

1 Introduction

Background:

- Identifying superior ingredients that provide distinct flavors (e.g. taste, aroma) is an important area of research. Brewers seek to identify raw ingredients to improve the stability of flavors during storage.
- There is a potential role for heirloom barley varieties to provide distinct attributes to the brewing process.
- Current research demonstrates a link between barley genetics and barley grain and malt chemistry.

Rationale and Hypothesis:

- Current barley lines contain relatively narrow genetic diversity. Recent efforts between the **Montana State University** (MSU) breeding program and the **Rocky Mountain Malting Barley Cooperative** are introgressing germplasm from heirloom barley to **expand the existing genetic and chemical diversity**.
- We therefore **hypothesize** that these new barley lines will contain unique chemical diversity, and this will impact malt flavor/aroma, fermentation quality, beer flavor, and beer flavor stability.

2 Experimental Design

An experiment was designed to profile metabolites for 160 heirloom varieties of malt, cultivated at Montana State University (MSU). Malts were analyzed for malting quality at MSU.

Metabolomics Workflow

Compound annotation using RamSearch and "ClassyFire" R package

Table 1. Diversity of compounds detected	
phenolics	vanillic acid, chlorogenic acid, caffeoyl acid, ferulic acid
aminoalcohols	serine, spermidine, histidine, homocysteine, glutamine, arginine
lipids	decanoic acid, dodecanoic acid, octadecanoic acid, phthalic acid, hexadecanoic acid, myristic acid
saccharides	fructose, sucrose, glucose, maltotriose
organic acids	malic acid, succinic acid, fumaric acid

3 Characterization of malt metabolome

- RP-UPLC-MS analysis detected 2,669 metabolites, 848 were annotated and assigned to a super and sub-class based on chemical ontology.
- ANOVA for genotype (with FDR adjusted $p < 0.05$) on the 848 metabolites revealed that 411 varied among the 160 lines. The metabolites that varied are indicated by the numerator in parenthesis.

4 Non-volatile Metabolites Contribute to Variation in Heirloom Malt Varieties

- Principal Component Analysis (PCA) was performed on 848 annotated malt metabolites to evaluate variation among the 130 2-row heirloom varieties. PCA can be used to characterize population structure based on **chemical similarities and differences**. PC scores plot for PC1 and PC3 explained 17.4% of chemical variation in malt.
- Hierarchical Cluster Analysis (HCA) was used to assign classes to varieties based on similarity, wherein the length of the branches that link two groups is related to this similarity.

5 Association of Malt Metabolites with Malting Quality Factors

- The associations between 848 annotated metabolites, 7 agronomic factors, and 12 malting quality factors were further evaluated using Spearman's rank correlation combined with hierarchical clustering and heat mapping.
- Horizontal black boxes indicate an example of the positive (dark blue, thin ellipses) or negative (dark red, thin ellipses) associations between metabolites and malting quality factors (here, malt protein is used).
- These data support that the unique profiles of each variety is the combination of many metabolites found in barley and affects malting quality factors.

6 O2PLS Association of Malt Metabolites with Malting Quality

- Two-way Orthogonal Partial Least Squares (O2PLS) analysis was performed on the 848 metabolites and 11 malting quality factors (malt protein, malt moisture, fine extract, turbidity, color, FAN, beta-glucan, alpha-amylase, S/T protein, and diastatic power (DP)).
- Biplot (top) displays the co-varying relationships, regarding protein, among variety (colored by protein level), metabolites (colored by chemical class), and malting quality factors (malt protein class). However, not all metabolites are associated with malting quality and may be associated with factors such as **flavor**.
- Variable Importance Projection plot (VIP) (bottom) displays the highest contributing (most important) metabolites which correlate to the malting quality/agronomic factors in this model.

7 Conclusions

- Malt chemistry was characterized through metabolomics.
- Variation in the malt chemistry, as well as associations between metabolite chemistry and malting quality/agronomic factors were observed.
- Heirloom barley genetics can provide new chemical diversity in malt.
- This malt chemical diversity is expected to impact flavor.

Future Directions

- To determine which **differences** in malt chemistry are reflected in the chemistry of the beer.
- To determine if the differences in the beer chemistry impact sensory attributes of beer, through **flavor and flavor stability**.
- To determine if there are barley and/or malt metabolites that can be **markers for beer flavor and/or flavor stability** for future breeding into lines adapted to dryland farming to the brewing process.

Acknowledgements

This work is supported by the USDA project #2017-68008-26209, Brewers Association, and the Colorado Agricultural Experiment Station.