Final Technical Report

Developing Quality Analysis Protocols for Non-Barley Grains Used in Craft Beverages (GG 23*2216)

Drs. James DeDecker[1] and Harmonie Bettenhausen[2]

1) Michigan State University – Upper Peninsula Research and Extension Center
2) Hartwick Center for Craft Food and Beverage

Abstract

Increasingly, non-barley grains are in demand by maltsters, brewers, and distillers seeking to create innovative, differentiated products that appeal to a wider base of consumers. Application of grain and malt quality testing protocols designed for malting barley to non-barley grains can generate misleading results. We adapted seven different ASBC quality analysis protocols to five different non-barley grain species of interest to the Michigan craft beverage industries. Our results suggest that ASBC barley methods are applicable to non-barley grains in some limited cases, but they generally require special handing to generate accurate quality assessments that maximize beverage yield, quality and marketability.

Background

Increasingly, non-barley grains are in demand by maltsters, brewers, and distillers seeking to create innovative, differentiated products that appeal to a wider base of consumers. In 2022, 21.6% of grain samples received by the Michigan State University – Upper Peninsula Research and Extension Center Grain Quality Lab (MSU-UPREC Lab) were species other than barley. The largest number of non-barley samples received consisted of wheat followed by rye, oats, corn and specialty grains like buckwheat. In Michigan, tax incentives under House Bill 4842 are further increasing demand for a wide range of local grains beyond malting barley that can be used in distilled spirits.

Crop quality for grains and other ingredients used in craft beverage production is a stated priority of the MI Craft Beverage Council. Currently, two university-based labs are providing grain and malt quality analysis services for the majority of craft beverage stakeholders in the Eastern U.S.; the MSU-UPREC Lab and the Hartwick College Center for Craft Food and Beverage (Hartwick Lab). Both labs follow protocols developed by the American Society of Brewing Chemists (ASBC) to test grain and malt against quality standards designed to enhance beverage product performance and protect consumer safety. However, quality standards and methods for the beverage industry have been developed by the ASBC with a primary focus on malting barley. Guidance on adjunct cereals (wheat, corn, rye, oats) is limited, and no information is available for specialty grains like buckwheat, which are commonly used in Gluten Free brewing.

Applying quality standards and methods developed for malting barley to non-barley grains can generate results that are incomplete or incorrect. Such information can be misleading for decision-makers looking to determine if a lot of grain is suitable for its intended beverage use and/or how the malting, brewing or distilling process can be optimized to leverage positive attributes of the grain while mitigating any quality challenges. For example, germination energy and capacity are critical variables estimating the ability of a grain lot to germinate quickly and completely in the malting process. Germination capacity (GC) in malting barley is measured by sprouting barley kernels in 100 mL of 0.75% H2O2 solution for 48 hours. Experience in our labs has shown that wheat generally takes longer to germinate than barley, up to 96 hours. Measuring the GC of wheat at 48 hours can lead to inaccurately low germination counts. This information could cause maltsters to unnecessarily reject a lot of wheat or incorrectly adjust the malting process with detrimental consequences across the craft beverage value chain.

Both the MSU-UPREC Lab and Hartwick Lab regularly receive samples of non-barley grains for quality testing. To date, we have worked individually with clients submitting these samples to identify relevant and meaningful quality factors to measure and then interpret the results to the best of our abilities. The relevance, validity and reproducibility of the ASBC methods for malting barley applied to non-barley grains in this way has not been formally studied. However, the growing volume and diversity of non-barley grains we are encountering suggests that a more formal and coordinated approach is necessary to protect and enhance the quality of craft beverage products in Michigan and beyond.

Goals and Objectives

1. Develop, optimize and validate the following grain and malt quality analysis standards and methods for non-barley grains. Hypothesis: Appropriate methods will vary significantly among non-barley grain species.

- Kernel assortment
- Germination energy & capacity (GE & GC)
- Pre-harvest sprout (RVA)
- β-glucan
- Free amino nitrogen (FAN)
- Enzyme activity (Diastatic Power & α-amylase)
- Fermentability & Predicted spirit yield (PSY)
- Sugar profile (Saccharides & Arabinoxylans)

2. Conduct a ring test to validate our methods by measuring accuracy and repeatability among participating labs. Hypothesis: The CV among labs will be less than 15.00.

3. Expand the analysis services offered by our labs to include non-barley grains. Publish new quality analysis protocols for non-barley grains to be used by other public labs and private industry.

Methods

Samples of high-quality barley and non-barley grains (ND Genesis, LCS Violetta and Buck Naked barley, Con V 22 V and VNS buckwheat, Ohio Blue corn, Rushmore and Streaker oats, KWS Serafino and ND Gardener cereal rye, and LCS 3334 wheat) were sourced from industry partners or fellow researchers and distributed among the collaborating labs. Efforts were made to select entries representing the diversity of grain types (species, variety, winter/spring, hulled/hulless, etc.) currently used by the MI craft beverage industries. Initially, each lot of grain was tested using ASBC methods designed for malting barley and the results compared between labs. Then, information from our literature review and experience was used to revise the ASBC protocols specifically for each of the five non-barley species and then compare results of these updated methods to results obtained using the standard ASBC protocols for barley.

Kernel assortment

Average seed size and established USDA assortment categories for the non-barley grains were obtained from the literature. This information was used to select seven appropriately sized Sortimat sieves/screens to segregate the largest, middle and thinnest thirds of an average grain sample, depending on species and habit. 100 g samples of each grain species/variety were run through each sieve size a minimum of four times recoding the weight and kernel count of each resulting fraction. Sieves similar in size to those used for malting barley were found to be appropriate for determining the percentage of plump kernels in other small grains (2.78 and 2.38 mm), but accurately segregating the thin fraction of wheat, oats and rye required a smaller 0.064 mm screen (vs. 1.98 mm in barley). Corn seed is much larger than the other species of interest, so entirely different sieves were required for kernel assortment in corn (8.5, 6.5 and 4.75 mm).

Germination energy & capacity (GE & GC)

Germination testing guidelines, including water volume, temperature and test duration, were obtained from the Association of Official Seed Analysts (AOSA) as a starting point for each species of interest. Water volumes of 4, 8, 10 and 12 ml per 100 seeds were compared for small grains and buckwheat, while corn samples received 8, 10 and 12 ml per 50 seeds. Triplicate samples were placed in petri dishes with two pieces of filter paper and the assigned volume of distilled water. Dishes were then placed in a germination chamber at 21 C with no light, with germination assessed visually at 24, 48, 72 and 96 hours (buckwheat and corn only). The number of germinated kernels was compared to the total number of kernels at each time interval to calculate percent germination energy (GE).

Pre-harvest sprout (RVA)

Due to the complexity of inducing and controlling PHS in model grain varieties/lots and the amount of basic research required to adapt RVA protocols for detecting PHS in non-barley grains, separate and significant research investments will be required to address this objective going forward. Furthermore, other PHS detection methods like falling number or direct grain enzyme activity testing may be appropriate as a supplement or alternative to RVA.

Enzyme activity (Diastatic Power & α-amylase), β-glucan, Free amino nitrogen (FAN), Fermentability & Predicted spirit yield (PSY)

Two mashing regimes were compared (EBC vs. IOB) and parameters including β-glucan, Free Amino Nitrogen (FAN), enzyme activity (Diastatic Power; α-amylase), and fermentability/PSY were measured for barley, rye, wheat, corn, and oats. The Hot Water (IOB) Extract Mash Procedure used 350mLs of DI water at 60°C, running for 60 minutes total. The EBC Extract Mash Procedure used 200mLs of DI water at 45°C initially, plus an additional 100mLs after 55 minutes when the mash reaches 70°C, running for 115 minutes total. Samples were prepped for fermentation and given 1.00 ± 0.001g of yeast per 100mLs of sample, then covered with aluminum foil. Samples were placed in an Incubator Shaker at 30°C and 150 RPMs to ferment over the course of 44 hours. After the 44 hours, samples that were fermented on-grain were filtered before being collected for analysis. A Gallery Discrete Analyzer for Automated Photometric Analysis, Skalar Segmented Flow Analyzer, and Megazyme Amylazyme Alpha-Amylase Assay were used to measure β-glucan, α-amylase and FAN.

Sugar profile (Saccharides & Arabinoxylans)

We conducted arabinoxylan/fructan/saccharide profiling using HPLC and verified the effectiveness of Dionex™ CarboPac™ PA100 IC Column in assessing sugar composition in non-barley grains to improve our understanding of sugar composition and its impact on fermentation. This data was used to establish correlations between sugar-to-nitrogen ratios and PSY outcomes, supporting the development of predictive models using linear and nonlinear regression techniques, including Random Forest and Support Vector Regression.

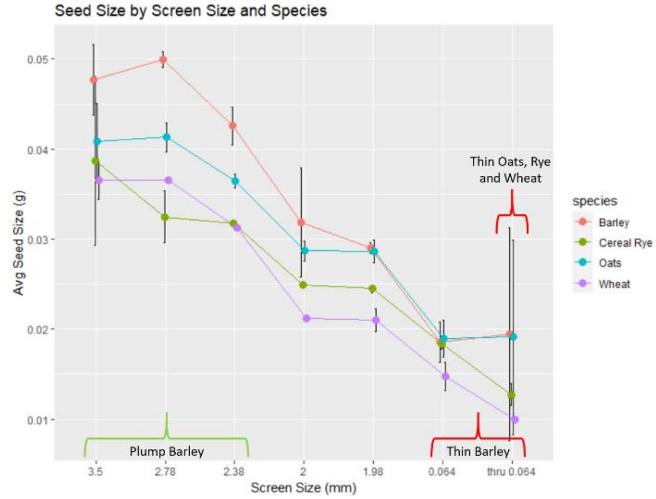
Results and Conclusions

Kernel assortment

The three barley control samples segregated predictably using the standard ASBC sieve/screen sizes of 7, 6, and 5/64ths (2.78, 2.38 and 1.98 mm) with significant differences in mean kernel weight between the plump and thin fractions ($p < 0.001$). Oats (esp. hulless), rye and wheat all required a smaller bottom screen size of 0.064 mm for accurate measurement of thins as defined by USDA and compared to barley (Fig. 1). The calculated thin fraction (%) of all these species was significantly higher using the ASBC 1.98 mm barley screen vs. the correct USDA 0.064 mm screen ($p < 0.01$), suggesting that ongoing application of the ASBC kernel assortment protocol for barley could lead to erroneous conclusions and unnecessary rejection of non-barley grain lots for malting. This is especially important given the finding noted below that malting may beespecially important for efficient fermentation of non-barley grains like rye. We also identified three novel round screen sizes specifically for corn to sort broken kernels and foreign matter on the Sortimat according to USDA protocols (8.5, 6.5 and 4.75 mm) and found that they reliably segregated our model variety with significant differences in mean kernel weight per fraction ($p < 0.001$).

Figure 1

Seed Size by Screen Size and Species

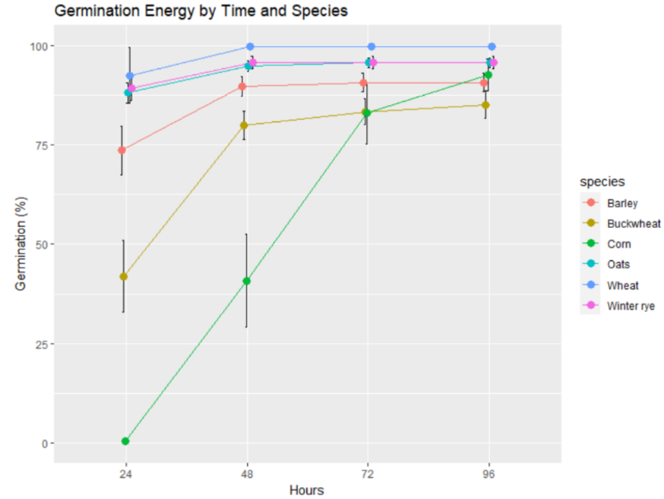


Germination energy & capacity (GE & GC)

Across the four durations of 24, 48, 72 and 96 hours, we found that 72 hours is the optimal duration for assessing GE in all species, as germination was either maximized at 72 hours and/or statistically similar to peak germination (Fig. 2). Only our corn sample gained appreciably in germination percentage from 72 to 96 hours, though the germinated fractions at those two durations were still statistically similar. The standard ASBC barley protocol (4 ml, 8ml) optimized barley germination and accurately segregated ND Genesis as water sensitive. We found that hulless oats and barley require higher

Figure 2

Germination Energy by Time and Species



water volumes for accurate GE determination (6-10 ml) as compared to their hulled counterparts. Cereal rye results differed between the OP and hybrid selections. KWS Serafino required higher water volumes of water than barley to maximize GE (8-10 ml), while ND Gardener was relatively resilient across the range of water volumes tested. It appears that the ASBC barley protocol (4 ml, 8ml) can be reliably applied to buckwheat. While corn germination was not statistically different across water volumes, 10 ml optimized numeric germination for 50 corn seeds of our model Ohion Blue dent corn variety.

Pre-harvest sprout (RVA)

Initial results suggest that using barley-based RVA protocols for other grains may lead to inaccuracies. The UPREC RVA machine was also out of service for required maintenance for a significant portion of the project period, limiting our ability investigate further. Additional research is needed to develop grain-specific RVA and/or alternative PHS protocols.

Enzyme activity (Diastatic Power & α-amylase), β-glucan, Free amino nitrogen (FAN), Fermentability & Predicted spirit yield (PSY)

EBC mash resulted in higher sugar extraction and fermentability for non-barley grains, particularly for raw rye. IOB mash, while reflecting practical brewing conditions, showed slightly lower efficiency for raw grains. Malted rye exhibited more stable enzymatic activity compared to raw rye, leading to consistent sugar extraction and higher PSY. Proteolytic enzyme additions significantly improved FAN availability and fermentability for non-barley grains, with varied impact based on grain type. Barley wort samples had a higher concentration of nitrogen compared to the rye wort samples when analyzed for FAN concentration. Barley wort therefore had more nitrogen that was available for yeast to consume during fermentation compared to the rye samples. A difference between FAN content of the wort and beer samples in the barley samples also indicated that most of the nitrogen was used by the yeast during the fermentation process. Rye samples presented a smaller difference between the wort and beer. AA testing showed that the barley wort also had a higher activity compared to the rye, indicating that barley has a greater ability to break down sugar compared to rye (Table 1).

Sample	BG - Wort (mg/L)	AA - Wort (mUnits/mL)	FAN - Wort (mg/L)	FAN - Beer (mg/L)
Cereal Rye	78	0.165	49	22
Cereal Rye	78	0.177	50	21
Spring Barley	67	1.065	204	57
Spring Barley	67	1.047	204	58

Table 1. Analysis of pure barley and rye samples using the Barley Method for mashing (IOB Mashing). Samples were evaluated using the Skalar for BG and FAN Concentrations and the Megazyme Assay methods. These samples were composed of 100% Rye or 100% Barley as a control and treated with the same enzymes.

Sugar profile (Saccharides & Arabinoxylans)

Results suggest that arabinoxylan content significantly influences wort viscosity, potentially affecting lautering performance. We found that sugar-to-nitrogen ratio was the most critical predictor of PSY, contributing 45.7% to variance in predictive models. BG levels were a major driver of fermentation efficiency, with malted rye performing more consistently than raw rye. FAN and AA had secondary but notable effects, particularly in raw rye samples where nitrogen was more limiting.

Communication Activities, Accomplishments, and Impacts

This project successfully advanced our understanding of non-barley grains, identifying improved analytical techniques for assessing their quality, malting and fermentation potential in craft beverage applications to maximize yield, efficiency and consumer acceptance. The findings will support maltsters, brewers and distillers in optimizing malting, mash protocols and fermentation processes for alternative grains. To-date this work has been presented as a research posted titled "Analysis of Enzymatic Activity in Malted Alternative Grains" at on industry/academic conference by Hartwick College student, Katelyn Sanzone.

Moving forward, we intend to complete and expand inter-lab ring testing of our updated protocols to share and validate our findings among partnering institutions and grain analysis professionals such as the Montana State University Barley, Malt & Brewing Quality Lab and the Cereal Crops Research Unit of USDA-ARS located in Madison, WI. The information gained from that exercise will support further outreach to stakeholders like ASBC members and private industry professionals through methods publications, conference presentations, webinars, etc.

Other ongoing research we intend to pursue includes: 1) further refinement of GE/GC and enzyme activity assessments for improved germination and PSY predictions, 2) development of grain-specific RVA or alternative protocols to improve sprout damage detection in non-barley grains, 3) investigation of additional variables, such as residual sugar and pH, to refine fermentation outcome predictions, and 4) extension of these methodologies to commercial-scale malting, brewing and distilling trials to assess the sensory impacts of alternative grains in final beverage products by correlating physical and chemical profiles with consumer preferences.

Budget Narrative

This project was conducted consistent with the budget proposed by the principal investigator and approved by the State of Michigan. The investments and results described herein have helped to leverage additional funding from several sources including separate MDARD CBC grant projects investigating OP corn and cereal rye varieties and agronomy. Michigan Crop Improvement Association is providing complimentary funding for barley, oat and cereal rye variety and agronomy studies in 2025 ($10,858). We have also partnered with College of Menominee Nation and LTBB Ziibimijwang Farm for northern OP flint corn variety and participatory breeding studies with support from the USDA NIFA Tribal Colleges Research grant program ($650,000).