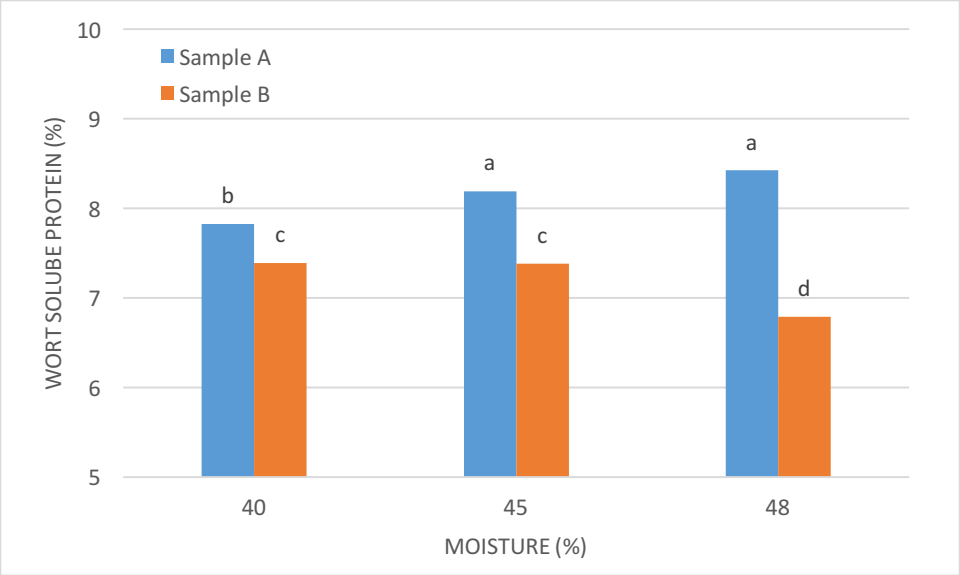


Fig. 12. Interaction of Sample and Germination Moisture for Soluble protein.
Columns within each sample level denoted by the same letter are not significantly different ($P \leq 0.05$).

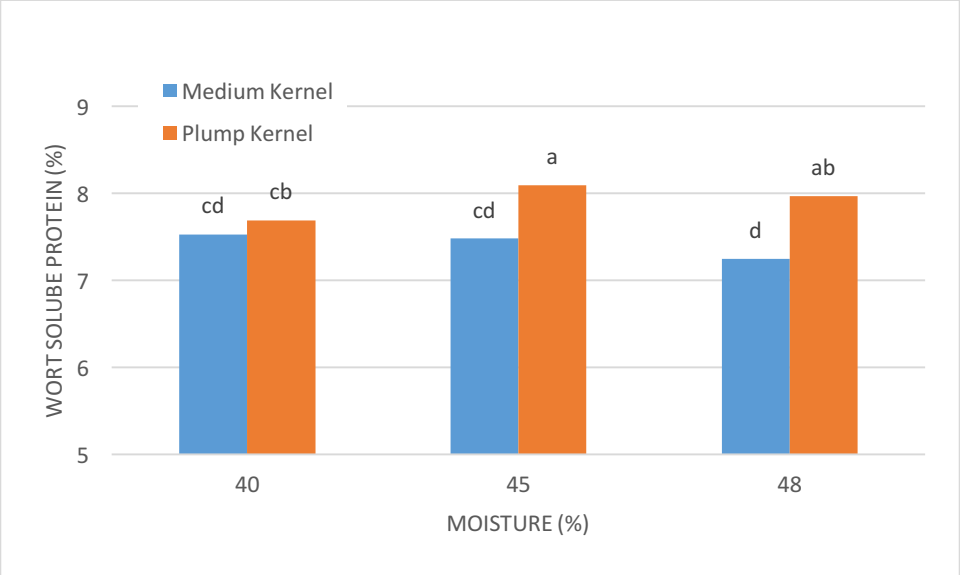


Fig. 13. Interaction of Grain Size and Germination Moisture for Soluble Protein.
Columns within each kernel size level denoted by the same letter are not significantly different ($P \leq 0.05$).

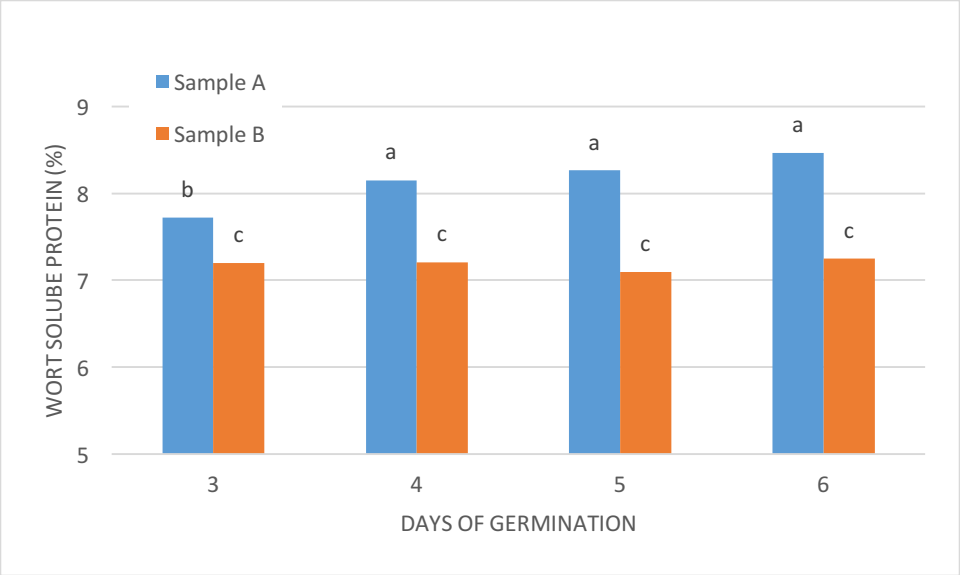


Fig. 14. Interaction of Sample and Germination Time for Soluble Protein.
Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

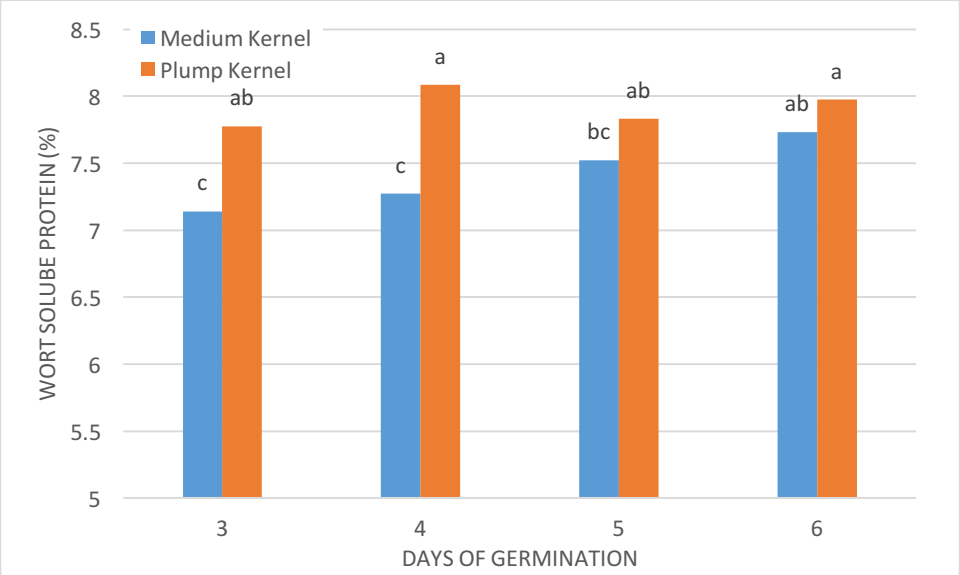


Fig. 15. Interaction of Grain Size and Germination Time for Soluble Protein.
Columns within each kernel size level denoted by the same letter are not significantly different (P≤0.05).

protein, which has previously been reported (Pomeranz, 1973). However, the modification or solubilization of rye endosperm protein appears to be less affected by germination time.

Free amino nitrogen (FAN) was significantly affected (P≤0.05) by grain size, germination moisture, and germination time modification (Table 1). The Interaction of sample × germination moisture was significant as well (Table 5). Figure 16 shows that the two varieties did respond uniformly across germination moisture levels. Sample B had increased FAN as the moisture level increased, while the sample A had an uneven response. However, as was the case for soluble protein and Kolbach index, the range of values observed was relatively small. Mean values of FAN ranged from 211 to 240, which is of minimal practical significance. Again it seems that the bulk of FAN is formed very early in germination, or may even partially exist in the raw grain. The highest levels were observed at 48% moisture. There was a linear response to germination days for both soluble protein, and Kolbach index. The contrasts of germination moisture for soluble protein and Kolbach index were non-significant in this study (Table 4). Table 5 also indicated the non-linear responses to both germination moisture and germination days for FAN.

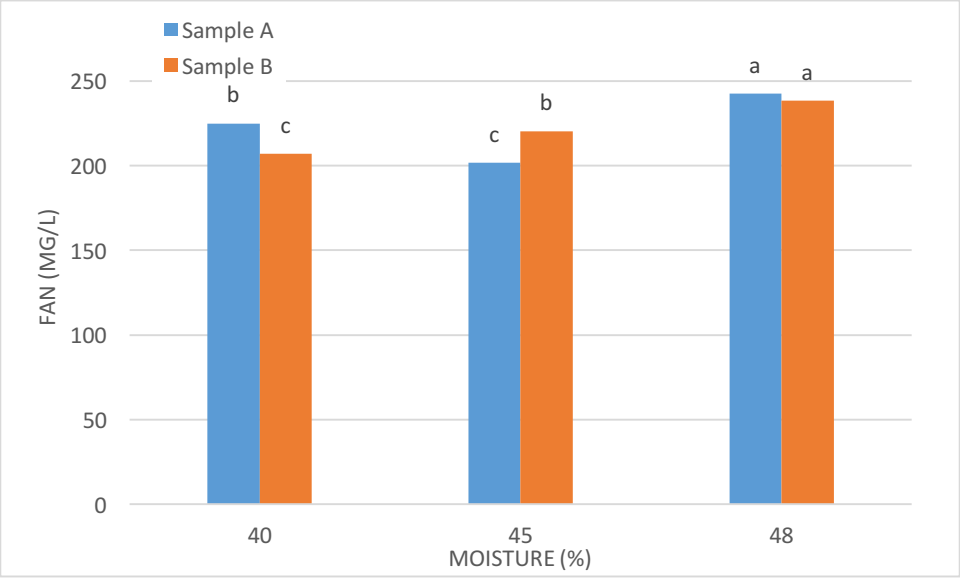


Fig. 16. Interaction of Sample and Germination Moisture for Wort Free Amino Nitrogen (FAN). Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

**Table 4. Analysis of Variance for Wort Soluble Protein and Kolbach Index (S/T)**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Wort Soluble Protein** | Sample (V) | 1 | 22.18 | 104.95 | <.0001 |
| | Size (S) | 1 | 5.98 | 28.30 | <.0001 |
| | Germination Moisture (G_M) | 2 | 0.34 | 1.60 | 0.2102 |
| | Linear G_M | 1 | 0.00 | 0.00 | 0.9903 |
| | Quadratic G_M | 1 | 0.67 | 3.19 | 0.0784 |
| | Germination Days (G_D) | 3 | 0.63 | 2.98 | 0.0373 |
| | Linear G_D | 1 | 1.684 | 7.97 | 0.0062 |
| | Quadratic G_D | 1 | 0.01 | 0.05 | 0.8185 |
| | Cubic G_D | 1 | 0.19 | 0.91 | 0.3428 |
| | V*S | 1 | 0.00 | 0.00 | 0.9589 |
| | V*G_M | 2 | 2.99 | 14.13 | <.0001 |
| | S*G_M | 2 | 0.70 | 3.34 | 0.0413 |
| | V*G_D | 3 | 0.60 | 2.85 | 0.0433 |
| | S*G_D | 3 | 0.44 | 2.07 | 0.1124 |
| | G_M*G_D | 6 | 0.20 | 0.96 | 0.4583 |
| | Error | 70 | 0.21 | | |
| **S/T** | Sample (V) | 1 | 75.61 | 5.36 | 0.0236 |
| | Size (S) | 1 | 435.15 | 30.84 | <.0001 |
| | Germination Moisture (G_M) | 2 | 28.15 | 1.99 | 0.1437 |
| | Linear G_M | 1 | 2.57 | 0.18 | 0.6711 |
| | Quadratic G_M | 1 | 53.73 | 3.81 | 0.0550 |
| | Germination Days (G_D) | 3 | 39.74 | 2.82 | 0.0453 |
| | Linear G_D | 1 | 104.86 | 7.43 | 0.0081 |
| | Quadratic G_D | 1 | 0.37 | 0.03 | 0.8723 |
| | Cubic G_D | 1 | 13.99 | 0.99 | 0.3228 |
| | V*S | 1 | 3.21 | 0.23 | 0.6347 |
| | V*G_M | 2 | 219.46 | 15.55 | <.0001 |
| | S*G_M | 2 | 41.18 | 2.92 | 0.0606 |
| | V*G_D | 3 | 37.84 | 2.68 | 0.004 |
| | S*G_D | 3 | 30.03 | 2.13 | 0.1044 |
| | G_M*G_D | 6 | 12.78 | 12.78 | 0.4960 |
| | Error | 70 | 14.11 | | |

### 5.6. Wort Viscosity, Arabinoxylan, and Beta-Glucan

Wort viscosity is used as an indicator of lautering and filtration problems in the brewery, and with barley malts is mainly attributed to high molecular weight beta-glucans. Values of greater than 1.5 cP are seen as potentially problematic (Birggs, 1998; De Clerk, 1957). The high viscosity of rye worts was previously mentioned (Briggs, 1998; Pomeranz, 1973), and is largely attributed to a high level of soluble arabinoxylans, although rye also contains some beta-glucan. Viscosity was significantly affected ($P \leq 0.05$) by sample, germination moisture, and germination time (Table 1). Interactions of sample × grain size, sample × germination moisture, and germination moisture × germination time were significant (Table 5). As expected, rye malt based worts had high viscosities when compared to barley based worts. The response of sample was uniform across kernel size (Fig 17), but differences were small. The viscosity of both samples declined as the germination moisture level increased, which indicated the possiblity of using a high germination moisture level to decrease the viscosity level (Fig 18). Figure 19 shows that the interaction of germination moisture x germination was due to magnitude. Viscosity was always highest at 40% moisture, but the viscosity differences between moisture became less pronounced as time progressed. High moisture levels were 1.79 cP lower in viscosity at 3 days. However, at six days the difference was only 0.43 cP. This indicates that the benefit of higher germination moisture was maximum when the malt endosperm matrix was less modified.

In this study, increased germination time or germination moisture level both had positive effects on decreasing rye wort viscosity. The influence of increased moisture was reduced as germination time increased. Decreases in viscosity should be reflected in β-glucan and arabinoxylans (AX) levels. Water-extractable AX (WEAX) can be cross-linked with the possibility of causing the filtration problems (Izydorcyk et al. 1990). Research by Schwarz and

**Table 5. Analysis of Variance for FAN and Wort Viscosity**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **FAN** | Sample (V) | 1 | 36.58 | 0.24 | 0.6275 |
| | Size (S) | 1 | 6418.10 | 41.68 | <.0001 |
| | Germination Moisture (G_M) | 2 | 7856.85 | 51.03 | <.0001 |
| | Linear G_M | 1 | 9498.45 | 61.69 | <.0001 |
| | Quadratic G_M | 1 | 6215.24 | 40.37 | <.0001 |
| | Germination Days (G_D) | 3 | 407.30 | 2.65 | 0.0558 |
| | Linear G_D | 1 | 412.06 | 2.68 | 0.1064 |
| | Quadratic G_D | 1 | 767.38 | 4.98 | 0.0288 |
| | Cubic G_D | 1 | 42.47 | 0.28 | 0.6011 |
| | V*S | 1 | 377.08 | 2.45 | 0.1221 |
| | V*G_M | 2 | 2694.63 | 17.50 | <.0001 |
| | S*G_M | 2 | 295.81 | 1.92 | 0.1541 |
| | V*G_D | 3 | 76.93 | 0.50 | 0.6837 |
| | S*G_D | 3 | 416.43 | 2.70 | 0.0519 |
| | G_M*G_D | 6 | 326.05 | 2.12 | 0.0619 |
| | Error | 70 | 153.98 | | |
| **Viscosity** | Sample (V) | 1 | 13.66 | 113.91 | <.0001 |
| | Size (S) | 1 | 0.05 | 0.38 | 0.5383 |
| | Germination Moisture (G_M) | 2 | 7.14 | 59.55 | <.0001 |
| | Linear G_M | 1 | 13.87 | 115.69 | <.0001 |
| | Quadratic G_M | 1 | 0.41 | 3.40 | 0.0694 |
| | Germination Days (G_D) | 3 | 4.00 | 33.33 | <.0001 |
| | Linear G_D | 1 | 11.52 | 96.11 | <.0001 |
| | Quadratic G_D | 1 | 0.39 | 3.27 | 0.0747 |
| | Cubic G_D | 1 | 0.07 | 0.62 | 0.4342 |
| | V*S | 1 | 0.47 | 3.89 | 0.0526 |
| | V*G_M | 2 | 2.58 | 21.54 | <.0001 |
| | S*G_M | 2 | 0.24 | 1.97 | 0.1475 |
| | V*G_D | 3 | 0.18 | 1.47 | 0.2290 |
| | S*G_D | 3 | 0.14 | 1.17 | 0.3293 |
| | G_M*G_D | 6 | 0.98 | 8.19 | <.0001 |
| | Error | 70 | 0.12 | | |

coworkers (2002) showed that β-glucan and AX had equivalently effects on viscosity and filterability with barley malts. As the enzymes that degrade AX are synthesized late in the germination stage (Banik et al, 1997), many AX can exist in final beer product, which also has been reported by Schwarz et al (2002). The amount of AX in commercial beer is approximately 10 times more than that of β-glucan.

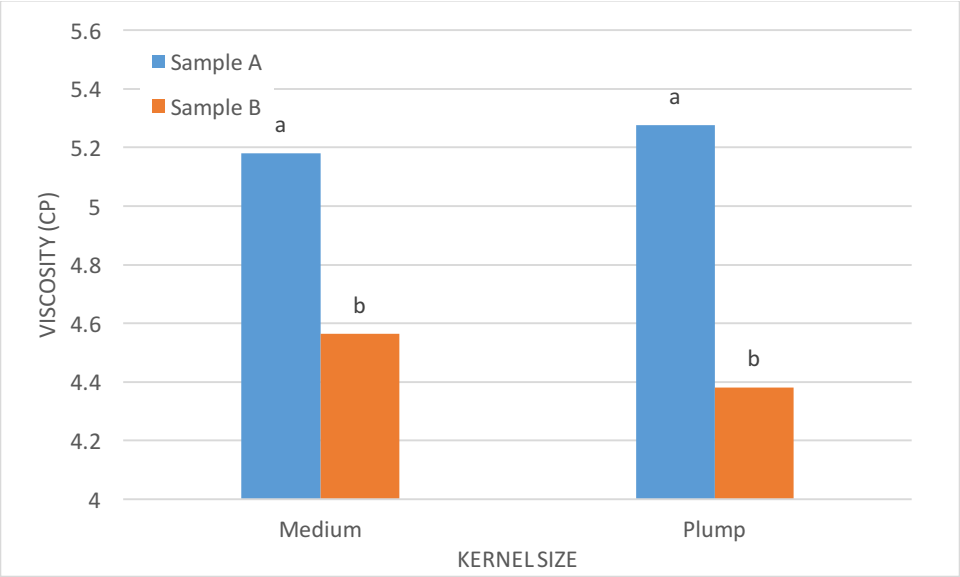


Fig. 17. Interaction of Sample and Grain Size for Wort Viscosity.
Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

The only factor to significantly affect wort (AX) content was germination time (Tables 1 and 6). A slight decrease was observed from 3 to 4 days, but no significant differences were seen after 4 days. The literature suggests that as the germination time progresses, enzymes that degrade AX are produced. However, a high percentage of AX survives and persists into the final beer (Coote and Kirsop, 1976), which contributes difficulties associated with the filtration. Li and coworkers (2004) studied on AX degradation in barley during malting and brewing, and reported that water-extractable AX had positive and higher correlation with wort viscosity than β-glucan.

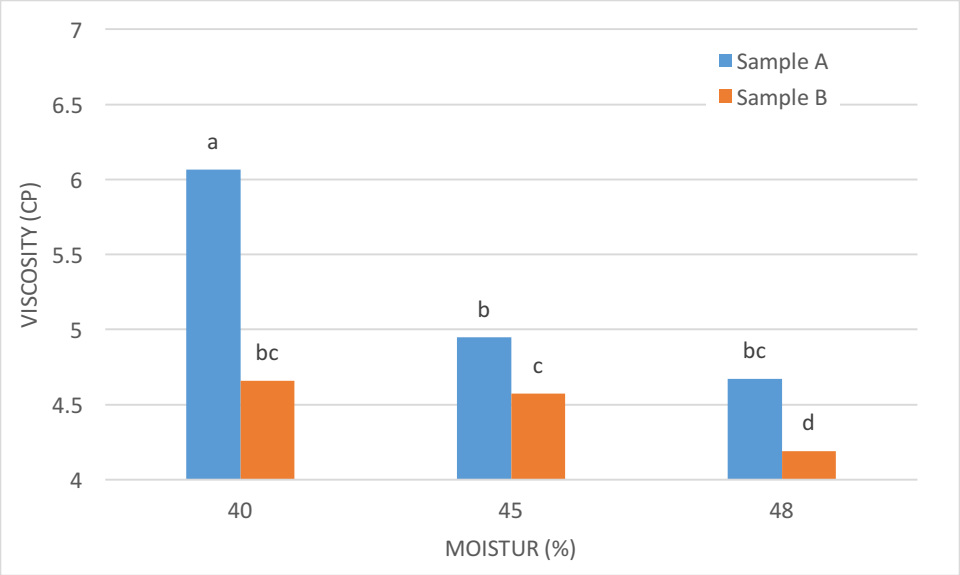


Fig. 18. Interaction of Sample and Germination Moisture for Wort Viscosity. Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

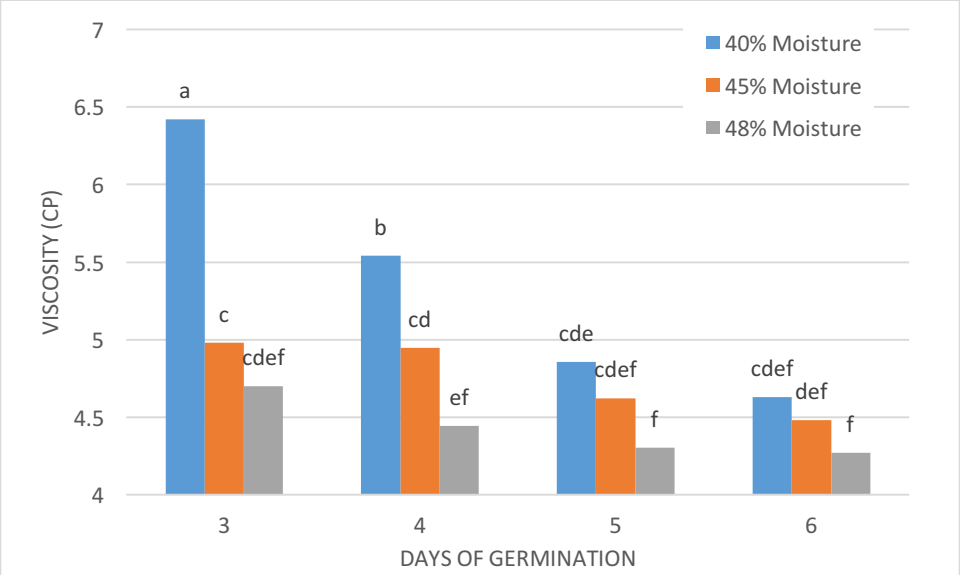


Fig. 19. Interaction of Germination Moisture and Germination Time for Wort Viscosity. Columns within each germination moisture level denoted by the same letter are not significantly different (P≤0.05).

Results for AX in the current study do not reflect molecular, as the wort samples were hydrolyzed, and total arabinose and xylose content (x 0.88) used to estimate AX. Xylose and arabinose measured could have been monosaccharides, part of oligosaccharides, in addition to

components of higher molecular weight AX. Thus, the observation that levels did not change after 4 days, or in response to moisture, is not surprising. Changes in AX molecular weight could have occurred, but were not measured.

Wort β-glucan content was significantly affected (P≤0.05) by sample, germination moisture, and germination time (Table 1). The interaction of germination moisture x – germination time was also significant (Table 6). Figure 20 shows the β-glucan level dcreased with both increasing germination times and moisture levels. Kernel size had no effect on β-glucan, but sample had a slight, but significant effect (Table 1).

Results in Table 5 and 6 show there were liner responses viscosity to both germination moisture and germination days,  non-linear response for both AX and β-glucan content to germination moisture and germination day.

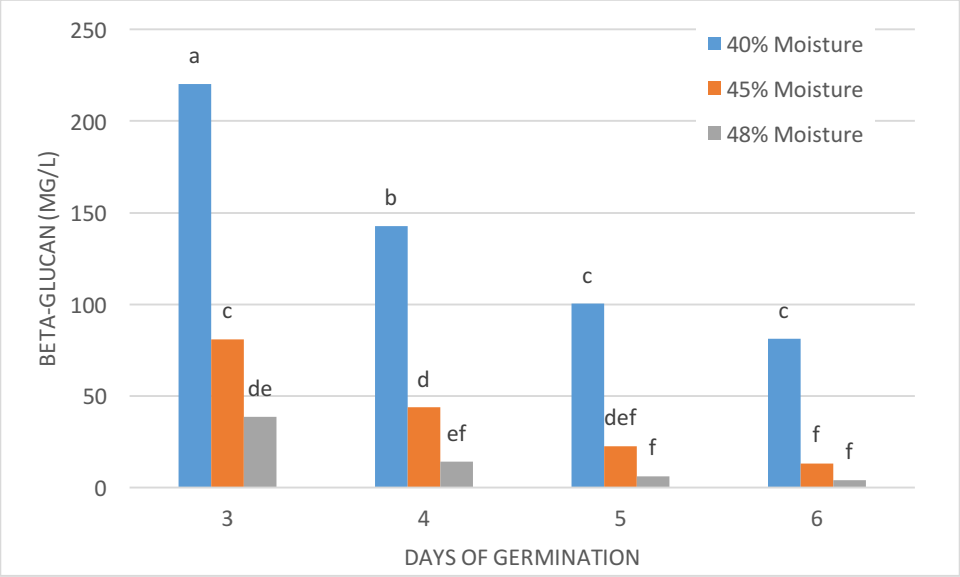


Fig. 20. Interaction of Germination Moisture and  Germination Time for Wort β-Glucan. Columns within each germination moisture level denoted by the same letter are not significantly different (P≤0.05).

**Table 6. Analysis of Variance for AX and β-Glucan**

| Dependent Viable | Source | DF | Mean Square | F value | Pr > F |
|---|---|---|---|---|---|
| **AX** | Sample (V) | 1 | 799.26 | 0.18 | 0.6761 |
| | Size (S) | 1 | 8.19 | 0.00 | 0.9662 |
| | Germination Moisture (G_M) | 2 | 2857.91 | 0.63 | 0.5359 |
| | Linear G_M | 1 | 5624.29 | 1.24 | 0.2696 |
| | Quadratic G_M | 1 | 91.53 | 0.02 | 0.8875 |
| | Germination Days (G_D) | 3 | 12235.60 | 2.69 | 0.0526 |
| | Linear G_D | 1 | 464.52 | 0.10 | 0.7500 |
| | Quadratic G_D | 1 | 26395.65 | 5.81 | 0.0185 |
| | Cubic G_D | 1 | 9846.62 | 2.17 | 0.1454 |
| | V*S | 1 | 440.91 | 0.10 | 0.7563 |
| | V*G_M | 2 | 158.77 | 0.03 | 0.9657 |
| | S*G_M | 2 | 2716.62 | 0.60 | 0.5526 |
| | V*G_D | 3 | 4109.32 | 0.90 | 0.4432 |
| | S*G_D | 3 | 3762.08 | 0.83 | 0.4826 |
| | G_M*G_D | 6 | 3653.18 | 0.80 | 0.5698 |
| | Error | 70 | 4541.08 | | |
| **β-Glucan** | Sample (V) | 1 | 2019.71 | 8.69 | 0.0043 |
| | Size (S) | 1 | 12.53 | 0.05 | 0.8170 |
| | Germination Moisture (G_M) | 2 | 129573.77 | 557.78 | <.0001 |
| | Linear G_M | 1 | 231883.54 | 998.19 | <.0001 |
| | Quadratic G_M | 1 | 27263.99 | 117.36 | <.0001 |
| | Germination Days (G_D) | 3 | 30653.36 | 131.95 | <.0001 |
| | Linear G_D | 1 | 84059.81 | 361.85 | <.0001 |
| | Quadratic G_D | 1 | 7803.47 | 33.59 | <.0001 |
| | Cubic G_D | 1 | 96.82 | 0.42 | 0.5207 |
| | V*S | 1 | 15.84 | 0.07 | 0.7947 |
| | V*G_M | 2 | 286.19 | 1.23 | 0.2980 |
| | S*G_M | 2 | 90.76 | 0.39 | 0.6781 |
| | V*G_D | 3 | 105.37 | 0.45 | 0.7156 |
| | S*G_D | 3 | 212.99 | 0.92 | 0.4374 |
| | G_M*G_D | 6 | 4436.55 | 19.10 | <.0001 |
| | Error | 70 | 232.30 | | |

### 5.7. Wort Phenolic Acids

Phenolic acids in wort were measured because of potential influence on a number of beer characteristics, including flavor. As an example, some brewing yeasts decarboxylate ferulic acid to form 4-vinylguaicol, which is associated with the spicy clove-like flavor in German wheat beers (Cui et al. 2015).

In this research, ferulic (58.6%), vanillic (32.1%), and p-coumaric acids (1.8%) were the most abundant phenolic acids present in rye wort. Gallic acid (1.4%), sinapinic acid (1.0%), syringic acid (1.0%), caffeic acid (1.0%), and a small amount of catechin (0.01%) were also found in this experiment. Rye wort phenolic acids were significantly affected ($P \leq 0.05$) by sample, days of germination, grain size, and germination moisture level. The sample variety × germination moisture interaction had a significant impact on ferulic and p-coumouric acids. Fig. 21 and fig. 22 shows the interaction effection on variety and germination moisture. Compared to Sample A, B had more ferulic and p-coumaric acids at a higher moisture levels. Sample A had the most ferulic and p-coumaric acids when the moisture level was 48%. Para-coumaric acid was also significantly affected by Sample-size interaction (Fig. 23). There was no significant difference for p-coumaric acid in Sample A, while the medium B kernels had almost 45% more p-coumaric acid than its plump kernels. This is not unexpected as phenolic acid content is often higher in the bran tissues. Levels of ferulic and vanillic acids increased with germination time, and levels were significant different between 4 and 5 days. Esterase enzymes may be involved in the release of bound phenolics during germination (Birggs, 1998).

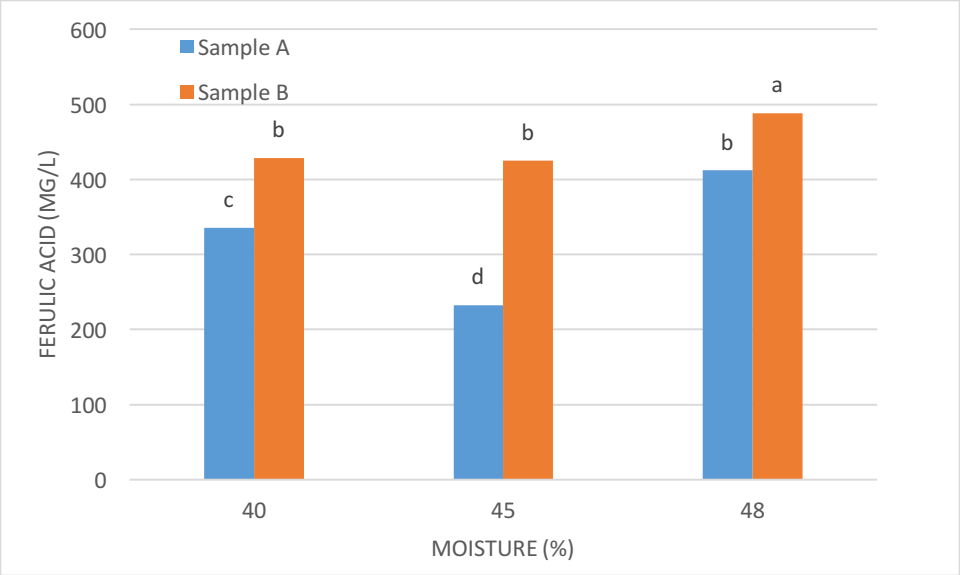


Fig. 21. Interaction of Sample and Germination Moisture for Wort Ferulic Acid. Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

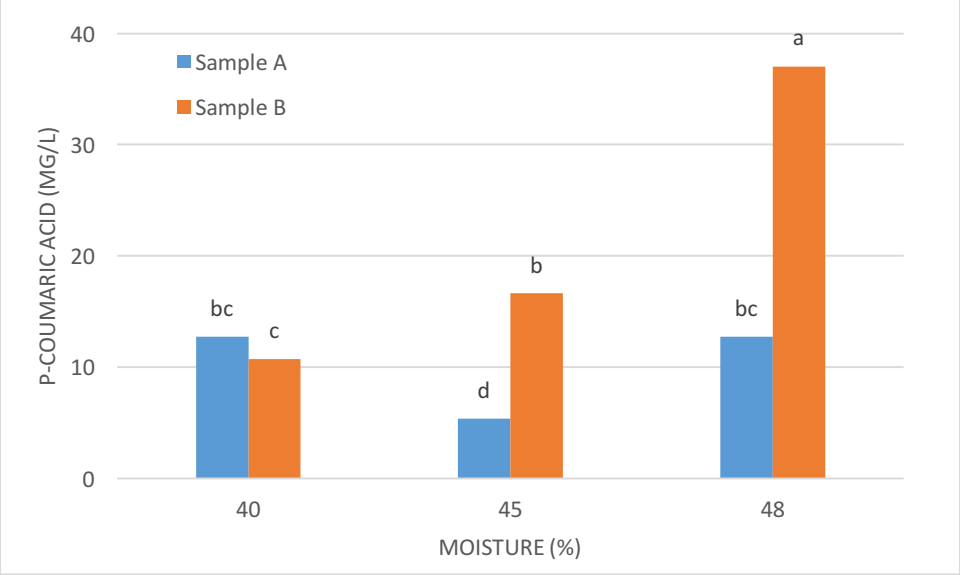


Fig. 22 Interaction of Sample and Germination Moisture for Wort p-Coumaric Acid. Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

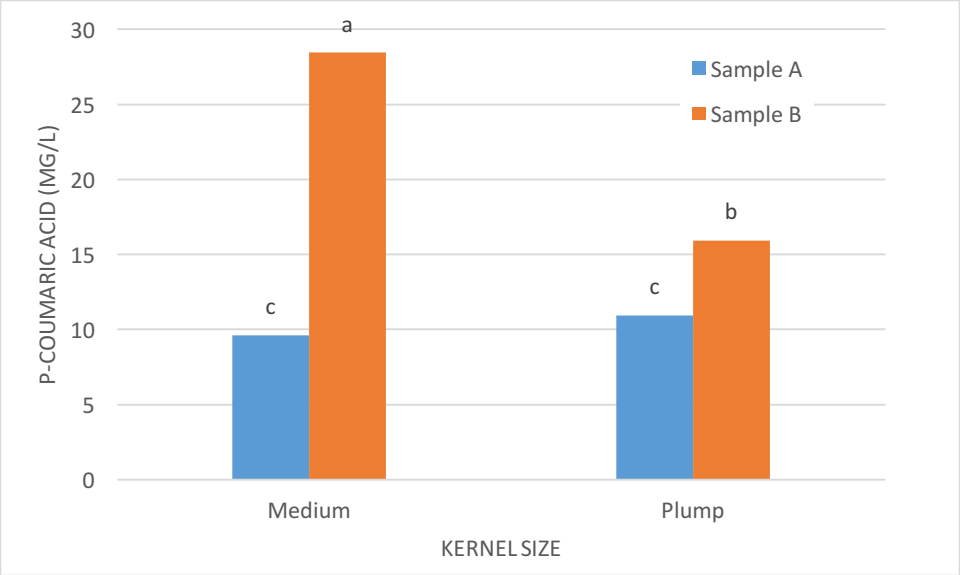


Fig. 23. Interaction of Sample and Grain Size for Wort p-Coumaric Acid.
Columns within each sample level denoted by the same letter are not significantly different ($P \leq 0.05$).

**Table 7. Mean of Phenolic Acids (mg/L) as Affected by Malting Operational Parameters [a]**

| | Caffeic | Ferulic | p-Coumaric | Sinapinic | Syringic | Vanillic | Gallic | Catechin | Total Phenolic Acids |
|---|---|---|---|---|---|---|---|---|---|
| **Sample** | | | | | | | | | |
| A | 10.96 a | 326.47 a | 10.28 a | 6.83 a | 6.47 a | 170.70 a | 22.71 a | 0.11 a | 554.89 a |
| B | 16.96 b | 448.64 b | 22.14 b | 8.13 b | 7.87 b | 257.42 b | 10.32 b | 0.06 a | 770.54 b |
| **Germination Time** | | | | | | | | | |
| Three day | 11.49 a | 323.77 a | 12.16 a | 6.76 a | 6.58 a | 183.23 a | 23.27 a | 0.04 a | 567.31 a |
| Four day | 12.29 a | 340.06 a | 13.39 a | 6.61 a | 6.99 ab | 190.21 a | 17.73 ab | 0.10 a | 587.39 a |
| Five day | 15.30 b | 426.06 b | 17.60 ab | 6.95 a | 8.04 bc | 245.33 b | 16.99 ab | 0.07 a | 736.35 b |
| Six day | 16.76 b | 460.31 b | 21.67 b | 7.09 a | 8.30 c | 237.48 b | 8.06 b | 0.14 a | 759.81 b |
| **Grain Size** | | | | | | | | | |
| Plump | 13.56 a | 366.96 a | 13.44 a | 6.61 a | 7.49 a | 186.57 a | 21.05 a | 0.09 a | 615.78 a |
| Medium | 14.36 a | 408.15 b | 18.98 b | 7.09 b | 7.46 a | 241.56 b | 11.97 a | 0.08 a | 709.65 b |
| **Moisture** | | | | | | | | | |
| 40% | 13.38 a | 384.00 a | 12.76 a | 6.56 a | 7.57 a | 256.27 a | 24.02 a | 0.19 a | 704.75 a |
| 45% | 12.12 a | 328.64 b | 10.98 b | 6.53 a | 5.95 b | 183.18 b | 9.12 b | 0.02 b | 556.55 b |
| 48% | 16.38 b | 450.01 a | 24.88 a | 7.47 b | 8.92 c | 202.74 b | 16.40 ab | 0.05 b | 726.84 a |

[a] Means followed by the same letter are not significantly different (P≤0.05).

**Table 8. Analysis of Variance for Phenolic Acids: Caffeic Acid and Ferulic Acid**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Caffeic** | Sample (V) | 1 | 844.60 | 26.86 | <.0001 |
| | Size (S) | 1 | 35.72 | 1.14 | 0.292 |
| | Germination Moisture (G_M) | 2 | 147.53 | 4.69 | 0.0139 |
| | Linear G_M | 1 | 13507.48 | 3.96 | 0.0505 |
| | Quadratic G_M | 1 | 15975.55 | 4.69 | 0.0339 |
| | Germination Days (G_D) | 3 | 159.09 | 5.06 | 0.0041 |
| | Linear G_D | 1 | 39381.01 | 11.55 | 0.0011 |
| | Quadratic G_D | 1 | 243.11 | 0.07 | 0.7902 |
| | Cubic G_D | 1 | 1680.93 | 0.49 | 0.4849 |
| | V*S | 1 | 21.63 | 0.69 | 0.4111 |
| | V*G_M | 2 | 55.41 | 1.76 | 0.1828 |
| | S*G_M | 2 | 1.30 | 0.04 | 0.9596 |
| | V*G_D | 3 | 23.57 | 0.75 | 0.5281 |
| | S*G_D | 3 | 45.04 | 1.43 | 0.2452 |
| | G_M*G_D | 6 | 4.48 | 0.14 | 0.9897 |
| | Errorr | 47 | 31.45 | | |
| **Ferulic** | Sample (V) | 1 | 343028.53 | 38.06 | <.0001 |
| | Size (S) | 1 | 52412.48 | 5.82 | 0.0198 |
| | Germination Moisture (G_M) | 2 | 117836.25 | 13.07 | <.0001 |
| | Linear G_M | 1 | 6543928.33 | 6.72 | 0.0117 |
| | Quadratic G_M | 1 | 16302127.98 | 16.73 | 0.0001 |
| | Germination Days (G_D) | 3 | 105822.33 | 11.74 | <.0001 |
| | Linear G_D | 1 | 27335600.97 | 28.06 | <.0001 |
| | Quadratic G_D | 1 | 185496.49 | 0.19 | 0.6640 |
| | Cubic G_D | 1 | 1755561.10 | 1.80 | 0.1839 |
| | V*S | 1 | 10357.66 | 1.15 | 0.2892 |
| | V*G_M | 2 | 29763.48 | 3.3 | 0.0455 |
| | S*G_M | 2 | 21070.09 | 2.34 | 0.1077 |
| | V*G_D | 3 | 5115.02 | 0.57 | 0.6391 |
| | S*G_D | 3 | 6190.91 | 0.69 | 0.5645 |
| | G_M*G_D | 6 | 7237.46 | 0.8 | 0.5726 |
| | Error | 47 | 9012.50 | | |

**Table 9. Analysis of Variance for Phenolic Acids: p-Coumaric Acid and Vanilic Acid**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **p-Coumaric** | Sample (V) | 1 | 2786.77 | 21.37 | <.0001 |
| | Size (S) | 1 | 569.46 | 4.37 | 0.0421 |
| | Germination Moisture (G_M) | 2 | 1803.50 | 13.83 | <.0001 |
| | Linear G_M | 1 | 220753.55 | 14.98 | 0.0002 |
| | Quadratic G_M | 1 | 128216.72 | 8.70 | 0.0044 |
| | Germination Days (G_D) | 3 | 372.66 | 2.86 | 0.0469 |
| | Linear G_D | 1 | 119300.63 | 8.09 | 0.0059 |
| | Quadratic G_D | 1 | 4629.35 | 0.31 | 0.5770 |
| | Cubic G_D | 1 | 1156.25 | 0.08 | 0.7803 |
| | V*S | 1 | 700.04 | 5.37 | 0.0249 |
| | V*G_M | 2 | 1200.55 | 9.21 | 0.0004 |
| | S*G_M | 2 | 212.47 | 1.63 | 0.2069 |
| | V*G_D | 3 | 76.41 | 0.59 | 0.6272 |
| | S*G_D | 3 | 39.28 | 0.3 | 0.8243 |
| | G_M*G_D | 6 | 67.80 | 0.52 | 0.7902 |
| | Error | 47 | 130.38 | | |
| **Vanilic** | Sample (V) | 1 | 162664.57 | 24.99 | <.0001 |
| | Size (S) | 1 | 74052.53 | 11.37 | 0.0015 |
| | Germination Moisture (G_M) | 2 | 40252.42 | 6.18 | 0.0041 |
| | Linear G_M | 1 | 4303836.91 | 6.46 | 0.0133 |
| | Quadratic G_M | 1 | 4479927.17 | 6.73 | 0.0116 |
| | Germination Days (G_D) | 3 | 24499.37 | 3.76 | 0.0168 |
| | Linear G_D | 1 | 5281801.01 | 7.93 | 0.0063 |
| | Quadratic G_D | 1 | 126560.23 | 0.19 | 0.6642 |
| | Cubic G_D | 1 | 1469269.27 | 2.21 | 0.1420 |
| | V*S | 1 | 751.47 | 0.12 | 0.7356 |
| | V*G_M | 2 | 20070.22 | 3.08 | 0.0552 |
| | S*G_M | 2 | 3478.48 | 0.53 | 0.5896 |
| | V*G_D | 3 | 4073.97 | 0.63 | 0.6019 |
| | S*G_D | 3 | 70.38 | 0.01 | 0.9984 |
| | G_M*G_D | 6 | 2980.65 | 0.46 | 0.8358 |
| | Error | 47 | 6510.36 | | |

**Table 10. Analysis of Variance for Total Phenolic Acids**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Total Phenolic Acids** | Sample (V) | 1 | 1058958.31 | 34.33 | <.0001 |
| | Size (S) | 1 | 250148.66 | 8.11 | 0.0065 |
| | Germination Moisture (G_M) | 2 | 278805.16 | 9.04 | 0.0005 |
| | Linear G_M | 1 | 733034.65 | 0.22 | 0.6376 |
| | Quadratic G_M | 1 | 52948155.21 | 16.17 | 0.0001 |
| | Germination Days (G_D) | 3 | 244714.21 | 7.93 | 0.0002 |
| | Linear G_D | 1 | 58729052.93 | 17.94 | <.0001 |
| | Quadratic G_D | 1 | 6586.08 | 0.00 | 0.9644 |
| | Cubic G_D | 1 | 7698067.74 | 2.35 | 0.1298 |
| | V*S | 1 | 29040.32 | 0.94 | 0.3369 |
| | V*G_M | 2 | 33495.57 | 1.09 | 0.346 |
| | S*G_M | 2 | 40298.41 | 1.31 | 0.2805 |
| | V*G_D | 3 | 15963.47 | 0.52 | 0.6723 |
| | S*G_D | 3 | 14608.55 | 0.47 | 0.7022 |
| | G_M*G_D | 6 | 15287.98 | 0.5 | 0.8084 |
| | Error | 47 | 30848.99 | | |

### 5.8. Wort Carbohydrates

Fructose, glucose, maltose, maltotriose and total wort carbohydrates were significantly affected (P≤ 0.05) by sample, germination time, and germination moisture level (Table 3). With the exception of maltose, longer germination times increased individual fermentable sugars and the total fermentable sugar. Maltose was observed to slightly decrease (1.61%) after five days' germination. Also, the content wort maltose was 1.9% more in plump grains than it was in medium kernels.

The interaction of sample variety × germination moisture had a significant impact on all individual and total fermentable sugars, except in fructose (Table 12, 13, and 14). Maltose also was significantly affected (P≤ 0.05) by interaction of germination days x germination moisture (Table 13).

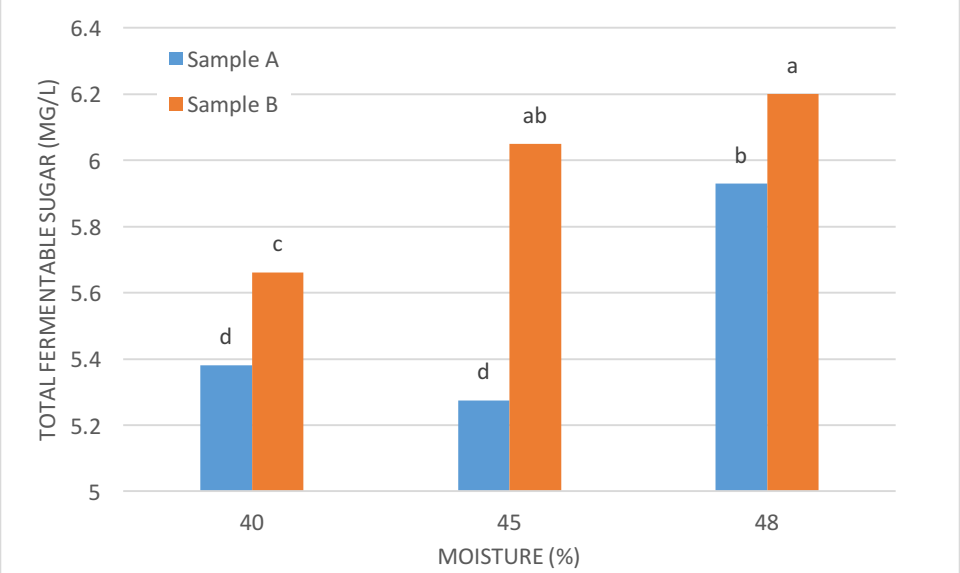


Fig. 24. Interaction between Sample and Germination Moisture for Wort Total Fermentable Sugar.
Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

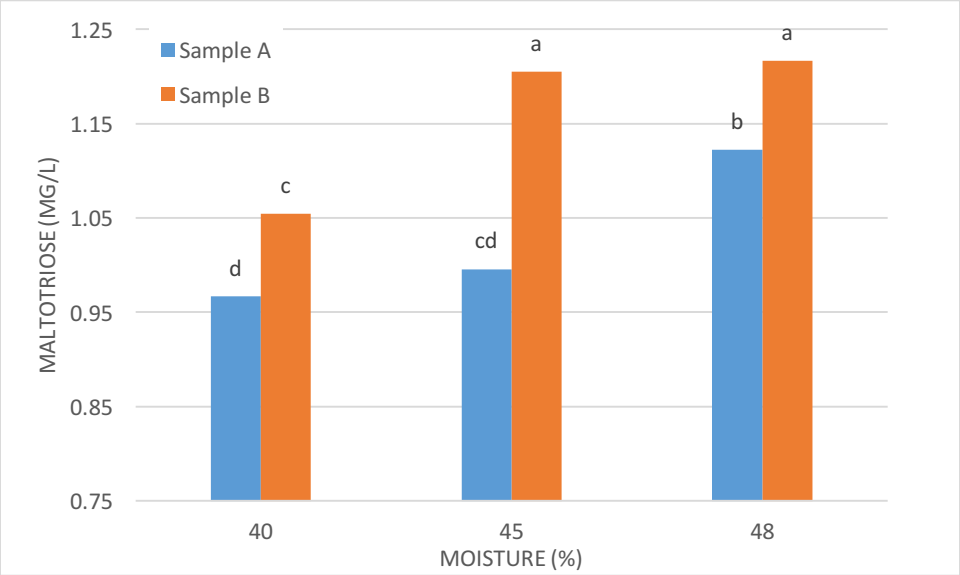


Fig. 25. Impact of Interaction between Sample and Germination Moisture for Wort Maltotriose. Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

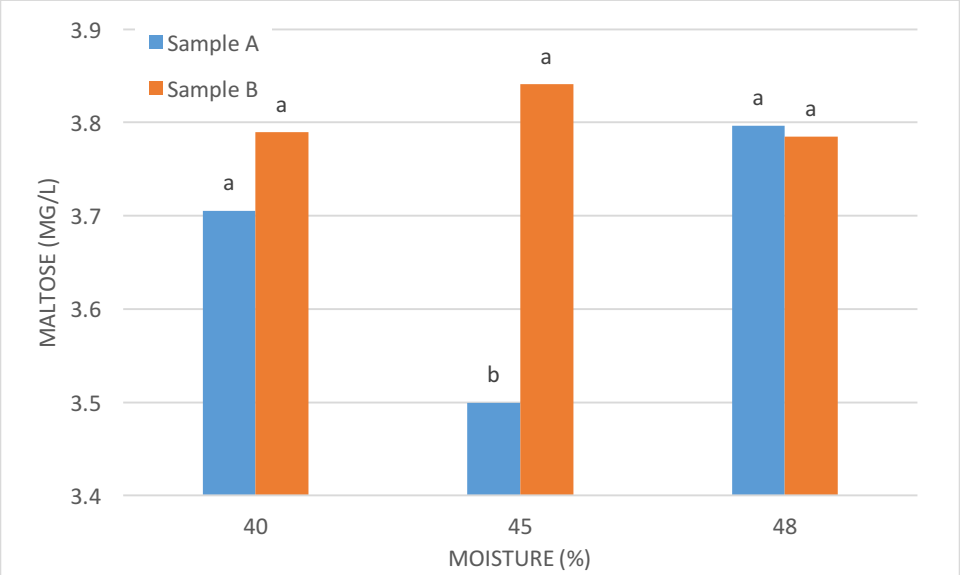


Fig. 26. Impact of Interaction between Sample and Germination Moisture for Wort Maltose. Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

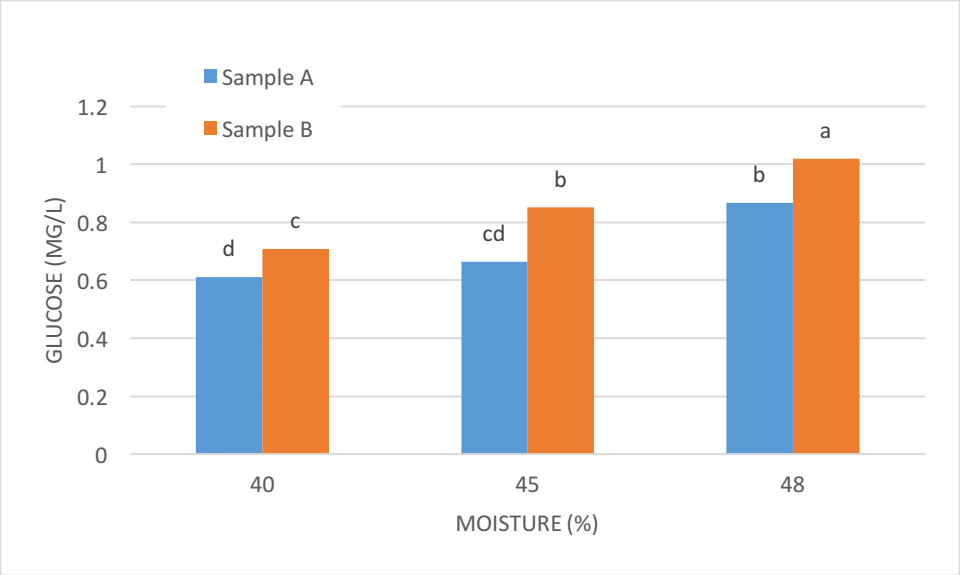


Fig. 27. Impact of Interaction between Sample and Germination Moisture for Wort Glucose. Columns within each sample level denoted by the same letter are not significantly different (P≤0.05).

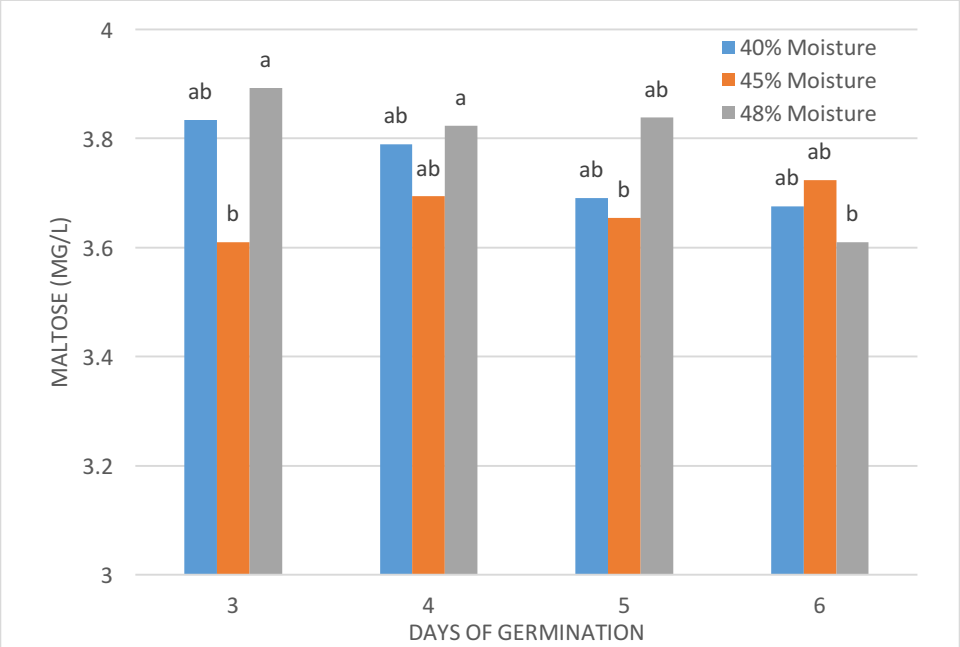


Fig. 28. Impact of Interaction between Germination Time and Germination Moisture for Wort Maltose.
Columns within each germination moisture level denoted by the same letter are not significantly different (P≤0.05).

**Table 11. Mean of Wort Fermentable Sugars (g/100mL) as Affected by Malting Operational Parameters[a]**

| | Fructose | Glucose | Maltose | Maltotriose | Total Fermentable Sugar |
|---|---|---|---|---|---|
| **Sample** | | | | | |
| A | 0.12 a | 0.71 a | 3.67 a | 1.03 a | 5.53 a |
| B | 0.15 b | 0.86 b | 3.81 b | 1.16 b | 5.97 b |
| **Germination Time** | | | | | |
| Three day | 0.11 a | 0.65 a | 3.78 a | 0.99 a | 5.53 a |
| Four day | 0.12 a | 0.75 b | 3.77 a | 1.10 b | 5.73 b |
| Five day | 0.14 b | 0.84 c | 3.73 ab | 1.14 c | 5.85 bc |
| Six day | 0.16 c | 0.91 d | 3.67 b | 1.15 c | 5.89 c |
| **Grain Size** | | | | | |
| Plump | 0.13 a | 0.80 a | 3.77 a | 1.09 a | 5.79 a |
| Medium | 0.14 a | 0.78 a | 3.70 b | 1.09 a | 5.71 a |
| **Moisture** | | | | | |
| 40% | 0.10 a | 0.66 a | 3.75 a | 1.01 a | 5.52 a |
| 45% | 0.13 b | 0.76 b | 3.67 b | 1.10 b | 5.66 b |
| 48% | 0.16 c | 0.94 c | 3.79 b | 1.17 c | 6.06 c |

[a] Means followed by the same letter are not significantly different ($P \leq 0.05$).

**Table 12. Analysis of Variance for Fermentable Sugars: Fructose and Glucose**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Fructose** | Sample (V) | 1 | 0.02 | 30.08 | <0.0001 |
| | Size (S) | 1 | 0.00 | 0.96 | 0.3300 |
| | Germination Moisture (G_M) | 2 | 0.03 | 41.53 | <0.0001 |
| | Linear G_M | 1 | 0.05 | 82.90 | <0.0001 |
| | Quadratic G_M | 1 | 0.00 | 0.16 | 0.6939 |
| | Germination Days (G_D) | 3 | 0.01 | 17.67 | <0.0001 |
| | Linear G_D | 1 | 0.03 | 52.30 | <0.0001 |
| | Quadratic G_D | 1 | 0.00 | 0.19 | 0.6655 |
| | Cubic G_D | 1 | 0.00 | 0.53 | 0.4678 |
| | V*S | 1 | 0.00 | 0.03 | 0.8727 |
| | V*G_M | 2 | 0.00 | 2.74 | 0.0713 |
| | S*G_M | 2 | 0.00 | 0.6 | 0.5505 |
| | V*G_D | 3 | 0.00 | 2.43 | 0.0722 |
| | S*G_D | 3 | 0.00 | 0.51 | 0.6739 |
| | G_M*G_D | 6 | 0.00 | 0.47 | 0.8251 |
| | Errorr | 70 | 0.0006 | | |
| **Glucose** | Sample (V) | 1 | 0.51 | 91.24 | <0.0001 |
| | Size (S) | 1 | 0.01 | 1.46 | 0.2316 |
| | Germination Moisture (G_M) | 2 | 0.66 | 118.76 | <0.0001 |
| | Linear G_M | 1 | 1.28 | 230.19 | <0.0001 |
| | Quadratic G_M | 1 | 0.04 | 7.33 | 0.0085 |
| | Germination Days (G_D) | 3 | 0.29 | 52.87 | <0.0001 |
| | Linear G_D | 1 | 0.88 | 157.75 | <0.0001 |
| | Quadratic G_D | 1 | 0.00 | 0.71 | 0.4035 |
| | Cubic G_D | 1 | 0.00 | 0.15 | 0.7027 |
| | V*S | 1 | 0.01 | 0.93 | 0.3372 |
| | V*G_M | 2 | 0.02 | 2.90 | 0.0616 |
| | S*G_M | 2 | 0.00 | 0.72 | 0.4898 |
| | V*G_D | 3 | 0.01 | 1.60 | 0.1968 |
| | S*G_D | 3 | 0.00 | 0.88 | 0.4543 |
| | G_M*G_D | 6 | 0.00 | 0.64 | 0.6994 |
| | Error | 70 | 0.0056 | | |

**Table 13. Analysis of Variance for Fermentable Sugars: Maltose and Maltotriose**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Maltose** | Sample (V) | 1 | 0.46 | 20.07 | <0.0001 |
| | Size (S) | 1 | 0.10 | 4.30 | 0.0418 |
| | Germination Moisture (G_M) | 2 | 0.24 | 5.26 | 0.0074 |
| | Linear G_M | 1 | 0.03 | 1.33 | 0.2528 |
| | Quadratic G_M | 1 | 0.21 | 9.19 | 0.0034 |
| | Germination Days (G_D) | 3 | 0.18 | 2.59 | 0.0596 |
| | Linear G_D | 1 | 0.16 | 7.15 | 0.0093 |
| | Quadratic G_D | 1 | 0.01 | 0.61 | 0.4362 |
| | Cubic G_D | 1 | 0.00 | 0.01 | 0.9267 |
| | V*S | 1 | 0.01 | 0.41 | 0.5263 |
| | V*G_M | 2 | 0.27 | 11.73 | <0.0001 |
| | S*G_M | 2 | 0.04 | 1.55 | 0.2195 |
| | V*G_D | 3 | 0.04 | 1.82 | 0.1511 |
| | S*G_D | 3 | 0.01 | 0.60 | 0.6148 |
| | G_M*G_D | 6 | 0.07 | 2.88 | 0.0145 |
| | Error | 70 | 0.0227 | | |
| **Maltotriose** | Sample (V) | 1 | 0.41 | 105.00 | <0.0001 |
| | Size (S) | 1 | 0.00 | 0.00 | 0.9987 |
| | Germination Moisture (G_M) | 2 | 0.20 | 52.17 | <0.0001 |
| | Linear G_M | 1 | 0.40 | 103.75 | <0.0001 |
| | Quadratic G_M | 1 | 0.00 | 0.58 | 0.4499 |
| | Germination Days (G_D) | 3 | 0.13 | 33.73 | <0.0001 |
| | Linear G_D | 1 | 0.34 | 86.41 | <0.0001 |
| | Quadratic G_D | 1 | 0.06 | 14.49 | 0.0003 |
| | Cubic G_D | 1 | 0.00 | 0.28 | 0.5986 |
| | V*S | 1 | 0.01 | 1.54 | 0.2186 |
| | V*G_M | 2 | 0.04 | 9.69 | 0.0002 |
| | S*G_M | 2 | 0.00 | 0.29 | 0.7508 |
| | V*G_D | 3 | 0.01 | 1.52 | 0.2177 |
| | S*G_D | 3 | 0.00 | 0.93 | 0.4323 |
| | G_M*G_D | 6 | 0.00 | 1.14 | 0.3473 |
| | Error | 70 | 0.0039 | | |

**Table 14. Analysis of Variance for Total Fermentable Sugars**

| Dependent Viable | Source | DF | Mean Square | *F* value | Pr > F |
|---|---|---|---|---|---|
| **Total Fermentable Sugar** | Sample (V) | 1 | 4.70 | 64.31 | <0.0001 |
| | Size (S) | 1 | 0.14 | 1.95 | 0.1668 |
| | Germination Moisture (G_M) | 2 | 2.54 | 34.82 | <0.0001 |
| | Linear G_M | 1 | 4.72 | 64.69 | <0.0001 |
| | Quadratic G_M | 1 | 0.36 | 4.95 | 0.0293 |
| | Germination Days (G_D) | 3 | 0.62 | 8.46 | <0.0001 |
| | Linear G_D | 1 | 1.69 | 23.12 | <0.0001 |
| | Quadratic G_D | 1 | 0.17 | 2.27 | 0.1360 |
| | Cubic G_D | 1 | 0.00 | 0.00 | 0.9980 |
| | V*S | 1 | 0.00 | 0.05 | 0.8316 |
| | V*G_M | 2 | 0.67 | 9.13 | 0.0003 |
| | S*G_M | 2 | 0.01 | 0.07 | 0.9314 |
| | V*G_D | 3 | 0.06 | 0.81 | 0.4933 |
| | S*G_D | 3 | 0.06 | 0.84 | 0.4777 |
| | G_M*G_D | 6 | 0.11 | 1.56 | 0.1721 |
| | Error | 70 | 0.0730 | | |

## 5.9. Stepwise Linear Regression

Stepwise linear regression analysis was used to determine the contribution of various traits to important malt quality parameters such as malt loss and extract (Table 15). Germination moisture was the most important factor for malt loss, diastatic power, α-amylase, wort viscosity, AX, and wort β-glucan, explaining 75%, 40%, 50%, 26%, 1%, and 64% of the variation observed in the operational parameters, respectively. Sample was the most important factor for extract and wort soluble protein, and it explained 31% and 39% of the variation

Germination moisture and time were able to explain 95% of the variation observed for malt loss. Only a small portion of the variability (36%) observed for extract could be explained using the parameters measured, and sample and germination time were most important. Sixty-

four persent of the variation observed in viscosity was explained by moisture, sample and germination time.

### 5.10. Relationships between Rye Malt Quality Parameters

Correlations were determined to relate all of the wort characteristics. In Table 18, malt loss was strongly and positively correlated with α-amylase (r=0.84), diastatic power (0.63), and FAN. Malt loss was correlated negatively with wort viscosity (r=-0.56) and wort β-glucan (r=-0.87). Malt losses are known to increases with germination time and increased endosperm modification. Beta-glucan is degraded to a greater extent as germination times increase, and α-amylase and proteolytic activies increase. Alpha-amylase was positively correlated with diastatic power, extract, and FAN, and negatively correlated with wort viscosity (r=0.77) and wort β-glucan (r=0.83). Diastatic power was been found have the positively correlated with FAN, and negatively correlation with wort viscosity, wort β-glucan, the ratio of wort soluble protein and total protein (S/T), and ratio of arabinose and xylose (A/X). As expected, extract was positively correlated with S/T, FAN, and negatively correlated with wort viscosity. It is possible because the higher extract increased the soluble protein and FAN in the wort, and decreased the rye wort viscosity. On the other hand, the wort viscosity was correlated positively with wort β-glucan (r=0.76), and negatively with the S/T.

**Table 15. Partial (Part.) and Cumulative (Cum.) $R^2$ Values from Stepwise Regression Analysis Across Operational Parameters for Malt Loss, Extract, DP, α-Amylase, Wort Soluble Protein, S/T, Wort Viscosity, FAN, AX, and Wort β-Glucan [a]**

| | Malt Loss | | Extract | | Diastatic Power | | Alpha-Amylase | | Wort Soluble Protein | | S/T | | Wort Viscosity | | FAN | | AX | | Wort β-Glucan | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** |
| **Sample** | NS | … | 28 | 28 (1) | 8 | 58 (2) | 1 | 85 (3) | 41 | 41 (1) | NS | … | 23 | 47 (2) | NS | … | 0 | 1 (2) | NS | … |
| **Germination time** | 20 | 95 (2) | 4 | 32 (2) | NS | … | 34 | 83 (2) | 3 | 51 (3) | NS | … | 18 | 65 (3) | NS | … | 0 | 1 (3) | 22 | 86 (2) |
| **Grain Size** | 0 | 95 (3) | NS | … | NS | … | 1 | 86 (4) | 7 | 48 (2) | 20 | 20 (1) | NS | … | 14 | 33 (2) | NS | … | NS | … |
| **Moisture** | 75 | 75 (1) | NS | … | 50 | 50 (1) | 50 | 50 (1) | NS | … | NS | … | 24 | 24 (1) | 19 | 19 (1) | 1 | 1 (1) | 64 | 64 (1) |

[a] NS indicated that the factor did not help explain the variation in the specified malt quality parameter at $P< 0.05$. Numbers in parentheses indicate the order in which parameters were added to the model.

**Table 16. Partial (Part.) and Cumulative (Cum.) $R^2$ Values from Stepwise Regression Analysis Across Operational Parameters for Phenolic Acids [a]**

| | Caffeic | | Ferulic | | p-Coumorre | | Sinapinic | | Syringic | | Vanillic | | Gallic | | Catechin | | Total Phenolic Acids | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** |
| **Sample** | 19 | 19 (1) | 19 | 19 (1) | 13 | 13 (1) | NS | … | 8 | 16 (2) | 17 | 17 (1) | 5 | 5 (1) | NS | … | 20 | 20 (1) |
| **Modification [b]** | 10 | 29 (2) | 17 | 35 (2) | 5 | 27 (3) | 2 | 15 (3) | 8 | 8 (1) | 6 | 35 (4) | 4 | 9 (2) | 2 | 8 (2) | 12 | 32 (2) |
| **Grain Size** | NS | … | 3 | 38 (3) | 3 | 29 (4) | 4 | 13 (2) | NS | … | 7 | 24 (2) | 3 | 15 (4) | NS | … | 4 | 36 (3) |
| **Moisture** | 2 | 31 (3) | 2 | 40 (4) | 9 | 22 (2) | 9 | 9 (1) | 2 | 18 (3) | 5 | 29 (3) | 3 | 12 (3) | 7 | 7 (1) | NS | … |

[a] NS indicated that the factor did not help explain the variation in the specified malt quality parameter at $P< 0.05$. Numbers in parentheses indicate the order in which parameters were added to the model.

[b] Days of germination.

**Table 17. Partial (Part.) and Cumulative (Cum.) R 2 Values from Stepwise Regression Analysis Across Operational Parameters for Fermentable Sugars [a]**

| | Fructose | | Glucose | | Maltose | | Maltotriose | | Total Fermentable Sugar | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** | **Part.** | **Cum.** |
| **Sample** | 12 | 64 (3) | 16 | 80 (3) | 14 | 14 (1) | 26 | 26 (1) | 24 | 24 (1) |
| **Modification [b]** | 19 | 52 (2) | 26 | 64 (2) | 3 | 17 (2) | 22 | 72 (3) | 9 | 55 (3) |
| **Grain Size** | NS | … | NS | … | 2 | 19 (3) | NS | … | NS | … |
| **Moisture** | 33 | 33 (1) | 38 | 38 (1) | NS | … | 24 | 50 (2) | 22 | 46 (2) |

[a] NS indicated that the factor did not help explain the variation in the specified malt quality parameter at P< 0.05. Numbers in parentheses indicate the order in which parameters were added to the model.

[b] Days of germination.

**Table 18. Correlation Coefficients of Rye Wort Characteristics (N=96) [a]**

| Factor | Alpha-Amylase | Diastatic Power | Extract | Wort Viscosity | Wort Soluble Protein | S/T | FAN | Wort β-Glucan | AX | A/X | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Malt Loss** | 0.84 *** | 0.63 *** | 0.04 | -0.56 *** | 0.12 | 0.06 | 0.39 *** | -0.87 *** | -0.10 | -0.04 | |
| **Alpha-Amylase** | 1 | 0.60 *** | 0.23 * | -0.77 *** | -0.01 | 0.10 | 0.33 *** | -0.83 *** | -0.06 | 0.00 | |
| **Diastatic Power** | | 1 | -0.07 | -0.22 * | 0.06 | -0.25 * | 0.49 *** | -0.48 *** | -0.10 | -0.22 | * |
| **Extract** | | | 1 | -0.22 * | -0.07 | 0.44 *** | 0.42 *** | -0.11 | 0.16 | 0.00 | |
| **Wort Viscosity** | | | | 1 | 0.15 | -0.25 * | -0.18 | 0.76 *** | 0.02 | -0.01 | |
| **Wort Soluble Protein** | | | | | 1 | 0.66 *** | 0.14 | -0.11 | -0.02 | 0.14 | |
| **S/T** | | | | | | 1 | 0.13 | -0.18 | 0.02 | 0.25 | * |
| **FAN** | | | | | | | 1 | -0.39 *** | 0.07 | -0.26 | * |
| **Wort β-Glucan** | | | | | | | | 1 | 0.03 | 0.01 | |
| **AX** | | | | | | | | | 1 | -0.43 | *** |
| **A/X** | | | | | | | | | | 1 | |

[a] *, **, ***, significative at 0.05, 0.001, and 0.0001probability levels respectively

# 6. SUMMARY AND CONCLUSIONS

In this study, grain size was found to have important influence on the quality parameters, with the exception of S/T. Germination moisture did not influence extract level, but increasing moisture was found to increase the malt loss and decrease viscosity. Viscosity was lowest (3.83 cP) after six days of germination at 48% moisture. However, there was no significant difference between viscosity for samples germinated for five or six days. Germination time increased extract values only up to 4 days. As expected, a longer germination times contributed to lower viscosity, but also greater malt loss. Significant interactions between some parameters confounded the interpretation of data, but in general high extract and lower viscosity were achieved by malting for at least 4 days at higher moisture. Based on the correlation coefficient analysis, the higher extracts were related to higher soluble protein and FAN in the wort, although viscosities were reduced. Malt loss was largest (22.16%) after six day of germination at 48% moisture. Moisture explained 75% variation, and germination time explained another 20% variation.

Based upon results of this study we recommend 5 days of germination at 45% moisture for the future evaluation of rye genotypes for malt quality. 5 days will provide adequate modification and extract for most types. While viscosity can be reduced at 48% moisture, mallt losses, and perhaps FAN, increase to unacceptable levels. In addition, conversations with craft maltsters have indicated that rye becomes “sticky” and very difficult to handle during germination, when steeped to the higher moisture level.

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# APPENDIX

## Table A1. 2014 Rye Grain Analysis

| Number | Variety | Sample Source | Crop Year | Growth Location | Variety Origin | Intended Use |
|---|---|---|---|---|---|---|
| 302 | Elbon | University of Minnesota-Crookston | 2014 | Crookston, MN | Samuel Noble Foundation | Cover Crop - Forage |
| 331 | Elbon | Oklahoma, Foavdation Seed | 2014 | Oklahoma | Samuel Noble Foundation | Cover Crop - Forage |
| 324 | Hancock | NDSU Carrington | 2014 | Carrington, ND | - | Cover Crop - Forage |
| 332 | Maton | Oklahoma, Foavdation Seed | 2014 | Oklahoma | Samuel Noble Foundation | Cover Crop - Forage |
| 303 | Maton II | University of Minnesota-Crookston | 2014 | Crookston, MN | OAES - Samuel Noble Foundation | Cover Crop - Forage |
| 333 | Maton II | Oklahoma, Foavdation Seed | 2014 | Oklahoma | Agriculture Canada | Cover Crop - Forage |
| 346 | Maton ll | Cornell University | 2014 | Ithica, NY [a] | OAES - Samuel Noble Foundation | Cover Crop - Forage |
| 305 | Oklon | University of Minnesota-Crookston | 2014 | Crookston, MN | Samuel Noble Foundation | Cover Crop - Forage |
| 334 | Oklon | Oklahoma, Foavdation Seed | 2014 | Oklahoma | Samuel Noble Foundation | Cover Crop - Forage |
| 309 | Wrens Abruzzi | University of Minnesota-Crookston | 2014 | Crookston, MN | University of Georgia | Cover Crop - Forage |
| 301 | Aroostook | University of Minnesota-Crookston | 2014 | Crookston, MN | USDA-NRCS | Cover Crop - Grain |
| 312 | Aroostook | Valley Malt-Andrea Stanley | 2014 | Benedicta, ME | USDA-NRCS | Cover Crop - Grain |
| 321 | Aroostook | NDSU Carrington | 2014 | Carrington, ND | USDA-NRCS | Cover Crop - Grain |
| 322 | Dacold | NDSU Carrington | 2014 | Carrington, ND | NDSU USDA | Cover Crop - Grain |
| 323 | Dacold old | NDSU Carrington | - | Carrington, ND | NDSU USDA | Cover Crop - Grain |
| 330 | DR02 (Dylan) | NDSU Carrington | 2014 | Carrington, ND | NDSU | Cover Crop - Grain |
| 307 | Rymin | University of Minnesota-Crookston | 2014 | Crookston, MN | University of Minnesota | Cover Crop - Grain |
| 327 | Rymin | NDSU Carrington | 2014 | Carrington, ND | University of Minnesota | Cover Crop - Grain |

**Table A1. 2014 Rye Grain Analysis (continued)**

| Number | Variety | Sample Source | Crop Year | Growth Location | Variety Origin | Intended Use |
|---|---|---|---|---|---|---|
| 308 | Spooner | University of Minnesota-Crookston | 2014 | Crookston, MN | University of Wisconsin | Cover Crop - Grain |
| 328 | Spooner | NDSU Carrington | 2014 | Carrington, ND | University of Wisconsin | Cover Crop - Grain |
| 329 | Wheeler | NDSU Carrington | 2014 | Carrington, ND | Michigan Agricultural Experiment Station | Cover Crop - Grain |
| 315 | AC Hazlet | Far North Spirits | 2014 | Hallock, MN | Agriculture and Agri-Food Canada | Grain |
| 349 | AC Hazlet | Cornell University | 2014 | Ithica, NY | Agriculture and Agri-Food Canada | Grain |
| 352 | AC Remmington | Cornell University | 2014 | Ithica, NY | Agriculture and Agri-Food Canada | Grain |
| 325 | AZ Hazlet | NDSU Carrington | 2014 | Carrington, ND | Agriculture and Agri-Food Canada | Grain |
| 313 | Danko | Valley Malt | 2014 | Ithaca, NY | Polish Plant Breeding Institute | Grain |
| 314 | Danko | Valley Malt | 2014 | Manitoba | Polish Plant Breeding Institute | Grain |
| 339 | Danko | Cornell University | 2014 | Ithica, NY | Polish Plant Breeding Institute | Grain |
| 304 | Musketeer | University of Minnesota-Crookston | 2014 | Crookston, MN | Agriculture Canada | Grain |
| 326 | Musketeer | NDSU Carrington | 2014 | Carrington, ND | Agriculture Canada | Grain |
| 306 | Prima | University of Minnesota-Crookston | 2014 | Crookston, MN | Agriculture Canada | Grain |
| 335 | Unknown, Winter Rye | Embden Grain Company, ND | 2014 | Iowa | - | Grain |
| 348 | AC Rifle | Cornell University | 2014 | Ithica, NY | Agriculture and Agri-Food Canada | Grain |
| 337 | Brasetto | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 340 | Brasetto | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 343 | Brasetto | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 336 | KWS Bono | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 345 | KWS H-139 | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |

**Table A1. 2014 Rye Grain Analysis (continued)**

| Number | Variety | Sample Source | Crop Year | Growth Location | Variety Origin | Intended Use |
|---|---|---|---|---|---|---|
| 350 | KWS H-140 | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 347 | KWS H-141 | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 353 | KWS H-142 | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 341 | KWS H-144 | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 344 | KWS Magnifico | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 338 | KWS Rhavo | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 351 | Palazzo | Cornell University | 2014 | Ithica, NY | KWS SAAT SE | Grain hybrid |
| 316 | unavaialble | Deer Creek Malt | 2014 | Pennsylvania | Germany | Grain hybrid |
| 317 | unavaialble | Deer Creek Malt | 2014 | Pennsylvania | Germany | Grain hybrid |
| 311 | (Unknown-heritage) | Grower | - | Calverton. Long Island, NY | - | unknown |

**Table A1. 2014 Rye Grain Analysis (continued)**

| Number | Moist. (%) | Kernel Assortment 7/64 | 6/64 | 5/64 | % Plump | % Thin | Test Weight lbs/bu | 1000 KWT | Protein (%) | Stirring Number |
|---|---|---|---|---|---|---|---|---|---|---|
| 302 | 6.6 | 0.1 | 1.1 | 32.6 | 1.2 | 66.2 | 50.1 | 15.2 | 19.6 | 91 |
| 331 | - | 0.2 | 21.1 | 64.8 | 21.3 | 13.9 | 56.1 | 22.9 | 16.0 | 146 |
| 324 | - | 1.4 | 40.2 | 50.6 | 41.6 | 7.8 | 57.0 | 27.3 | 11.2 | 174 |
| 332 | - | 0.0 | 3.6 | 52.0 | 3.6 | 44.4 | 54.3 | 17.1 | 14.0 | 168 |
| 303 | 7.0 | 0.2 | 9.3 | 61.6 | 9.5 | 28.9 | 54.5 | 21.3 | 16.2 | 141 |
| 333 | - | 0.2 | 8.4 | 63.9 | 8.6 | 27.5 | 55.8 | 19.2 | 17.2 | 146 |
| 346 | - | 1.0 | 28.8 | 58.0 | 29.8 | 12.2 | 52.9 | 29.9 | 13.9 | 73 |
| 305 | 7.3 | 0.1 | 2.7 | 50.7 | 2.8 | 46.5 | 55.3 | 19.1 | 17.5 | 112 |
| 334 | - | 0.0 | 2.6 | 48.7 | 2.6 | 48.7 | 55.1 | 16.9 | 16.7 | 139 |
| 309 | 7.5 | 0 | 3.9 | 41.9 | 3.9 | 54.2 | 50.4 | 17.3 | 17.2 | 94 |
| 301 | 7.4 | 0.7 | 11.6 | 57.6 | 12.3 | 30.1 | 53.3 | 22.2 | 13.7 | 67 |
| 312 | 14.4 | 5.7 | 55.7 | 35 | 61.4 | 3.6 | 54.0 | 28.7 | 11.4 | 46 |
| 321 | - | 0.0 | 7.9 | 61.8 | 7.9 | 30.3 | 55.1 | 19.8 | 12.9 | 131 |
| 322 | - | 1.9 | 27.5 | 51.3 | 29.4 | 19.3 | 54.6 | 21.4 | 12.6 | 140 |
| 323 | - | 0.9 | 23.5 | 55.9 | 24.4 | 19.7 | NE [a] | 19.9 | 9.8 | 171 |
| 330 | - | 6.1 | 57.0 | 32.8 | 63.1 | 4.1 | 57.9 | 28.5 | 12.7 | 147 |
| 307 | 8.1 | 5.9 | 51.3 | 35.3 | 57.2 | 7.5 | 57.5 | 24.6 | 10.5 | 90 |
| 327 | - | 0.0 | 7.2 | 58.3 | 7.2 | 34.5 | NE | 18.7 | 13.7 | 166 |
| 308 | 7.5 | 0.9 | 31.2 | 57.6 | 32.1 | 10.3 | 56.4 | 26.3 | 13.9 | 125 |
| 328 | - | 0.7 | 27.6 | 59.0 | 28.3 | 12.7 | 56.6 | 25.4 | 13.4 | 146 |
| 329 | - | 5.3 | 63.0 | 28.8 | 68.3 | 2.9 | NE | 32.8 | 12.8 | 177 |
| 315 | - | 32.2 | 57.7 | 9.8 | 89.9 | 0.3 | 58.1 | 34.4 | 12.1 | 19 |
| 349 | - | 33.6 | 56.4 | 8.8 | 90 | 1.2 | 55.1 | 32.8 | 9.1 | 53 |
| 352 | - | 2.3 | 30.3 | 53.6 | 32.6 | 13.8 | 51.8 | 25.4 | 11.2 | 66 |
| 325 | - | 16.8 | 61.7 | 18.7 | 78.5 | 2.8 | NE | 32.1 | 10.5 | 72 |
| 313 | 14.6 | 16.7 | 62.9 | 17.5 | 79.6 | 2.9 | 54.6 | 34.4 | 11.3 | 41 |
| 314 | 13.4 | 7.2 | 61.4 | 30.2 | 68.6 | 1.2 | 59.8 | 30.9 | 11.1 | 105 |
| 339 | - | 7.7 | 64.0 | 26.7 | 71.7 | 1.6 | 55.6 | 29.9 | 9.3 | 92 |
| 304 | 7.9 | 4.4 | 49.5 | 44.2 | 53.9 | 1.9 | 58.6 | 32.3 | 12.1 | 132 |
| 326 | - | 4.7 | 47.7 | 39.4 | 52.4 | 8.2 | NE | 30.6 | 11.4 | 161 |
| 306 | 7.7 | 11.9 | 54.5 | 30.6 | 66.4 | 3 | 57.3 | 30.8 | 12.0 | 131 |
| 335 | - | 5.8 | 36.3 | 42.6 | 42.1 | 15.3 | 55.4 | 25.1 | 10.9 | 87 |
| 348 | - | 3.9 | 33.6 | 46.7 | 37.5 | 15.8 | 51.2 | 22.8 | 12.3 | 32 |
| 337 | - | 7.9 | 66.4 | 23.5 | 74.3 | 2.2 | 54.2 | 30.8 | 7.7 | 133 |
| 340 | - | 12.0 | 67.2 | 19.9 | 79.2 | 0.9 | 54.1 | 31.3 | 8.1 | 151 |
| 343 | - | 8.1 | 67.8 | 22.1 | 75.9 | 2.0 | 54.1 | 31.6 | 8.1 | 158 |
| 336 | - | 3.2 | 57.7 | 36.5 | 60.9 | 2.6 | 55.9 | 30.2 | 7.9 | 136 |
| 345 | - | 9.3 | 66.9 | 22.3 | 76.2 | 1.5 | 54.4 | 31.1 | 8.3 | 114 |
| 350 | - | 7.7 | 68.7 | 22.2 | 76.4 | 1.4 | 54.0 | 30.5 | 8.5 | 121 |
| 347 | - | 9.2 | 66.0 | 22.9 | 75.2 | 1.9 | 54.0 | 30.6 | 8.2 | 120 |
| 353 | - | 7.2 | 63.3 | 28.0 | 70.5 | 1.5 | 55.0 | 30.9 | 8.5 | 109 |
| 341 | - | 2.1 | 56.2 | 40.0 | 58.3 | 1.7 | 54.3 | 27.6 | 7.6 | 132 |
| 344 | - | 7.1 | 61.2 | 29.4 | 68.3 | 2.3 | 54.9 | 30.7 | 8.0 | 116 |

[a] Not Enough

**Table A1. 2014 Rye Grain Analysis (continued)**

| Number | Moist. (%) | Kernel Assortment 7/64 | 6/64 | 5/64 | % Plump | % Thin | Test Weight lbs/bu | 1000 KWT | Protein (%) | Stirring Number |
|---|---|---|---|---|---|---|---|---|---|---|
| 338 | - | 6.5 | 64.7 | 27.0 | 71.2 | 1.8 | 55.3 | 31.3 | 8.8 | 155 |
| 351 | - | 11.0 | 66.0 | 21.4 | 77 | 1.6 | 54.3 | 32.1 | 8.0 | 135 |
| 316 | - | 8.7 | 53.7 | 29.1 | 62.4 | 8.5 | 50.9 | 27.1 | 11.3 | 144 |
| 317 | - | 16.9 | 62.1 | 19.5 | 79 | 1.5 | 57.5 | 37.7 | 9.4 | 153 |
| 311 | 12.8 | 0.6 | 27.8 | 58.8 | 28.4 | 12.8 | 56.9 | 22.9 | 10.3 | 127 |

**Table A1. 2014 Rye Grain Analysis (continued)**

| Number | Germination as is (%) | Germination Thin Kernels Removed | Germination difference | DON (mg/L) | Seed Available [a] (g) |
|---|---|---|---|---|---|
| 302 | 88 | 92 | 4 | 0.00 | 116.28 |
| 331 | - | 100 | - | 0.00 | 258.30 |
| 324 | - | 96 | - | 0.01 | 111.00 |
| 332 | - | 93 | - | 0.01 | 166.80 |
| 303 | 85 | 91 | 6 | 0.00 | 295.87 |
| 333 | - | 98 | - | 0.00 | 217.50 |
| 346 | - | 91 | - | 0.09 | 263.40 |
| 305 | 88 | 81 | -7 | 0.00 | 201.30 |
| 334 | - | 92 | - | 0.63 | 153.90 |
| 309 | 90 | 93 | 3 | 0.00 | 108.97 |
| 301 | 91 | 92 | 1 | 0.49 | 262.72 |
| 312 | 94 | 95 | 1 | 2.99 | 289.20 |
| 321 | - | 98 | - | 0.15 | 111.00 |
| 322 | - | 98 | - | 0.00 | 103.00 |
| 323 | - | 73 | - | 0.01 | 97.00 |
| 330 | - | 96 | - | 0.00 | 7414.84 |
| 307 | 97 | 98 | 1 | 0.17 | 300.00 |
| 327 | - | 100 | - | 0.37 | 102.00 |
| 308 | 93 | 96 | 3 | 0.20 | 300.00 |
| 328 | - | 98 | - | 0.01 | 116.00 |
| 329 | - | 88 | - | 0.09 | 106.00 |
| 315 | - | 83 | - | 0.37 | 5833.75 |
| 349 | - | 88 | - | 1.32 | 297.73 |
| 352 | - | 91 | - | 0.37 | 258.60 |
| 325 | - | 98 | - | 0.00 | 99.00 |
| 313 | 78 | 74 | -4 | 0.10 | 1395.33 |
| 314 | 93 | 98 | 5 | 0.02 | 1252.78 |
| 339 | - | 98 | - | 0.43 | 296.41 |
| 304 | 91 | 96 | 5 | 0.00 | 300.00 |
| 326 | - | 96 | - | 0.00 | 93.00 |
| 306 | 99 | 97 | -2 | 0.01 | 300.00 |
| 335 | - | 91 | - | 0.70 | 16174.48 |
| 348 | - | 93 | - | 0.32 | 252.60 |
| 337 | - | 98 | - | 0.27 | 294.29 |
| 340 | - | 96 | - | 0.60 | 296.41 |
| 343 | - | 95 | - | 1.30 | 294.99 |
| 336 | - | 95 | - | 0.40 | 293.53 |
| 345 | - | 93 | - | 1.74 | 295.94 |
| 350 | - | 93 | - | 0.49 | 296.50 |
| 347 | - | 94 | - | 1.02 | 296.64 |
| 353 | - | 99 | - | 0.64 | 295.50 |
| 341 | - | 96 | - | 1.20 | 290.62 |
| 344 | - | 96 | - | 0.53 | 293.10 |
| 338 | - | 96 | - | 0.27 | 294.97 |
| 351 | - | 92 | - | 1.23 | 295.20 |
| 316 | - | 96 | - | 0.01 | 568.22 |
| 317 | - | 80 | - | 0.05 | 532.89 |
| 311 | 96 | 97 | 2 | 0.14 | 429.90 |

[a] After removed the dockage