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Statistically significant difference between the aroma profiles of beer brewed from sorghum and malt

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Introduction

Gluten-intolerance in the form of Coeliac disease affects about 1-2% of the general population of Western countries.[1] The recent influx of gluten-free foodstuff variety and research is resulting from this growing consumer market. Sorghum–based beer is a widely produced gluten-free alcoholic beverage, but the aroma profile of these beers has yet to be fully developed. An analytical comparison was made between beers brewed from either barley or sorghum malt to identify the chemical differences between the chemical aroma profiles. The chemical analysis of the beer was based on the optimized conditions described by Saison *et al.* using solid phase microextraction (SPME) coupled with by gas chromatography with mass spectra detection (GCMS).[2]

Brewing

The Malt Extract Syrup (either Maillard Malts® Sorghum Extract Syrup, Maillard Malts® Amber Malt Extract Syrup) was added to approximately 3 gallons of water and boiled for one hour. Wort was cooled, then diluted with DI water to a volume of 5 gallons. From which three 3 L aliquots were transferred into clean, sterile one gallon fermentors. Two grams of Safale US-05 Ale Dry Yeast was added to each of the aliquots and the fermentors were sealed with an airlock. Each fermentor was maintained at room temperature during fermentation.

Beer Analysis

From each fermentor, a 10 mL of beer was taken and placed into a 20 mL headspace sample vial containing 3 g NaCl and 50 µL internal standard (200 mg/L 2-heptanol). The sample was thermally conditioned at 35 ˚C for 10 minutes then a 50/30 DVB/CAR/PDMS Stableflex fiber was exposed to the headspace for 30 minutes with agitation at 250 RPM. Fibers were thermally desorbed into in a Shimadzu QP 2010 SE GCMS. Analysis conditions are based on optimized conditions described by Saison *et al.*[2]

Figure 1: Semi-volatile compounds that show highly significant change in median amount relative to internal standard after fermentation.

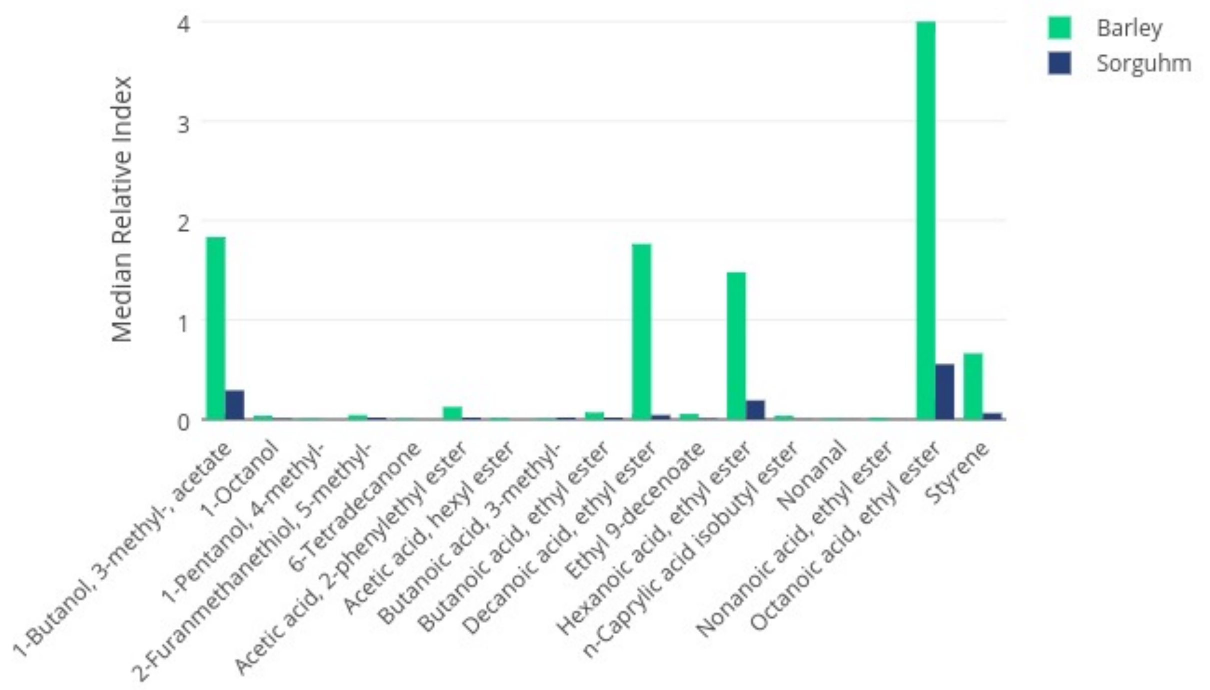

Figure 2: Confidence intervals for statistically significant median differences (Barley – Sorghum) in relative amount after fermentation.

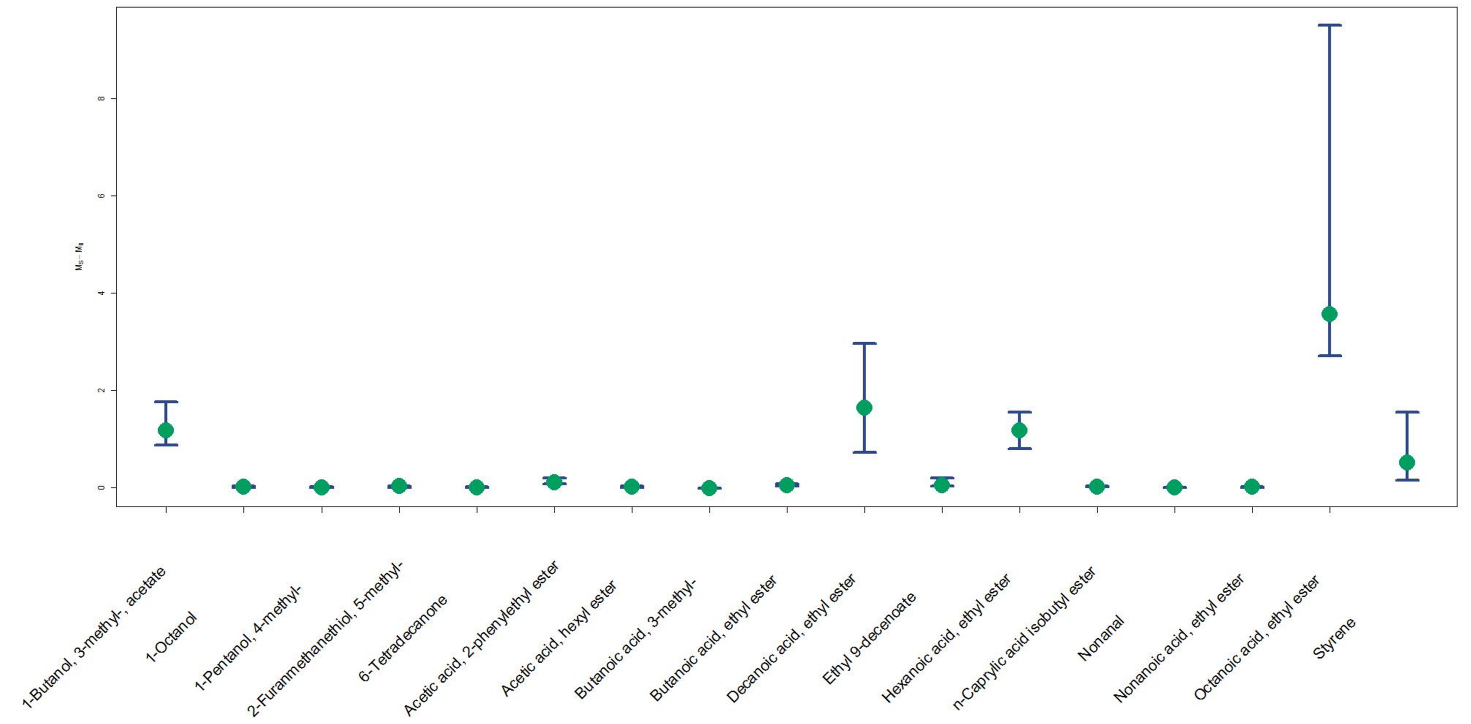

Table 1: Semi-volatile compounds that show highly significant change in median amount relative to internal standard regardless of grain material.

Compound	Initial	Final	P-value	Compound	Initial	Final	P-value	Compound	Initial	Final	P-value
1-Butanol, 2-methyl-	0.0657	2.0574	0.005	Acetic acid, butyl ester	0.0009	0.0726	0.008	Ethyl Acetate	0.0005	0.4686	<0.001
1-Butanol, 3-methyl-	0.0014	4.4535	<0.001	Ammonium acetate	0.0042	0.1486	<0.001	Furan, 2,5-dimethyl-	0.0002	0.0056	<0.001
1-Butanol, 3-methyl-, acetate	0.0011	1.0757	<0.001	Benzophenone	0.0012	0.0353	0.007	Heptane, 5-ethyl-2,2,3-trimethyl-	0.0010	0.0064	0.003
1-Pentanol	0.0010	0.0665	<0.001	Butanal, 3-methyl-	0.0198	0.0045	0.003	Hexadecanoic acid, ethyl ester	0.0007	0.0183	0.008
1-Propanol, 2-methyl-	0.0295	0.5106	<0.001	Butanoic acid, 2-methyl-	0.0005	0.0097	0.002	Hexanoic acid, ethyl ester	0.0084	0.6685	<0.001
1,2-Dimethyl-cyclopent-2-enecarboxylic acid	0.0019	0.0276	0.001	Butanoic acid, 3-methyl-	0.0009	0.0112	<0.001	Nonanal	0.0018	0.0056	<0.001
1,6-Octadien-3-ol, 3,7-dimethyl-	0.0015	0.0098	0.002	Cyclohexane, 1,1,3,5-tetramethyl-, cis-	0.0008	0.0018	0.007	Nonane, 3-methyl-5-propyl-	0.0025	0.0064	0.005
2-Butanol, 3-(1,3,3-trimethylbutoxy)-	0.0021	0.0083	0.005	Decanal	0.0011	0.0037	0.002	Nonane, 5-(2-methylpropyl)-	0.0003	0.0046	<0.001
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-	0.0018	0.0112	<0.001	Decane, 2,2-dimethyl-	0.0008	0.0041	<0.001	Octanoic acid	0.0022	0.0769	<0.001
2-Methoxy-4-vinylphenol	0.0020	0.0112	0.004	Decanoic acid, ethyl ester	0.0064	0.5295	<0.001	Octanoic acid, 3-methylbutyl ester	0.0006	0.0466	<0.001
4-Octen-3-ol, 2,2-dimethyl-	0.0005	0.0054	0.005	Dodecane, 2,2,11,11-tetramethyl-	0.0010	0.0047	<0.001	Octanoic acid, ethyl ester	0.0105	2.6329	<0.001
Acetic acid	0.0016	0.0311	<0.001	Dodecanoic acid, ethyl ester	0.0043	0.1026	<0.001	Pentadecane	0.0009	0.0525	0.002
Acetic acid, 2-phenylethyl ester	0.0008	0.0631	<0.001	Ethane, 1,1-diethoxy-	0.0009	0.0096	<0.001	Phenylethyl Alcohol	0.0049	0.5571	<0.001
								Propanoic acid, ethyl ester	0.0006	0.0076	<0.001

Table 2: Semi-volatile compounds that show highly significant change in median amount relative to internal standard per grain material.

Barley				Sorghum			
Compound	**Initial**	**Final**	**P-value**	**Compound**	**Initial**	**Final**	**P-value**
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	0.0003	0.0021	0.009	1-Butanol, 3-methyl-	0.0014	5.7332	<0.001
1,6-Octadien-3-ol, 3,7-dimethyl-	0.0015	0.0116	0.003	1-Butanol, 3-methyl-, acetate	0.0011	0.2913	<0.001
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-	0.0019	0.0120	0.002	1-Pentanol	0.0010	0.0152	<0.001
2-Furanmethanol	0.0037	0.0106	0.002	1-Propanol, 2-methyl-	0.0389	0.6642	<0.001
Ammonium acetate	0.0042	0.1451	<0.001	Acetic acid	0.0007	0.0201	<0.001
Butanal, 3-methyl-	0.0416	0.0022	<0.001	Acetic acid, 2-phenylethyl ester	0.0010	0.0157	<0.001
Butanoic acid, 3-methyl-	0.0009	0.0097	<0.001	Acetic acid, butyl ester	0.0006	0.0516	<0.001
Decanal	0.0014	0.0052	0.002	Butanal, 2-methyl-	0.0016	0.0005	0.007
Decane, 2,2-dimethyl-	0.0011	0.0044	0.003	Cyclohexane, 1,1,3,5-tetramethyl-, cis-	0.0008	0.0018	0.009
Decanoic acid, ethyl ester	0.0028	1.7641	<0.001	Ethane, 1,1-diethoxy-	0.0008	0.0108	<0.001
Dodecane, 2,2,11,11-tetramethyl-	0.0010	0.0054	<0.001	Ethyl Acetate	0.005	0.4049	<0.001
Ethyl Acetate	0.0006	0.5310	<0.001	Hexanoic acid, ethyl ester	0.0074	0.1915	<0.001
Furan, 2,5-dimethyl-	0.0002	0.0072	0.002	Octanoic acid	0.0038	0.0464	<0.001
Furfural	0.0534	0.0148	0.005	Octanoic acid, 3-methylbutyl ester	0.0006	0.0148	<0.001
Mequinol	0.0198	0.1050	<0.001	Octanoic acid, ethyl ester	0.0365	0.5552	<0.001
Nonanal	0.0018	0.0097	<0.001	Phenylethyl Alcohol	0.0062	0.4792	<0.001
Nonane, 5-(2-methylpropyl)-	0.0002	0.0042	0.002	Propanoic acid, ethyl ester	0.0006	0.0056	0.005
Octanoic acid, ethyl ester	0.0061	3.9988	<0.001	Styrene	0.0203	0.0628	0.009

Table 3: Semi-volatile compounds only observed in beer brewed from either barley or sorghum.

Compound	Barley	Sorghum
2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	X	
3(2H)-Furanone, dihydro-2-methyl-	X	
Acetic acid, decyl ester	X	
Acetic acid, heptyl ester	X	
Decanoic acid, propyl ester	X	
Hexanoic acid, propyl ester	X	
n-Capric acid isobutyl ester	X	
Pentanoic acid, 4-methyl-, ethyl ester	X	
3(2H)-Thiophenone, dihydro-2-methyl-		X

Statistical Analysis

All two sample comparisons were made using the Wilcoxon rank sum test (Mann-Whitney U test). This nonparametric test is markedly more efficient for non-normal distributions and more robust against outliers than standard t-tests. When assumptions for the t-procedures are satisfied, the rank sum has only a negligible loss in efficiency. Therefore, nonparametric procedures are more appropriate here. Estimates provided represent the median of the distribution for relative index as opposed to the mean. All p-values were adjusted to minimize false discovery rate due to multiple testing. The open source software, R was used for analysis. There were 2,345 unique compounds detected in the samples, but only 152 had enough occurrences for statistical inference. Of those 152, only those significant at the 0.01 level are provided in this summary.

Discussion

The major results of the study can be summarized as follows.

- 152 compounds determined to show significant differences following fermentation.
- Of these 42 compounds show highly significant differences (P-value < 0.01)
  - 18 present in Barley samples
  - 18 present in Sorghum samples.
- Only nine found to be unique to either Sorghum or Barley.
- 40 compounds were found to be present in both Barley and Sorghum but at statistically significant levels.
  - 17 of these compounds were highly significant (Pvalue <0.01)

It should be noted that this is not an exhaustive list of compounds as the fiber is somewhat elective in extraction but these compounds should be investigated further to understand the contributions to favor and aroma differences of these two beer types.

References:

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3. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. 2017. https://www.R-project.org/.

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