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Comprehensive Analysis of Different Contemporary Barley Genotypes Enhances and Expands the Scope of Barley Contributions to Beer Flavor

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ABSTRACT

Recent research has demonstrated contributions of barley genotype to beer flavor based on the progeny of a cross between an heirloom and a more contemporary barley variety. To advance this line of research, the current study used two independent sets of barley germplasm to address the contributions of different barley genotypes to beer flavor. Pedigree, quality of malt and beer, and beer metabolomic profiles were compared within and between the two sets. Utilizing both laboratory and consumer panels, differences in sensory attributes of malt hot steeps and lager beers that are attributable to barley genotype were investigated. Genotype, in this context, is defined in the broadest sense to include experimental germplasm and released varieties. Results concur with previous studies: the two sets of barley germplasm were found to have, both within and between, distinct but subtle differences in flavor profiles of malt hot steeps and finished lager beers. Distinct metabolomic profiles, attributable to barley genotype, were detected. Further, covariation of metabolomic profiles and sensory attributes were identified using data from both sensory panels. These observations lead to the conclusion that the variable metabolites observed among the two sets of barley germplasm are a direct result of genetic differences that lead to differential chemical responses within the malting and brewing processes.

KEYWORDS
Barley; malt; beer; flavor; chemistry; hot steeps; quality; breeding

Introduction

Malted barley is the primary source of fermentable sugars used to ferment most beers. Until recently, barley contributions to beer flavor were mostly attributed to Maillard Reaction Products (MRPs) developed during malt kilning and the interactions of malts with hops. However, recent research exploring the relationship between genetic variation of barley and beer flavor has shown that genotype does impact beer flavor.[1–3] Genotype, in this context, is defined in the broadest sense to include experimental germplasm and released varieties. The degree of malt modification and growing environment were also determined to impact the sensory characteristics of beer, based on a large number of nano-brews, malt analytics, and a research sensory panel.[1,2] Bettenhausen et al.[3] carried this research a step further with (i) larger, pilot scale malts and beers, (ii) brewery, consumer, and laboratory sensory panels, and (iii) measurement of volatile and non-volatile metabolites.

The interactions between malt chemistry traits and genotypes have been demonstrated to contribute unique beer flavor characteristics. Genetic differences and resulting metabolite composition differences lead to variation in the amount and composition of precursor amino acids and saccharides within the barley kernel. Through the process of malting, these precursors have the potential for biochemical reactions during germination to produce metabolites and MRPs vital for flavor characteristics. Our previous research on the contributions of barley to beer flavor was based on the progeny of a cross between an heirloom (Golden Promise) and a more contemporary barley variety (Full Pint) with a unique malting quality profile.[1–4] By expanding the scope of the evaluated germplasm, the current study addresses the next question: what are the contributions of other, different, and contemporary barley genotypes to beer flavor?

To address this question, two different sets of barleys were chosen: (1) winter two-row commercially available malting varieties and (2) spring two-row potential varieties with Full Pint as one parent and varieties other than Golden Promise as the other parent. Pedigree, malt quality, beer quality, sensory attributes, and metabolomic profiles were compared within and between the two sets. The commercially available varieties were grown near Condon, Oregon in collaboration with the Western Rivers Conservancy

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Table 1. Pedigree and developer or provider of barley lines per project/set: Western Rivers Conservancy (WRC) and Next Pint (NP).

Project/set	Variety/selection	Pedigree	Developer/Provider
WRC	Wintmalt	(Opal*3087/96, F1)*(8751/Magie)	Ackermann Saatzucht GmbH & Co. KG
	Thunder	Wintmalt/Charles	Oregon State University
	Violetta	Opal x Br 2324b616	Saatzucht Josef Breun GmbH & Co.
	Flavia	(((Carrrero * NIKS.2230) * Aquarelle) * Metaxa) * Wintmalt	Ackermann Saatzucht GmbH & Co. KG
	Calypso	Sunbeam/Suzuka	Limagrain Cereal Seeds
NP	DH131756	Violetta/Full Pint	Oregon State University
	DH131144	Full Pint/Violetta	Oregon State University
	DH120270	Maris Otter/Full Pint	Oregon State University
	Full Pint	Orca/Harrington	Oregon State University

Pedigree based on breeding annotated method mother/father. DH, doubled haploid, experimental barley selection that has not been released.

(WRC; http://www.westernrivers.org/) within the framework of a project designed to enhance riparian habitat around the John Day River and its tributaries. The acquisition of the Rattray Ranch, historically used to produce dryland winter wheat, allowed for assessing the potential for winter malting barley as an alternative crop. Strips of four commercially available barley varieties were embedded within a commercial field of Wintmalt. The second set was derived from the Next Pint (NP) project, a collaboration between Mecca Grade Estate Malt (MGEM; https://www.meccagrade.com/) and Oregon State University to develop a variety to replace Full Pint, the current MGEM estate variety. Three advanced lines and Full Pint were grown, with irrigation, near Madras, Oregon at MGEM facilities.

The two sets of barley lines followed an experimental pipeline similar to that described in Bettenhausen et al. (2020).[3] Briefly, each line underwent i) pilot scale malting and brewing, (ii) quality analysis of malts and beers, (iii) sensory analysis of beer by a trained laboratory panel and a consumer panel, and (iv) metabolomic profiling of finished beer. In addition, sensory analysis of malt hot steeps was conducted. Since its development, the hot steep malt sensory evaluation method has piqued the interest of brewing and malting industries as an improved approach to evaluate malt sensory, as well as predict beer sensory characteristics derived from malts.[5,6] Though widely used and discussed, there are few formal comparisons of hot steep malt and beer sensory. Therefore, the potential of hot steep malt sensory evaluation as an economical, effective tool for assessing barley/malt impacts on beer flavor was investigated. The current study advances research examining contributions of barley genotype to sensory characteristics of malt and finished beer.

Materials and methods

Plant material

Two independent sets of barley germplasm were used in this experiment, designated WRC set and NP set (Table 1). The WRC set included five released cultivars all of which are two-row winter growth habit types, four of European origin and one developed at Oregon State University (https://barleyworld.org/). Three of the five cultivars are approved by the American Malting Barley Association (AMBA) (Wintmalt, Thunder, Violetta; https://ambainc.org/2020-amba-recommended-malting-barley-varieties/). The NP set included three advanced lines and a Full Pint "check", all of which are two-row spring growth habit types developed by the Oregon State University barley breeding program. None of the barleys in the NP set are on the AMBA approved list. The three advanced lines were bred and selected over three years of testing from a larger set of 126 doubled haploid progeny derived from crosses with Full Pint.

The WRC set was grown at the Rattray Ranch, near Condon, Oregon (45°14′8″N 120°11′6″W). Briefly, the varieties were planted in the fall of 2017 and harvested in the summer of 2018. No irrigation was applied, as is standard practice in this summer-fallow dryland production area. Each variety, except Wintmalt, was grown in a 1.6 ha strip. The strips were embedded in a 197 ha field of Wintmalt. The strips were planted, maintained, and harvested using commercial equipment. The NP set was grown at the Klann Farm, near Madras, Oregon (44°46'29.3"N 121°10'17.0"W). Briefly, the three advanced lines were planted in the spring of 2018 in 0.05 ha strips. Irrigation was applied following regular practices. The strips were embedded in a commercial field of wheat. The strips were planted and harvested using OSU Barley Project research equipment. Full Pint grain was sourced from an adjoining field managed by Oregon State University. Additional details on growing the WRC and NP sets, including agronomic practices, are provided in Supplemental File 1.

Malting and malt quality

Approximately 230 kg subsamples of grain were obtained for each of the barley lines in the WRC and NP sets. Each barley line was malted independently in 90 kg batches, using the OSU mini-malter (https://barleyworld.org/), as previously utilized by Bettenhausen et al. (2020).[3] Steeping conditions were the same for both sets and supplemental moisture was provided during the first day of germination by spraying if required. In order to optimize modification of the grain, the WRC set had a target moisture of 46% and the target for the NP set ranged from 45-51% based on results from micro-malting. Both sets were germinated for four days (WRC at 16 °C and NP at 18 °C) and had identical kilning conditions. Detailed malt protocols are available in Supplemental File 2. Malt quality analyses were conducted by the Hartwick Center for Craft Food & Beverage (https://www.hartwick.edu/about-us/centers-institutes/center-for-craft-food-and-beverage/) following standard ASBC testing methods.[3,4] The malting quality traits (and results) are shown in Table 2.

Table 2. Malt quality of barley lines per project/set.

Project/set	Variety	Moisture	Friability	Extract	Color	β-glucan	SP	TP	S/T	FAN	DP	AA	Filtration	Clarity	pH
		%	%	%	°SRM	mg/L	%	%	%	mg/L	°L	DU	Time		
WRC	Wintmalt	4.6	91.2	80.3	1.56	128	3.78	10	37.8	123	102	43.4	normal	hazy	6.07
	Thunder	4.8	97.0	83.9	1.97	58	4.89	9.1	53.7	202	124	78.7	normal	clear	5.91
	Violetta	4.6	95.2	80.3	1.69	29	3.89	9.5	40.9	141	113	40.2	normal	clear	6.06
	Flavia	4.6	96.8	80.0	1.57	33	3.64	9.2	39.6	127	111	44.1	normal	clear	6.06
	Calypso	4.3	99.2	81.3	1.73	31	3.83	8.8	43.5	150	114	46.6	normal	clear	6.04
NP	DH131756	4.6	82.5	82.5	1.94	77	5.8	13.8	42	237	163	70.2	normal	clear	5.83
	DH131144	4.7	84.7	81.4	2.22	38	5.62	12.2	46.1	236	174	83.9	normal	clear	5.98
	DH120270	4.5	72.1	78.5	1.41	272	4.35	13.1	33.2	150	161	58.5	normal	clear	5.98
	Full Pint	4.7	69.4	82.9	1.84	110	5.32	12.9	41.2	220	208	91.9	normal	clear	5.99
Adjunct Malt Criteria		NA	NA	> 81%	0.812-1.27	< 100	4.8-5.6%	≦ 13%	40-47%	> 210	> 140	> 50	NA	NA	NA
All-malt Criteria		NA	NA	> 81%	0.812-1.42	< 100	< 5.3%	≦ 12%	38-45%	140-190	110-150	40-70	NA	NA	NA

All-malt and Adjunct malt criteria are based on parameters suggested by American Malting Barley Association (https://ambainc.org/wp-content/uploads/2019/10/Malting_Barley_Breeding_Guidelines_June_2019.pdf) Color is measured using Standard Reference Method (SRM); SP, soluble protein; TP, total protein; S/T, soluble/total percentage of protein; FAN, free amino nitrogen; DP, diastatic power in degree Lintner; AA, alpha amylase.

Brewing

Using an Esau and Hueber 2.5 hl brewery at Oregon State University (OSU), lager beers were prepared in collaboration with the OSU Brewing Science Lab. Each malt variety/selection was mashed and brewed separately in two different batches 1) WRC malts in May 2019, 2) NP malts in July 2019, yielding 1.2hl each of German Pilsener-style, malt-forward lager. The brewing recipe and protocol were adapted from a single-malt, lager protocol supplied by Rahr Malting intended to emphasize malt forward characteristics and achieve a drinkable, "commercial style" lager. Key ingredients were the neutral yeast (Bohemian Lager Strain 2124, Wyeast Labs), hop extracts (Isohop, John I. Haas, Inc.) and hop pellets (Kazbek hops, Brewers Supply Group). The brewing protocol was similar to Bettenhausen et al.[3] but with modifications, and the full protocol is provided in Supplemental File 2. Analysis of the beer was performed by the OSU Brewing Science Lab as described in Table 3.

Beer sensory

A beer sensory pipeline was performed as described in Bettenhausen et al. (2020),[3] and two types of sensory studies were conducted (1) a consumer panel and (2) a laboratory panel.

The consumer panel testing was performed in collaboration with the Oregon State University Center for Sensory & Consumer Behavior Research (http://agsci-labs.oregonstate.edu/sensoryresearch/). WRC beers were tested in August 2019 while NP beers were tested in January 2020. The procedures were performed as described by Bettenhausen et al.[3] and detailed in Supplementary material 3. Briefly, participants (WRC n = 152; NP n = 155) were asked to answer a series of questions per beer, including (1) overall liking (scale from 1 to 9), (2) Check All That Apply (CATA) for sensory characteristics, (3) "ideal lager" attributes, and 4) demographics.

The laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in October 2019. Thirteen panelists (6 M, 7 F; 22–55 years old), who had prior experience on beer and wine descriptive analysis sensory panels, were trained over three separate sessions with the beers in question using the Projective Mapping with Ultra Flash Profiling sensory method[7,8] and detailed in Supplementary material 4. WRC beers and NP beers were assessed for sensory attributes on two separate days, with each beer being presented in duplicate (WRC n = 10; NP n = 8). During each testing session, panelists assessed the orthonasal aroma and flavor by mouth of the beer in two separate tests, with new blind codes for the samples.

Hot steep malt sensory

Sensory analysis was performed on liquid extract produced from hot steeps of all malts in the experiment, prepared in accordance with ASBC Methods of Analysis – Sensory Analysis 14.[6] Descriptive data were collected using Projective Mapping (PM) combined with Ultra Flash Profiling.[7,8] Due to changes in panelist availability between the beer and hot steep malt sensory analyses, a new laboratory panel was recruited and trained over four, one-hour sessions, detailed in Supplementary material 5. This 15-member panel (8 M, 7 F; 23–68 years old) consisted of some of the same members as the beer sensory panel, but also included some new members, most of which had prior experience performing sensory analyses on other foods such as wine. Laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in March 2020. Malt hot steeps from five WRC malts and four NP malts were assessed for sensory attributes on separate days. During each testing session, panelists assessed both the orthonasal aroma and the flavor by mouth of the malt hot steeps in two separate tests. Half the panel carried out the orthonasal testing session followed by a five-minute break and then the flavor session, while the other half of the panel proceeded in the opposite order. Unique blind codes were used for each test, and the serving order was randomized for each panelist. The WRC malt hot steep sessions were carried out with 15 panelists held over two days, while the NP malt hot steep session was carried out with ten panelists on a single day.

Sensory data analysis

All sensory data were collected via Compusense Cloud Software (Version 20.0.7404.31336, Guelph, Ontario,

Table 3. Beer quality of barley lines per project/set.

Project/set	Sample Name	ABV (%)	OG (°P)	RE (%w/w)	AE (°P)	Color (EBC)	RDF (%)	IBU
WRC	Wintmalt	5.12	12.14	4.38	2.52	3.79	65.44	22.94
	Thunder	5.41	12.05	3.82	1.87	4.01	69.64	23.6
	Violetta	5.42	12.27	4.04	2.09	3.17	68.47	20.74
	Flavia	5.40	12.31	4.11	2.16	3.16	68.03	21.35
	Calypso	5.31	12.06	3.99	2.07	4.09	68.29	23.88
NP	DH131756	5.21	12.08	4.18	2.30	6.57	66.85	21.11
	DH131144	5.34	12.12	4.01	2.08	7.89	68.33	23.94
	DH120270	4.99	11.70	4.11	2.30	4.72	66.29	22.33
	Full Pint	5.10	11.64	3.86	2.01	6.21	68.17	22.1
BA Guidelines	German Pilsener	4.6-5.3	11.0-12.9	NA	NA	3-4	NA	25-50

From beer produced from each malt; ABV, alcohol by volume; OG, Original Gravity of wort (°P, Degrees Plato); RE, real extract, based on attenuation of wort; AE, apparent extract, RDF, real degree of fermentation; Color, based on EBC method; IBU, international bittering units based on dissolved solids. German Pilsener guidelines provided by the Brewers Association (https://www.brewersassociation.org/edu/brewers-association-beer-style-guidelines/#Lager%20Styles).

Canada). Projective Mapping combined with Ultra Flash Profiling provides both attribute counts and coordinate data for each sample evaluated. Coordinate data was analyzed using XLSTAT Multiple Factor Analysis (MFA) (Addinsoft, New York, NY). Individual MFA plots for aroma and flavor were created for both WRC and NP sample sets in both beer and malt hot steeps. Attribute data was processed in order to combine specific descriptors under the more broad descriptors, in accordance with the Base Malt Flavor Map (Supplemental File 6). Post processing, descriptor data were then analyzed by Correspondence Analysis (CA) in XLSTAT. Attributes were ranked according to frequency of use summed across all of the samples in the set. As there is no standard cutoff for attribute inclusion, it is up to the researcher to determine the appropriate threshold.[8] In this case, the cutoff was set in order to display pertinent attributes, while filtering out attributes that do not help further explain the relationship between the samples. Those attributes that were used at a rate of at least 45% of the most frequently used attribute were included in the CA plot for the laboratory panel beer aroma sensory data. For the malt hot steeps, aroma and flavor CA plots were created individually before being combined and plotted together with the attributes used being those that were used by the overall panel with a frequency of >25% of that of the most frequently used top attribute.

Detection of the metabolome in beer

Volatile metabolites in beer were detected using a non-targeted metabolomics approach. The methods included analysis of volatiles using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS/SPME-GC-MS) with methods as previously described[3] and detailed in Supplementary material 7. Briefly, mass spectra from the MS platform was converted to the .cdf file format and processed and annotated using the workflow described in Bettenhausen et al.[3,4] Metabolite quantities were established as previously described.[4] Briefly, each sample resulted in a matrix of molecular features (defined by retention time and mass (m/z)) generated using XCMS software in R v. 3.2.4.[9] Mass spectra were deconvoluted using the RamClust algorithm[10] and normalized to total ion current (TIC); the relative abundance and variance of each molecular feature was determined by the mean area of the pooled quality control (QC) injection. Volatile metabolites were annotated by spectral matching in RamSearch software[11] to an in-house database of ∼1,500 compounds and to external and theoretical databases including NIST v14 (http://www.nist.gov), Metlin,[12] Golm Metabolome Database,[13] MSFinder software (v. 3.26, RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, Japan),[14,15] Human Metabolome Database (HMDB),[16] and FooDB;[17] Spectra were also evaluated using the findMAIN function of the interpretMSSpectrum R package[18] and chemical ontologies were established using HMDB and the ClassyFire package in R.[19]

Statistics (metabolomics)

Volatile metabolite abundances for each dataset (WRC and NP) were compared independently. Principal Components Analysis (PCA) was conducted on unit-variance (UV) scaled metabolites and sensory traits from each panel with SIMCA software v. 15 (Sartorius Stedim Biotech, Umea, Sweden).[20] Respective sensory attributes of each independent sensory panel were integrated with the volatile metabolites for further multivariate analysis. Orthogonal projection to latent squares (OPLS) analysis was conducted for the WRC set on two PCA-reduced and UV-scaled components for sensory (one for the Violetta/Calypso trend, a second component for the Thunder/Wintmalt trend) and the 130 UV-scaled volatile metabolites. OPLS analysis was conducted for the NP set on two PCA-reduced and UV-scaled components for sensory (one for the Full Pint/DH120270 trend, a second component for the DH131144/DH131756 trend) and 160 UV-scaled volatile metabolites, both with SIMCA software. The 20 sensory attributes from the consumer panel (y) were regressed on the UV-scaled metabolite data (x). Predictive power (Q^2) was determined via cross-validation, by which the data was divided into seven parts and 1/7th of the data was removed, and the model was built on the remaining 6/7th of data remaining, and the removed 1/7th of data are predicted from the model. Heat maps were created after z-transformation of the metabolite data. The resulting z-scores were converted into colors and grouped using hierarchical clustering on the Spearman's rank correlation (r_s) between metabolite and sensory trait values.[21]

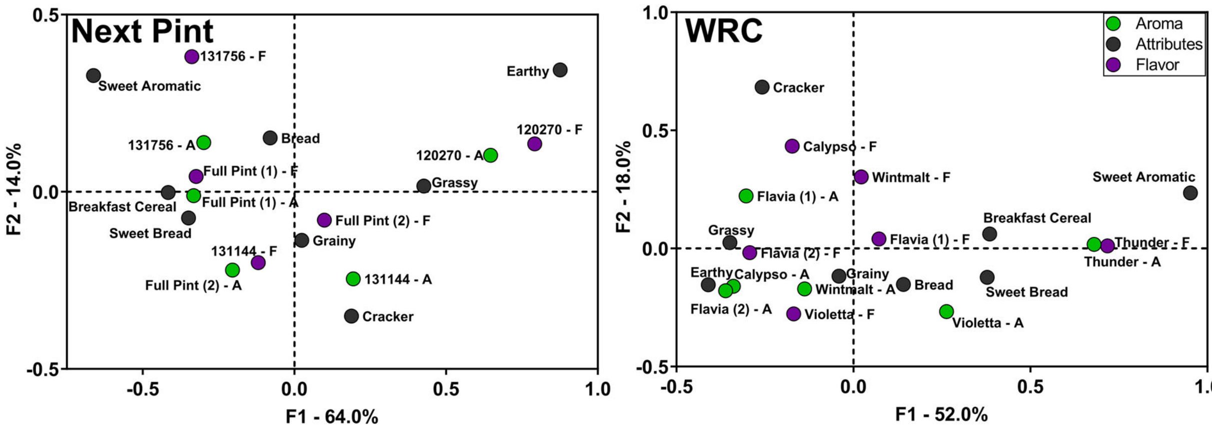

Figure 1. Correspondence Analysis from hot steep Projective Mapping (left pane: Western Rivers Conservancy samples, right pane: Next Pint samples). "1" and "2" designates duplicate observations of the same samples with different blind codes. CA plots show which attributes (black squares) are used to describe the samples (indicated by green and purple circles). Samples that are close together are described similarly, while samples far apart were described differently. Both Aroma and Flavor evaluations are plotted together with the top eight most frequently used attributes.

Results

Barley, malting quality, and beer quality associated with barley genetics

As shown in Table 1, and in greater detail in Supplementary Figure 1, there were genetic relationships among the barley varieties/selections used in this study. Varieties were selected based on logistical constraints: the WRC set chosen from commercially available winter malting barleys with sufficient seed availability; the NP set chosen within the scope of work of the project with Mecca Grade Estate Malt. In the WRC set of winter growth habit two-row varieties, Opal is a parent shared by Wintmalt and Violetta. Wintmalt, in turn is a parent shared by Thunder and Flavia. Calypso does not have Wintmalt or Opal in its pedigree. Both of its parents have Puffin in their pedigrees, and Puffin has Maris Otter in its pedigree. Thunder has Charles, the first North American two-row malting barley approved by AMBA, as its other parent. Thunder is unique in this set in having European and North American parentage. The NP set, comprised of spring growth habit two-row experimental varieties and the variety Full Pint, has an unusual genetic structure in that the three selections are derived from "wide" crosses between European winter two-rows (Violetta and Maris Otter) and a North American two-row (Full Pint). Two of the selections, DH131144 and DH131756, are sisters derived from the cross of Full Pint x Violetta; Violetta was the male parent of the former and female parent of the latter. In this set, DH120270 is unique in having Maris Otter as a parent. Violetta and Maris Otter are, therefore, genetic commonalities between the WRC and NP sets.

There were notable similarities and some key differences in malting quality within and between the WRC and NP sets (Table 2), using the AMBA specifications for adjunct and all-malt quality. Within the WRC, all varieties were highly friable. Calypso, Flavia, and Violetta were well-modified and the most similar to each other. They met most criteria for the all-malt specifications but were too low in free amino nitrogen (FAN), diastatic power (DP), and alpha-amylase (AA) for the adjunct specifications. Wintmalt was the least modified of the set, with the highest β-glucan and lowest S/T (soluble/total protein), not meeting all-malt or adjunct criteria. Thunder was the most modified and notable for its high extract, FAN, AA, and S/T. Entries within the NP set came closest to meeting adjunct criteria, rather than all-malt criteria. DH131756 and DH131144 were well-modified and met most if not all AMBA adjunct specifications. DH120270 was under-modified, with low friability, high ß-glucan, lower extract, S/T, FAN, DP, and AA. It did not meet all-malt or adjunct criteria. Full Pint was less modified than DH131756 and DH131144, with lower friability and higher ß-glucan. It met AMBA adjunct specifications for most criteria but was slightly over specifications for ß-glucan and total protein (TP). Comparisons between the two sets show that the WRC malts were more friable and - except for Thunder - had lower extracts, TP, FAN, DP, and AA than the NP set. Overall, Calypso came closest to meeting the all-malt criteria and DH131144 met all the criteria for adjunct malting.

All beers fell within range for German lager-style, Pilsener beer guidelines – except for color and ABV, as described by the Brewers Association Beer Style Guidelines (https://www.brewersassociation.org/edu/brewers-association-beer-style-guidelines/#Lager%20Styles). All the NP beers were darker in color and fell outside of the style guidelines. The IBU values were similar for all beers, but below the BA guidelines for this beer style (Table 3). With each set of malts (WRC and NP), Wintmalt and DH120270 had the lowest alcohol by volume (ABV) and real degrees of fermentation (RDF), respectively, while Thunder and DH131144 had the highest. Compared collectively across both data sets, ABV ranged from 4.99% to 5.42% while RDF ranged from 65.44% to 69.64%.

Sensory characteristics for malt hot steeps

Projective Mapping was used to evaluate both aroma and flavor attributes of malt hot steeps made from the WRC (15 panelists) and NP (10 panelists) samples. In each sample set, one malt was randomly selected to be presented as a duplicate. For the WRC malts, Flavia was replicated giving six total malt hot steep samples. Based on aroma evaluation only, panelists grouped duplicates closely together, implying perceived

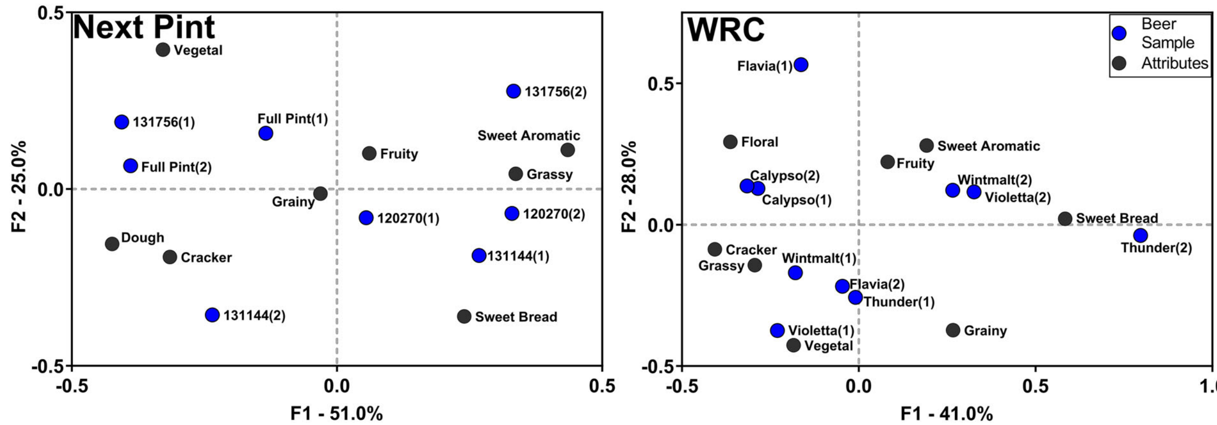

Figure 2. Correspondence Analysis of top 8 most used aroma attributes from beer Projective Mapping with Laboratory Panel (right pane: Next Pint beers; left pane: Western Rivers Conservancy beers). 1 and 2 designates duplicate observations of the same samples with different blind codes. CA demonstrates which aroma attributes (indicated by black squares) are used to describe the beer samples (indicated by blue circles).

similarities between them, and dissimilarities between other samples. During the flavor evaluation, the Flavia duplicates were not placed as close to one another. *Thin body* was the only mouthfeel attribute used frequently enough to be plotted. Coordinate data from aroma evaluation showed that Thunder and Violetta were different from the other samples (Supplementary Figure 3). During the aroma evaluation, *grainy* was used consistently among the samples but showed more variable usage during flavor evaluation (Figure 1). In both aroma and flavor evaluations, *grassy* had a large variation in usage among the samples, with Calypso being described as *grassy* most frequently. Additionally, Calypso's aroma was described by *vegetal*, while its flavor was described by *cracker*. Both Flavia samples were high in *grassy*, and on average were high in *earthy*. Thunder and Violetta were each much lower in *grassy* than the rest of the samples. Thunder was consistently described by *sweet aromatic, breakfast cereal*, and *sweet bread*. Violetta was also more closely associated with *bread*. Descriptors used for Wintmalt varied between aroma and flavor, but *grassy* was used in both.

For the NP malts, Full Pint was replicated, giving five malt hot steep samples. The coordinate data showed similar configurations between aroma and flavor evaluations, with the exception of a Full Pint duplication moving positions (aroma data shown in Supplementary Figure 3, flavor data not shown). In both the MFA and CA plots, DH120270 appeared distinct from the other malt steep samples. *Grainy* was the most used descriptor for the NP aroma and flavor evaluations and was not helpful in the discrimination of samples, hence its location near the center of the samples (Figure 1). There were large differences in usage across samples for *grassy* in both flavor and aroma, and *sweet aromatic* via aroma only (attribute count data not shown for concision). Additionally, *sweet bread, earthy*, and *breakfast cereal* highlighted the differences between the samples during the flavor evaluation. In both aroma and flavor evaluations, Full Pint was described by *breakfast cereal*, with the exception of one Full Pint flavor replication. DH120270 was the most unique sample of the group and was highly *grassy* and *earthy* across both evaluations. DH131144 and DH131756 were both described attributes within the *bread* category, though DH131144 was described with *cracker* and DH131756 with *sweet aromatic*.

Beer sensory – consumer panel

The consumer panel noted differences in flavor between the WRC beers, but these were not significant. Violetta was liked more than Calypso (Tukey's Post Hoc HSD test $p = 0.06$; Supplementary Table 1A). Consumers were able to distinguish significant differences in attributes *citrus, floral, hoppy*, and *sweet* between the five WRC beers (Cochran's Q test, $p < 0.05$, Supplementary Table 2A). Thunder was significantly less *citrus* than the other four varieties, more *hoppy* than Violetta, and more *toasted* than Wintmalt; Violetta was found to be significantly more *sweet* and *floral* than Calypso, Flavia, and Wintmalt, and more *refreshing* than Calypso; And Wintmalt and Violetta were significantly more *crisp* than Thunder (McNemar's multiple pairwise comparison, $p < 0.05$, Supplementary Table 3A).

There were no significant differences in "Overall Liking" for the NP beers (ANOVA, $p = 0.72$; Supplementary Table 1B). However, consumers were able to distinguish significant differences in the *bitter* attribute between the four beers (Cochran's Q test, $p < 0.05$, Supplementary Table 2B). Full Pint was found to be significantly less bitter than DH120270; DH120270 was found to be significantly more *light* in mouthfeel than DH131756; and DH131144 and more *thin/watery* than either DH131756 and Full Pint (McNemar's multiple pairwise comparison, $p < 0.05$, Supplementary Table 3B).

Consumer panelists identified important attributes for an "ideal lager" from the list of common descriptors given in the CATA. *Crisp* and *refreshing* were selected as key attributes for an "ideal lager" in both the WRC and NP sets. *Citrus* and *light* were also selected as key attributes for the WRC and NP sets, respectively (Supplementary Figure 2 A and B).

Beer sensory – laboratory panel

Projective Mapping was used to assess both aroma and flavor attributes of the WRC (13 panelists) and NP (10 panelists)

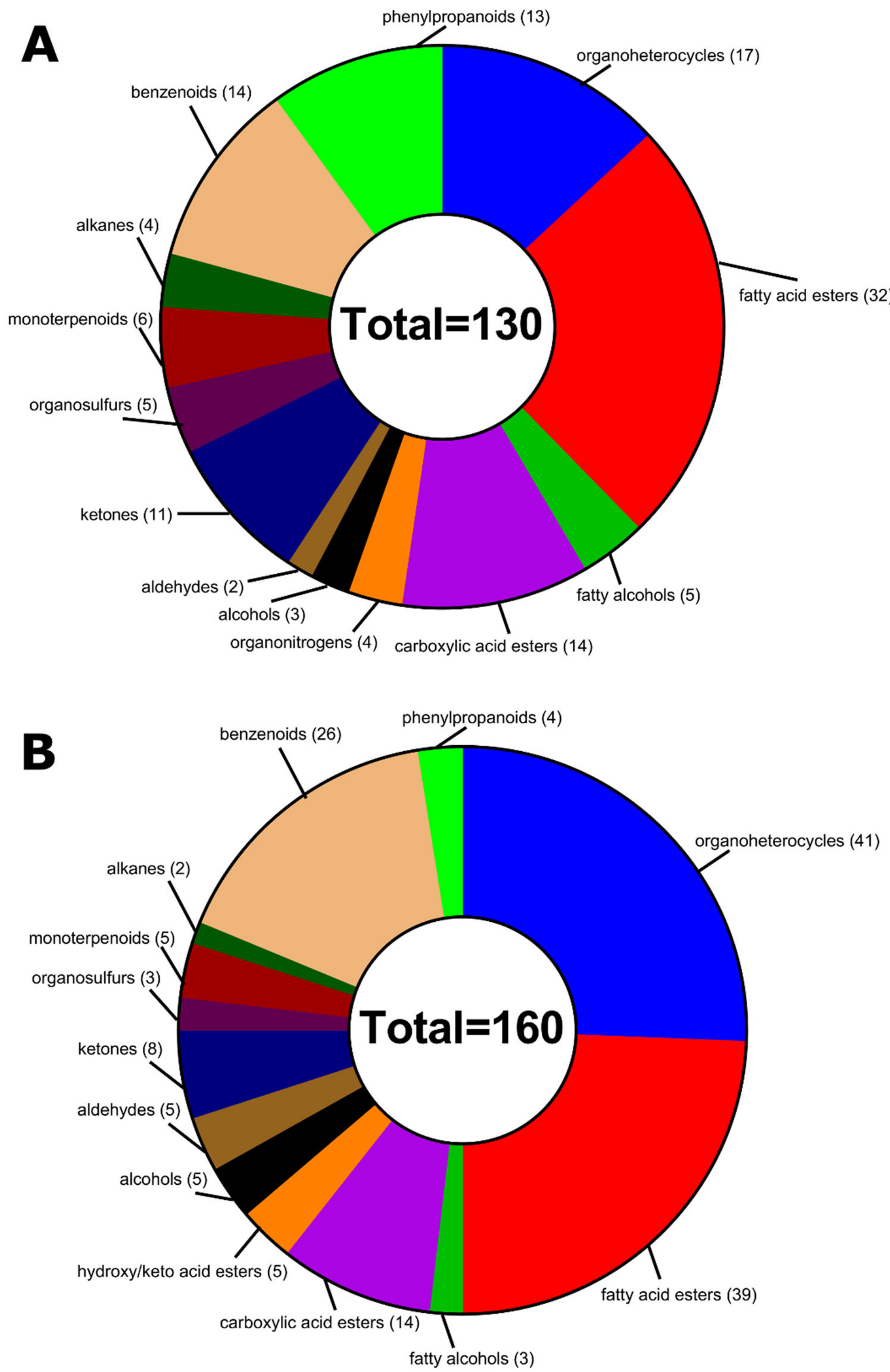

Figure 3. Annotated beer metabolites and the corresponding chemical classes for WRC and NP datasets. A total of 130 and 160 metabolites were annotated for (A) WRC and (B) NP, respectively. Pie charts display metabolites, by broad class (black text).

beers in duplicate (10 and 8 beers per set, respectively). Multifactor Analysis (MFA) plots of the WRC aroma coordinate data showed separation of the duplicates, which indicates that differences between the beers were subtle (Supplementary Figure 4). This pattern was also present in the coordinate data from the WRC flavor test, with the exception of Calypso and Violetta duplicates, which were closer together (data not shown). Correspondence Analysis (CA) with attribute data showed Calypso duplicates were close together and were described by *fruity* and *floral* in aroma (Figure 2), and *fruity* in flavor (data not shown for concision). Aroma attribute data showed differences between duplicates for the other 4 beer samples. *Fruity* was the most commonly used descriptor for this sample set, while *earthy*, *grainy*, and *floral* helped discriminate the samples from one another. Additionally, the flavor data showed

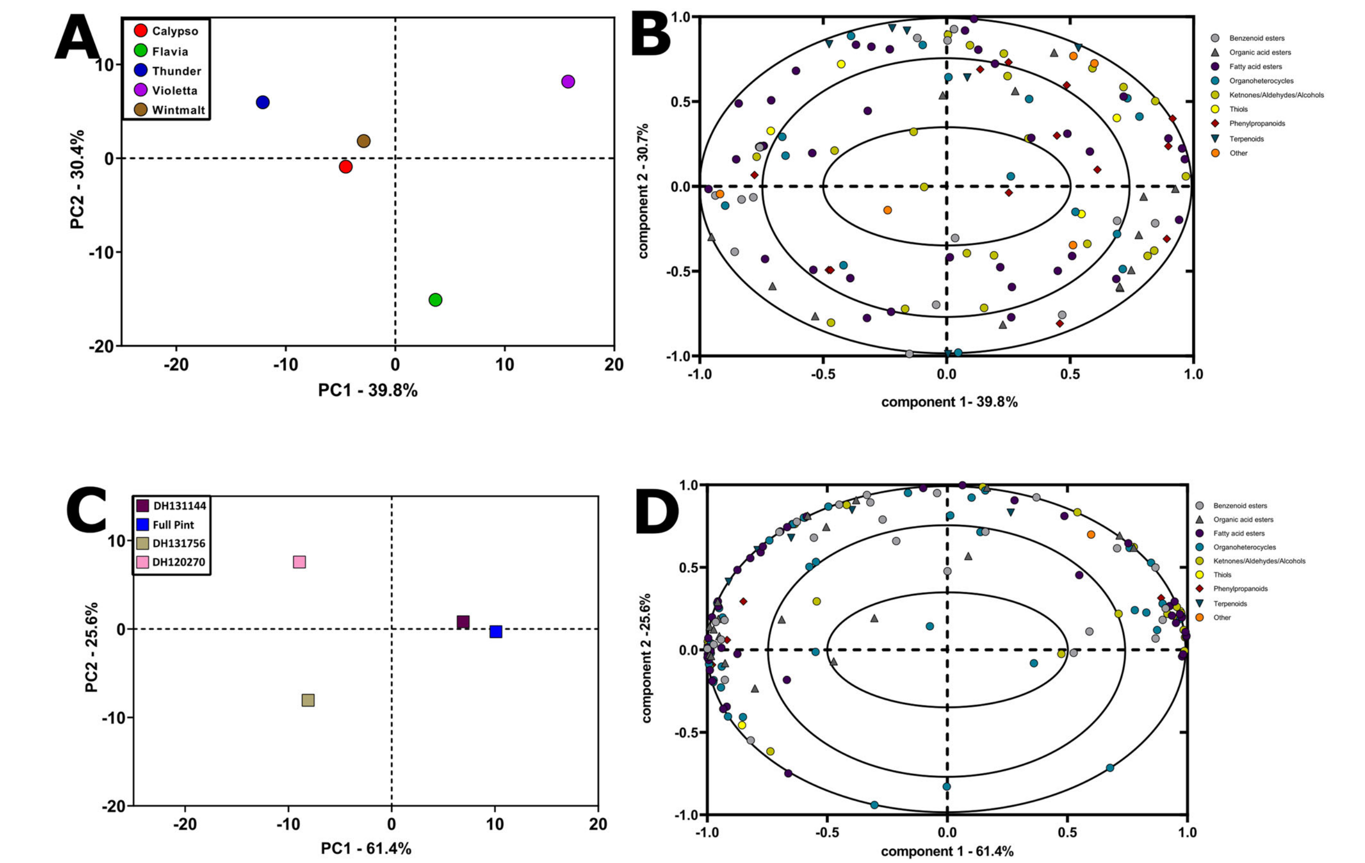

Figure 4. Principal component analysis (PCA) of beer metabolites of the 9 beers from WRC and NP, performed on the annotated metabolites for those datasets. PCA scores plots were produced based analysis of the 130 and 160 volatile metabolites, respectively (A) PC1 and PC2 for WRC and (B) corresponding correlation-scaled loadings plot, (C) PC1 and PC2 for NP and (D) corresponding correlation-scaled loadings plot. Loadings were colored according to broad sensory trait.

Flavia duplicates were similar and described by *grainy* and *grassy*. Wintmalt duplicates were close together and described by *sweet aromatic*, *floral* and *vegetal*. On average, Violetta duplicates were higher in *dough* and *sweet bread* than the other samples, which did not match its description by orthonasal evaluation. Thunder duplicates showed differences in use of *sweet bread* and *sweet aromatic* between them. In summary, there were inconsistencies in describing the WRC samples and with grouping the duplicate beer samples.

The MFA plots for the NP aroma sample set (8 beers) showed that, with the exception of DH131756, the duplicates are placed closely together, indicating that they were perceived as similar by the panel (Supplementary Figure 4). In the plot for the NP flavor sample set, DH131756 and DH131144 duplicates were mixed together, indicating that panelists were confusing these four beer samples. For both aroma and flavor evaluation, *grainy* was the most frequently used attribute for the sample set and thus unhelpful for discriminating samples (Figure 2). In both aroma and flavor, both *sweet bread* and *vegetal* had high variation in usage frequency between the samples (attribute count data not shown). DH120270 was described by *grassy* via orthonasal evaluation but was described by *vegetal* via taste evaluation (flavor data not shown). In both the aroma and flavor evaluations, the duplicates for DH131144 varied somewhat. In general, they were described with both *sweet aromatic* or *sweet bread*, as well as *dough*, *pasta,* or *cracker*. Although there were differences between the DH131756 duplicates they were both high in *fruity* in the aroma evaluation, and high in *sweet aromatic* in the flavor evaluation. Full Pint duplicates varied in their attribute counts for various descriptors but were consistently associated with *dough* in both aroma and flavor. Overall, duplicates were more similarly described for the NP sample set than the WRC sample set, indicating that there were greater differences between samples within the NP set.

Metabolomics

Metabolite variation among beers within the WRC and NP sets

From HS/SPME-GC-MS, 1,342 metabolites were detected and 130 were annotated within the WRC set and within the NP set, 676 metabolites were detected and 160 were annotated (Figure 3). Volatile beer metabolites were annotated and assigned to a super and sub-class based on chemical ontology (Tables 4 and 5). Classes of metabolites varied between WRC and NP datasets (Figure 3A,B).

PCA was conducted on the 130 volatile compounds with the five WRC beers and this demonstrates that variation was attributed to the *barley variety* (Figure 4A). PCA generated three principal components and was able to explain 86.6%

Table 4. WRC metabolite data.

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC0490	alkaloids	alkaloids	piperine	animal, pepper	0.26	0.91	0.71
WRC1013	benzenoids	benzaldehydes	benzaldehyde	almond, bitter, burnt sugar, cherry, sweet	0.74	−0.34	0.62
WRC0487		benzamines	benzoic acid, 2-amino-4-methyl-	NA	−0.80	0.09	0.57
WRC0437		benzenoids	1-phenyl-2-pentanol	earthy, green, mild, sweet	−0.83	−0.41	0.87
WRC0118			4-hydroxybenzyl alcohol	astringent	0.79	−0.12	< 0.05
WRC0058			benzamide, 4-ethyl-n-butyl-n-tetradecyl-	NA	−0.55	−0.13	< 0.05
WRC0303			n,n-dimethyl-3-methylaniline	NA	−0.61	0.40	0.93
WRC0031			phenylethyl alcohol	alcoholic	−0.03	−0.35	0.69
WRC0153		benzoic acid esters	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	almond, floral, herb, lettuce, phenolic, prune, sweet, wintergreen	−0.51	0.61	0.02
WRC0240			benzoic acid, 3-amino-	bitter	−0.49	0.54	0.78
WRC0081			m-anisic acid, cyclobutyl ester	NA	0.26	−0.84	0.43
WRC0485			phenoxyacetic acid	sour, sweet	0.66	−0.23	0.75
WRC0481			4-hydroxybenzyl alcohol	almond, bitter, coconut, fruity, sweet	−0.74	−0.55	< 0.05
WRC0195		phenols	phenol	phenolic	−0.62	−0.64	0.78
WRC0162		xylenes	2-thiophenecarboxaldehyde	NA	0.20	−0.45	0.99
WRC0632	hydrocarbons	alkanes	pentane	alkanes	−0.65	−0.36	0.60
WRC0298		hydrocarbons	(+/-)-n,n-dimethyl menthyl succinamide	cool, minty	0.14	0.59	0.35
WRC0087			2-butene, 2-methyl-	NA	0.78	0.06	0.03
WRC0183			3-octen-1-yne	NA	0.01	0.07	0.48
WRC0549	lipids	Fatty acids/fatty acid esters	2-hexenyl valerate	apple, banana, cognac, fruity, green, pineapple	−0.66	0.38	0.42
WRC0017			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	−0.68	0.27	0.73
WRC0038			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	−0.50	0.68	0.83
WRC1280			butanedioic acid ester	apple, apricot, chocolate, cooked, cranberry, fruty, grape, musty	0.71	0.54	0.50
WRC0395			cis-3-hexenyl 3-methylbutanoate	apple, fresh, fruity, green, pineapple, tropical	0.03	−0.39	0.61
WRC0042			decanoic acid, 2-methylbutyl ester	apple, brandy, fruity, grape, pear, sweet, waxy	−1.00	0.08	0.56
WRC0054			dodecanedioic acid ester	clean, floral, soapy, sweet	−0.66	0.70	0.00
WRC0016			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	−0.87	0.17	0.36
WRC0012			ethyl dodecanoate	clean, floral, soapy, sweet	−0.77	0.17	0.99
WRC0010			ethyl nonanoate-like	fruity, rose, rum, tropical, wine	0.83	0.05	0.91
WRC0021			ethyl nonanoate-like 1	fruity, rose, rum, tropical, wine	−0.33	0.88	0.18
WRC0034			ethyl nonanoate-like 2	fruity, rose, rum, tropical, wine	−0.45	0.35	0.72
WRC0037			ethyl nonanoate-like 3	fruity, rose, rum, tropical, wine	0.51	−0.62	0.10
WRC0838			hexanedioic acid ester	NA	−0.34	−0.80	0.31
WRC1258			hexanoic acid, 2-ethyl-, 1,1-dimethylethyl ester	apple peel, banana, fruity, pineapple, sweet	−0.07	−0.97	0.55
WRC0044			hexanoic acid, ethyl ester	apple peel, banana, fruity, green, pineapple, sweet	0.89	−0.04	0.84
WRC0055			hexanoic acid, ethyl ester	apple peel, banana, fruity, green, pineapple, sweet	−0.60	−0.61	0.05
WRC0850			hexyl butyrate	apple, apple peel, fruity, green, soapy, sweet, waxy	−0.72	0.23	0.88

(continued)

Table 4. Continued.

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC0502			methyl stearate	oily, waxy	−0.44	0.13	0.65
WRC0428			methylglutaric acid	NA	−0.81	−0.01	0.11
WRC0025			n-capric acid isobutyl ester	green, herbal, aldehydic, orange, sweet, vegetable	−0.76	−0.45	0.55
WRC0018			n-decanoic acid	apple, brandy, fruity, grape, pear, sweet, waxy	−0.13	0.46	0.14
WRC0056			octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	fatty, waxy	−0.26	0.56	0.58
WRC0053			octanoic acid, 3-methylbutyl ester	coconut, fruity, green, pineapple, soapy, sweet	−0.95	0.00	0.88
WRC0039			pentadecanoic acid ester	NA	−0.75	0.44	0.85
WRC0033			pentadecanoic acid, ethyl ester	NA	0.13	0.56	0.19
WRC0048			pentanoic acid, 3-methyl-, ethyl ester	apple, fruity, green, nutty, pineapple, sweet	0.37	−0.64	0.34
WRC0152			picolinyl 2,5-octadecadienoate	NA	0.13	0.42	0.34
WRC1067			propionic acid, ethyl ester	fruity, grape, juicy, pineapple, rum, sweet	0.07	−0.61	0.99
WRC0011			stearic acid	NA	0.56	0.71	< 0.05
WRC0098			tetradecanoic acid, ethyl ester	ether, soapy, sweet, violet, waxy	0.41	−0.83	0.74
WRC0036		fatty alcohols	1,2-hexanediol	NA	0.25	0.62	< 0.05
WRC0288			5-hexenol	green	0.60	0.12	0.44
WRC0028			octadecane-1,2-diol	NA	0.39	0.14	< 0.05
WRC0045			octadecane-1,2-diol	NA	0.03	−0.98	< 0.05
WRC1044		fatty amides	butyramide	nutty	0.49	0.70	1.00
WRC0638	organic acids	carboxylic acid esters	4-isopropylphenylacetic acid	cumin	0.58	−0.41	0.44
WRC0035			acetic acid, 2-phenylethyl ester	acidic, vinegar	0.20	0.68	0.94
WRC0149			acetic acid, hydroxy-, ethyl ester	vinegar, acetic	0.67	−0.24	0.21
WRC0679			chicoric acid	NA	−0.64	−0.53	0.39
WRC0063			cyclohexanecarboxylic acid, hexyl ester	NA	0.79	−0.31	0.17
WRC0384			dimethyl malonate	fruity	0.14	0.80	0.23
WRC0188			heptyl 2-methylpropanoate	apple, apricot, cherry, floral, fruity, grape, green, orange, pear, raspberry	−0.18	−0.95	0.37
WRC0390			isopentyl acetate	banana, bitter, fruity, solvent, sweet	−0.08	0.83	0.49
WRC0813			methoxyphenylacetic acid	NA	0.94	0.03	0.64
WRC0194			propanoic acid, ethyl ester	fruity, grape, juicy, pineapple, tropical, rum, sweet	0.79	0.41	0.64
WRC0047			triethyl citrate	acidic	0.98	−0.14	0.80
WRC0375			triethyl citrate	fruity, wine	−0.43	−0.88	0.99
WRC0806		thioesters	ethanethioic acid, s-(1-methylethyl) ester	coffee, fruity, garlic, meaty, onion, sulfur	0.77	0.56	0.75
WRC0061	organoheterocycles	benzodiazines	quinoxaline	NA	−0.80	0.11	0.51
WRC0304		benzopyrans	9h-xanthene-9-carboxylic acid 4-iodo-phenyl ester	NA	−0.76	−0.40	< 0.05
WRC0299		furanones	2,5-dimethyl-4-(1-pyrrolidinyl)-3(2h)-furanone	cereal	−0.78	0.11	< 0.05
WRC0228		furans	2-pentylfuran	butter, green bean	0.70	−0.18	0.75
WRC0020		indoles	1h-indole	NA	0.38	0.51	0.09
WRC0187		lactones	4-hydroxybutanoic acid lactone	NA	−0.18	−0.38	0.64
WRC0483		lactones	5-methyl-delta-valerolactone	herbal, sweet	0.49	−0.76	0.61
WRC0621		pyridines	2-methyl-5-(methylthio)pyrazine	NA	0.63	0.07	0.23

WRC0113			2-pyridinecarboxaldehyde	orange, beer	0.47	0.72	0.99
WRC0374			3-acetoxypyridine	NA	−0.67	0.30	0.28
WRC0198			3-butenoic acid, 2-oxo-4-phenyl-	caramel, green, radish, sweet, walnut	−0.56	0.26	0.69
WRC0514			3-pyridinecarboxamide	NA	−0.04	0.10	0.95
WRC0493			4-pyridinecarboxylic acid	NA	0.34	−0.16	0.28
WRC0489			pyridine	amine, fishy, putrid, rancid, sour	0.34	0.40	0.21
WRC0144		pyrimidines	6-amino-4-phenyl-1h-quinazolin-2-one	NA	−0.21	0.76	0.25
WRC0015		quinolines	4,8-dimethylquinoline	tea	−0.54	0.44	< 0.05
WRC0027			quinoline	tea	−0.72	0.68	< 0.05
WRC0626	organonitrogen compounds	amines	2-diethylaminoethanol	NA	−0.10	0.02	0.53
WRC0231		aminoalcohols	ethanol, 2-mercapto-	meaty, sulfur	−0.76	0.04	< 0.05
WRC0146		aminoxides	trimethylamine n-oxide	NA	0.82	−0.25	< 0.05
WRC0049		monoalkylamines	1,2-diamino-2-methylpropane		−0.04	0.78	0.69
WRC0377	organooxygen compounds	alcohols	1,3-propanediol	bitter	0.47	0.14	0.42
WRC0095			1-pentanol	balsam, balsamic, fusel, oil, sweet, vanilla	0.13	0.52	0.33
WRC0079			2,3-butanediol	buttery, creamy, fruit, fruity, onion	−0.34	−0.51	0.63
WRC0492		sugar alcohols	maltitol	NA	0.63	0.62	0.91
WRC0672		aldehydes	5-hydroxymethyl-2-furancarboxaldehyde	caramel, cardboard, musty, waxy	0.32	−0.54	0.55
WRC0154			pyrrole-2-carboxaldehyde	ethereal	0.48	0.70	0.59
WRC0041		alkenes	1,2-dimethoxy-ethene	NA	−0.05	0.86	0.98
WRC0050		cyclic ketones	5h-inden-5-one, 1,2,3,3a,4,7a-hexahydro-7a-methyl-, trans-	NA	−0.01	−0.69	0.40
WRC0686		enals	2-butenal, 3-methyl-	almond, cherry, fruity, nutty, sweet	0.99	0.13	0.29
WRC0522			2-propenal	almond, cherry	−0.24	0.69	0.54
WRC0642			2-propenal-like	almond, cherry	0.53	−0.06	0.56
WRC0110		ketones	2,4,6-tri-isopropylacetophenone	NA	0.49	0.63	0.43
WRC0376			5-methyl-3-hexen-2-one	berry, cheese, sweet	−0.38	0.28	0.79
WRC0184			benzyl ethyl ketone	tea	−0.74	−0.24	0.76
WRC0631			p-pentylacetophenone	NA	−0.33	0.90	0.41
WRC0378		monosaccharide phosphates	.alpha.-d-mannose 1-phosphate	NA	0.02	−0.61	0.35
WRC0156		o-glycosyl compounds	isomaltose	sweet	0.21	−0.40	0.33
WRC0088			hydroxylamine, o-methyl-	NA	0.43	0.59	0.99
WRC0089	organosulfur compounds	sulfonyls	methyl methanethiosulfonate	sulfur	−0.72	0.40	0.37
WRC0013		thiols	1-propene-1-thiol	sulfur	0.62	0.60	< 0.05
WRC0285			3-mercapto-3-methyl-1-butanol	meat, meat broth, roasted, spicy, sweet, vegetable	−0.80	0.39	0.25
WRC0503			ethanethiol	sulfur	0.73	0.45	0.80
WRC0604	phenylpropanoids	chalcones	2,2′,4′-trihydroxychalcone	bitter	−0.27	−0.79	0.63
WRC1022		cinnamaldehydes	3-(4-methylphenyl)-2-propenal	cinnamon, spicy	0.04	−0.03	0.72
WRC0230		cinnamic acid esters	isoamyl cinnamate	cocoa, floral, musty, orchid	0.26	0.35	< 0.05
WRC1015		coumarin glycosides	7-diethylaminocoumarin		−0.64	0.02	0.79
WRC0383		coumarins	3,4-dihydro-2h-1-benzopyran-2-one	almond, cinnamon, coconut, coumarin, creamy, herbal, sweet, tobacco	−0.31	0.23	0.52
WRC0496			3-hydroxycoumarin	NA	0.32	0.77	0.52
WRC0817			7-methoxycoumarin-4-acetic acid	NA	0.66	−0.56	0.36
WRC0125		curcuminoids	curcumin	NA	0.42	0.72	0.73

(continued)

Table 4. Continued.

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC0173		flavonoids	quercetin 3′-methyl ether	NA	0.02	0.20	0.96
WRC0830			kaempferol 3-o-rutinoside	NA	0.89	0.18	0.71
WRC0207			quercetin 3,5,7,3′,4′-pentamethyl ether	orange, oregano	0.03	0.58	0.29
WRC0266		hydroxycinnamic acid esters	trans-ferulic acid	NA	0.35	0.40	0.53
WRC0322		phenols	phenol	NA	−0.54	−0.72	0.32
WRC0071	prenol lipids	monoterpenoids	linalool	citrus, floral, green, lavender, lemon, orange, sweet	−0.02	0.92	0.37
WRC1030			p-menthan-1-ol	NA	−0.66	0.56	0.60
WRC0182			trans-geranic acid methyl ester	tea	0.08	0.81	0.82
WRC0284		sesquiterpenoids	alpha-cadinol	herb, woody	−0.68	0.59	0.22
WRC0196			alpha-cubebene	herbal	−0.82	0.52	0.51
WRC0155			epicubenol	NA	0.43	−0.84	0.57

a = Predicted flavor attribute based on information in FooDB [17]; NA = No flavor information found.
b = Correlation-scaled loadings examine the strength and direction of the relationship between the metabolite(s) and the sensory component (X) metabolites shown are those which met the threshold for this analysis, |< 0.75|.
c = From ANOVA supporting variation among the n = 5 beers.

of the variation in the data for the WRC varieties. In this scores plot, PC1 (39.8%) explained the separation between Wintmalt, Flavia, and Violetta vs. Thunder and Calypso. The loadings plot (Figure 4B) of volatile metabolites attributed to these WRC varieties did not explain any trends among the varieties.

PCA was conducted on the 160 volatile compounds detected in the NP set resulting in three principal components (Figure 4C) which explained 87.0% of the variation in the data for the three selections and Full Pint. In this scores plot, PC1 (61.4%) explained the separation between DH120270 and DH131756 vs. DH131144 and Full Pint. The loadings plot (Figure 4B) of volatile metabolites attributed to these varieties demonstrates a high content of lipids (fatty acid esters), terpenoids, and organoheterocyclic compounds (potential MRPs), specifically for DH120270.

OPLS modeling

To investigate relationships between the beer volatiles and each of the beer descriptors from the consumer panel (Figure 5A,B), an orthogonal projection to latent structures (OPLS) model was developed for two sensory attribute principal components (correlation-scaled PC1 scores for Violetta and traits such as *crisp, overall liking, refreshing, citrus,* and *floral,* with orthogonally correlated traits such as *astringent, bitter* (associated with Calypso) and correlation-scaled PC2 scores for *hoppy, honey,* and *toasted* (such as are associated with Thunder). The OPLS algorithm for the WRC set resulted in one predictive and two orthogonal component that explained 76.8% of the variation, with a predictive power of Q^2 = 98.8% to support that the model was not over-fit. Metabolites were considered to be associated to the "Violetta" trend if the correlation-scaled Component 1 loading > |0.75| and < |0.25| for the correlation-scaled orthogonal component (Figure 5A and C, Table 4). Furthermore, the OPLS algorithm which regressed PC2 scores resulted in one predictive and two orthogonal components that explained 76.4% of the variation with a predictive power of Q^2 = 94.8%. The metabolites associated with the "Thunder" trend (correlation-scaled PC2 scores) were subject to the thresholds previously mentioned (Figure 5E, Table 4).

For the NP set, an OPLS model was developed for two sensory attribute principal components (correlation-scaled PC1 scores for Full Pint and traits such as *toasted, molasses, caramel,* and *honey* with orthogonally correlated traits such as *citrus, bitter* (associated with DH120270) and correlation-scaled PC2 scores for *malty* and *non-tropical fruity* (such as are associated with DH131144) (Figure 5B and D, Table 5). The OPLS algorithm for the NP set resulted in one predictive and one orthogonal component that explained 81.9% of the variation, with a predictive power of Q^2 = 65.0% to support that the model was not over-fit). Metabolites were considered to be associated to the "Full Pint" trend if the correlation-scaled Component 1 loading > |0.75| and < |0.25| for the correlation-scaled orthogonal component (Table 4). Furthermore, the OPLS algorithm which regressed PC2 scores resulted in one predictive and two orthogonal

Table 5. NP metabolite data.

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP163	alkane	alkane	18-methyl-nonadecane-1,2-diol	alkane, bland	−0.30	−0.94	0.82
NP110	benzenoids	benzaldehydes	benzaldehyde-like	almond, bitter, burnt sugar, cherry, sweet	−0.69	−0.67	0.32
NP225			benzaldehyde-like	almond, bitter, burnt sugar, cherry, sweet	−0.92	−0.10	0.12
NP496		benzenoids	1-(3,4-dimethylphenoxy)-4-(3,4-dimethylphenylsulfonyl)benzene	benzene	−0.58	0.71	0.80
NP034			2-phenylethanol	bitter, floral, honey, lilac, rose, spice	−0.93	0.24	0.31
NP105		benzoic acid esters	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	almond, floral, herb, lettuce, phenolic, prune, sweet, wintergreen	0.13	−0.69	0.18
NP397			4-methoxybenzyl phenylacetate	anise, balsam, honey, woody	0.66	−0.17	0.82
NP083			allyl benzoate	berry, cherry, floral, sweet	−0.96	0.09	0.76
NP045			amyl salicylate	azalea, chocolate, clover, floral, green, herbal, sweet	−0.28	0.61	0.54
NP046			benzamide-like	bitter	0.62	0.05	0.23
NP041			butyl salicylate	clover, bitter, harsh	−0.32	0.59	< 0.05
NP390			ethyl benzoate	anise, balsam, banana, berry, bitter, cherry, cranberry, fruit, grape, minty, musty, sweet	−0.52	0.81	0.29
NP035			octyl benzoate	lemon balm, balsam, fruity	−0.52	0.52	0.66
NP226			phenylacetate	flower, honey	−0.14	0.32	0.24
NP113			salicylic acid ester	azalea, chocolate, clover, floral, green, herbal, sweet	0.69	−0.02	0.20
NP354			4-hydroxybenzoic acid	nutty, phenolic	−0.83	0.49	0.73
NP298			benzoic acid-like	bitter	−0.76	0.57	0.77
					−0.20	−0.93	
NP146		phenlyacetaldehydes	(e)-2-phenyl-2-butenal	phenolic, black tea	0.66	0.15	< 0.05
NP407		phenols	1,2-benzenediol	NA	−0.57	−0.04	0.35
NP122			2-ethylphenol	coffee	0.20	0.62	0.44
NP381			2-methoxy-4-vinylphenol	clove, curry, peanut, smoky, spicy	−0.78	0.54	0.09
NP091			phenol-like	phenolic, bitter	−0.84	0.44	< 0.05
NP221			phenol-like	phenolic, bitter	−0.86	0.48	< 0.05
NP379			phenol-like	phenolic, bitter	−0.43	0.89	< 0.05
NP348			vanillylmandelic acid	sweet, vanilla	0.08	−0.99	0.88
NP565		thiophenols	2,6-dimethylbenzenethiol	meaty, metallic, phenolic, roasted, sulfurous	−0.72	0.67	0.63
NP062	dithioles	1,2-dithioles	dithiole-like	sulfur	0.06	0.77	0.90
NP068	hydrocabons	alkanes	2-methylheptane	NA	0.34	−0.87	0.45
NP013	lipids	fatty acid esters	10-undecenoic acid, ethyl ester	clean, cognac, creamy, fruity, musty, soapy, waxy	−0.71	0.73	0.43
NP642			2-butenoic acid, phenyl ester	caramel, green, radish, sweet, walnut	0.27	−0.94	0.07
NP014			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	−0.90	0.39	0.45
NP398			3-nonenoate	fruity, green, melon, pear, watermelon	−0.74	0.70	0.76
NP416			butanoic acid, butyl ester	apple, banana, berry, fruity, peach, pear, pineapple, sweet	−0.80	0.61	0.98
NP024			decanoic acid ester	citrus, fatty, rancid, sour	−0.13	0.75	0.32
NP047			decyl propionate	cognac, ether, fatty, fruity, rum	0.30	0.49	0.80
NP375			diethyl decanedioate	fruity, melon, quince, wine	0.02	−0.51	0.28
NP477			diethyl maleate	banana	−0.39	0.81	0.90
NP011			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	0.46	−0.76	0.81
NP033			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	−0.44	0.19	0.72
NP012			ethyl decanoate-like 1	apple, brandy, fruity, grape, pear, sweet, waxy	−0.88	0.51	0.46
NP021			ethyl decanoate-like 2	apple, brandy, fruity, grape, pear, sweet, waxy	0.23	0.54	0.43

(*continued*)

Table 5. Continued.

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP031			ethyl dodecanoate	clean, floral, soapy, sweet	−0.35	−0.92	< 0.05
NP061			ethyl nonanoate-like 1	fruity, rose, rum, tropical, wine	−0.80	0.63	0.59
NP020			ethyl nonanoate-like 2	fruity, rose, rum, tropical, wine	−0.77	−0.05	0.41
NP016			ethyl nonanoate-like 3	fruity, rose, rum, tropical, wine	−0.23	0.79	0.50
NP044			ethyl nonanoate-like 4	fruity, rose, rum, tropical, wine	0.69	−0.09	0.39
NP096			ethyl propionate-like 1	fruity, grape, juicy, pineapple, rum, sweet	−0.85	0.49	0.65
NP295			ethyl propionate-like 2	fruity, grape, juicy, pineapple, rum, sweet	−0.87	−0.05	0.11
NP325			glutaric acid ester	NA	−0.53	0.81	0.19
NP165			glutaric acid, 2-ethylphenyl decyl ester	NA	0.17	−0.98	0.07
NP065			heptanoic acid, ethyl ester-like 1	berry, floral, fruit, green, sweet, waxy	0.62	−0.48	0.64
NP066			heptanoic acid, ethyl ester-like 2	berry, floral, fruit, green, sweet, waxy	−0.45	0.87	0.64
NP051			hexadecanoic acid, ethyl ester	balsam, creamy, fruity, milky	0.49	−0.53	< 0.05
NP048			hexanoic acid, ethyl ester-like 1	apple peel, banana, fruity, green, pineapple, sweet	−0.44	0.82	0.16
NP023			hexanoic acid, ethyl ester-like 2	apple peel, banana, fruity, green, pineapple, sweet	0.63	−0.44	0.30
NP025			hexanoic acid, ethyl ester-like 3	apple peel, banana, fruity, green, pineapple, sweet	−0.47	0.80	0.47
NP027			hexanoic acid, ethyl ester-like 4	apple peel, banana, fruity, green, pineapple, sweet	−0.85	0.49	0.48
NP302			isopropyl 2-methylbutanoate	ethereal, fruity, green, pineapple, sweet, tropical	0.38	−0.85	0.65
NP197			methyl caprylate-like 1	green, herbal, aldehydic, orange, sweet, vegetable	−0.30	0.81	0.43
NP019			methyl caprylate-like 2	green, herbal, aldehydic, orange, sweet, vegetable	0.56	−0.61	0.56
NP026			methyl caprylate-like 3	green, herbal, aldehydic, orange, sweet, vegetable	−0.30	0.76	0.60
NP154			octadecanoic acid, 17-methyl-, methyl ester	fatty, waxy	0.21	−0.95	< 0.05
NP028			pentadecanoic acid, ethyl ester	NA	−0.04	−1.00	0.48
NP018			pentanoic acid ester	fruity	−0.40	0.81	0.06
NP145			pentanoic acid, 2,4-dimethyl-, methyl ester	apple, fruity, green, nutty, pineapple, sweet	−0.92	0.43	0.56
NP222			pentanoic acid, 2-methyl	apple, berry, fruity, hazelnut, tropical	−0.02	0.02	0.45
NP218			tetradecanoic acid, ethyl ester	ether, soapy, sweet, violet, waxy	−0.13	0.71	< 0.05
NP194		fatty alcohols	1,2-hexanediol	NA	0.10	0.99	0.11
NP064			2-nonen-1-ol	cardboard	−0.72	0.71	< 0.05
NP288			cis-4-decenol	fatty, fruity, waxy	−0.82	0.56	< 0.05
NP097	organic acids	carboximidic acid esters	acetamide	mousy	−0.99	0.14	0.23
NP007		carboxylic acid esters	3-mercaptohexyl acetate	floral, fruity, passion fruit, pear, tropical	−0.58	−0.68	0.17
					0.00	−0.79	
NP558			3-mercaptopropionic acid	roasted, sulfurous	−0.70	0.71	0.27
NP253			acetic acid, 2-methylphenyl ester	vinegar, acetic	−0.62	0.73	0.27
NP037			acetic acid, 2-phenylethyl ester	vinegar, acetic	−0.54	0.84	0.27
NP038			acetic acid, methyl ester	vinegar, acetic	−0.48	0.74	0.38
NP077			acetic acid-like	vinegar, acetic	−0.67	0.67	0.44
NP206			acetic acid-like	vinegar, acetic	−0.72	−0.16	0.59
NP200			ethyl acetate	anise, balsam, ethereal, fruity, green, pineapple, sweet	0.14	0.95	0.91
NP003			ethyl lactate	butter, butterscotch, fruity, tart	−0.67	0.53	0.94
NP216			fumarate	NA	−0.07	−0.53	< 0.05

NP101			oxalic acid ester	NA	−0.90	0.34	0.75
NP040			1-butanol, 2-methyl	banana, fruity, juicy, overripe fruit, peanut, sweet	−0.54	0.49	0.97
NP030			isopentyl acetate	banana, bitter, fruity, solvent, sweet	−0.47	0.73	0.83
NP022		hydroxy acids	beta-hydroxypyruvic acid	cabbage, sour, radish	−0.85	0.45	0.10
NP141			ethyl 2-(methylthio)acetate	apricot, citrus, earthy, floral, fruity, green, herbaceous, meaty, nutty	−0.02	0.46	0.81
NP001			ethyl (±)-3-hydroxybutyrate	NA	−0.82	0.04	0.56
NP008			hydroxybutyric acid	NA	−0.71	−0.67	0.46
NP002			malic acid	NA	−0.55	0.73	0.50
NP454		keto acids	ketobutyric acid	NA	0.32	−0.88	< 0.05
NP056		benzodiazines	5-methylquinoxaline-;like	burnt, coffee, corn, nutty, roasted, toasted	−0.31	0.81	< 0.05
NP150			5-methylquinoxaline-;like	burnt, coffee, corn, nutty, roasted, toasted	0.86	0.43	0.64
NP213		benzopyrans	3,4-dihydro-6-methoxy-2,2-dimethyl-2h-1-benzopyran-4-ol	mushroom	−0.60	0.65	0.14
NP036			4-methylene-3,4-dihydroisocoumarin	NA	−0.10	−0.98	0.44
NP220		benzothiazoles	benzothiazole	coffee, gasoline, meat, nutty, rubber, sulfur, vegetable	−0.07	0.74	< 0.05
NP195		furanones	2(5h)-furanone, 5-methyl-5-phenyl-	NA	−0.78	0.64	0.82
NP387			5-methyl-3(2h)-furanone	NA	−0.44	0.78	0.60
NP198		furans	2-furoic acid ester	fruity, fungal, mushroom, sweet, tobacco	0.67	0.30	0.13
NP148			2-pentylfuran	NA	0.01	−1.00	0.97
NP259			3,4-furandicarboxylic acid	Maillard	0.27	−0.74	0.65
NP464		heteroaromatic compounds	2-(methoxymethyl)furan	coffee, roasted	−0.78	−0.26	0.77
NP393			2-(methylthiomethyl)furan	garlic, horseradish, onion, sulfur, vegetable	−0.42	0.84	< 0.05
NP076			2,5-dimethyl-3-(methylthio)furan	coffee, roasted	−0.87	0.39	0.96
NP563			2-propylthiophene	NA	−0.43	0.62	0.72
NP049			5-ethyl-(3h)-furan-2-one	spice	−0.35	0.35	0.09
NP289			dimethyl furan	onion	−0.15	0.34	0.98
NP052			furfuryl ethyl ether-like	coffee, roasted	−0.06	0.98	0.24
NP069			furfuryl ethyl ether-like	coffee, roasted	−0.88	0.42	< 0.05
NP497			furfuryl ethyl ether-like	coffee, roasted	0.45	−0.75	0.29
NP564			thiophene	garlic, onion	0.79	−0.59	0.55
NP545		isocoumarans	isobenzofuranone-like	celery, herbal	−0.50	0.19	< 0.05
NP515		lactones	6-butyloxan-2-one	coconut, coumarin, milky, sweet	−0.83	0.57	0.27
NP006		purines	hypoxanthine	NA	−0.76	0.52	0.57
NP306			purine-like	Maillard	−0.04	0.50	0.08
NP102		pyrazines	isopropyl pyrazine	green, honey, minty, nutty	−0.21	0.64	0.14
NP541			pyridine-4-carboxylic acid, 2,2,6,6-tetramethyl-4-oxo-1-piperidinyl ester	NA	−0.52	−0.70	0.98
NP088		pyrazoles	3-nonyl-1h-pyrazole	NA	−0.94	0.10	0.43
NP336		pyridines	3-butenoic acid	NA	−0.84	0.49	0.40
NP629			4-methylpyridine	tea, fig	0.16	−0.43	< 0.05
NP189			4-vinylpyridine	tea	0.39	0.35	0.55
NP050			4-vinylpyridine-like	tea	−0.73	0.70	0.43
NP300			5-methoxypyrimidine	NA	0.07	0.63	0.46
NP278		pyrimidines	2,4-diamino-5,6-dihydroxypyrimidine	NA	−0.22	0.72	0.38
NP512		pyrrolidines	2-pyrrolidinone	NA	−0.89	0.42	0.81
NP461		pyrrolines	3-acetyl-1h-pyrroline	NA	−0.04	−0.98	0.31
NP391			1-(4-methyl-1h-pyrazol-1-yl)ethanone	bread, nut, walnut	0.55	−0.22	0.46
NP396		quinolines	4,8-dimethylquinoline	tea	−0.79	0.45	0.71
NP094		thiazolidines	4,4-dimethyl-thiazolidine	NA	0.11	−0.98	0.59
NP333	organooxygen compounds	alcohols	1-(2-furyl)-3-buten-1-ol	fruity, sweet	0.45	−0.66	< 0.05
NP132			1-pentanol	balsam, balsamic, fusel, oil, sweet, vanilla	0.59	−0.48	0.51
NP147			2,3-butanediol	buttery, creamy, fruit, fruity, onion	−0.50	0.80	0.64

(continued)

Table 5. Continued.

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP262			2-buten-1-ol	NA	0.58	−0.53	0.67
NP455			shikimate	NA	−0.19	0.90	< 0.05
NP427		aldehydes	2-methyl-2-heptenal-like 1	almond, fatty, fresh, green, pungent, soap, vegetable	0.69	−0.01	0.22
NP560			2-methyl-2-heptenal-like 2	almond, fatty, fresh, green, pungent, soap, vegetable	−0.61	0.78	0.69
NP426			5-hydroxymethyl-2-furancarboxaldehyde	caramel, cardboard, musty, waxy, fatty	−0.39	−0.53	0.47
NP386			5-methyl-2-furancarboxaldehyde	almond, burnt sugar, caramel, maple, spice	0.35	−0.88	0.94
NP493			nonanal	citrus, fatty, fishy, fresh, grapefruit, lime, orange peel	0.33	−0.89	0.30
NP126		aryl alkyl ketones	2-acetylfuran	almond, balsam, beef, caramel, cocoa, coffee, peanut, potato, sweet	0.21	−0.96	0.79
NP478		carbonyl compounds	1-phenyl-1-pentanone	balsam, valerian	−0.84	0.48	0.34
NP042			2,5-dihydroxybenzaldehyde	NA	−0.14	−0.98	0.49
NP106			2-acetyl-3-(1-methyl-2-pyrrolyl)-1,4-benzenediol	bread, nut, walnut	0.16	0.44	0.30
NP255			1-hexene	caraway, celery, green, pepper, rooty, spicy	−0.05	−0.72	0.53
NP176		ethers	1-hexene, 4-methyl-	earthy, green, leafy, mushroom, violet	−0.66	−0.07	0.54
NP638		ketones	2-nonen-4-one	fruity	−0.54	0.80	0.42
NP428			3-penten-2-one	acetone, fishy, fruity, phenolic	−0.83	0.50	0.44
NP060			9-heptadecanone	NA	−0.31	0.81	< 0.05
NP269		sugar alcohols	galactitol	NA	0.40	−0.67	0.25
NP231	organosulfur compounds	thioethers	3-(methylthio)thiophene	NA	−0.32	0.82	0.58
NP373		thiols	3-mercapto-3-methyl-1-butanol	meat broth, roasted, spicy, sweet, vegetable	−0.37	0.93	0.54
NP299	phenylpropanoids	chalcones	2,4-dihydroxychalcone	NA	−0.07	−0.99	0.52
NP109		cinnamic acid esters	1-(m-methoxycinnamoyl)pyrrolidine	NA	−0.88	0.42	0.39
NP134			propyl cinnamate	amber, musty, vine	−0.60	0.64	0.33
NP205			ferulic acid	NA	−0.25	0.16	0.19
NP004		flavonoids	epicatechin	NA	−0.81	0.62	0.58
NP072	prenol lipids	monoterpenoids	4-isopropylbenzoic acid	NA	−0.98	0.25	0.41
NP131			alpha-terpineol	anise, citrus, floral, lilac, mint, oil, pine, terpene, woody	−0.98	0.08	0.10
NP634			citral	citrus, lemon, mint	−0.82	0.57	< 0.05
NP039			linalool	citrus, floral, green, lavender, lemon, orange, sweet			< 0.05
NP559			p-menthan-2-one	herbal, minty, spearmint	−0.98	0.25	0.09

a = Predicted flavor attribute based on information in FooDB [17]; NA = No flavor information found.
b = Correlation-scaled loadings examine the strength and direction of the relationship between the metabolite(s) and the sensory component (*X*) metabolites shown are those which met the threshold for this analysis, |< 0.75|.
c = From ANOVA supporting variation among the n = 4 beers.

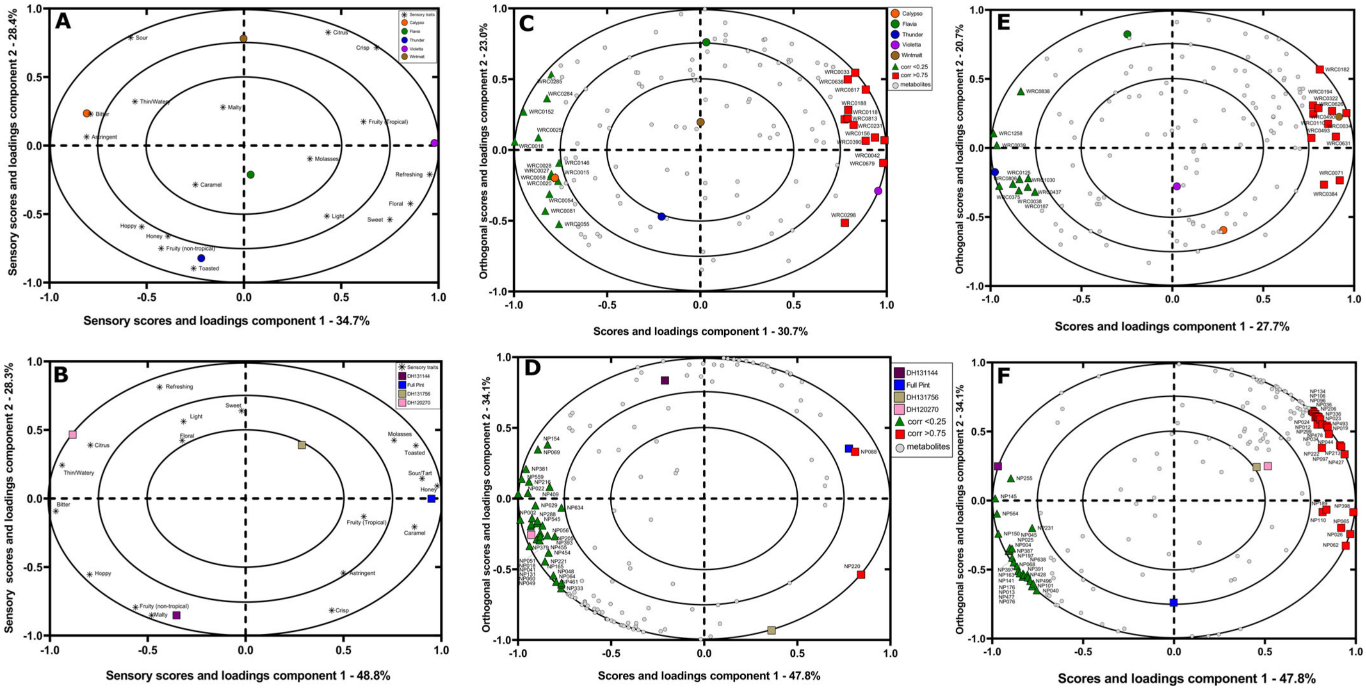

Figure 5. Multivariate association of beer metabolites with consumer panel sensory traits. PCA was performed on data for 14 sensory traits quantified for the 12 malt hot steeps (A) WRC PCA scores and correlation-scaled loadings biplot based on consumer panel data. (B) NP PCA scores and correlation-scaled loadings biplot based on consumer panel data. The association between beer metabolites and consumer panel sensory traits was evaluated with orthogonal projection to latent structures (OPLS) and performed on 130 and 160 volatile metabolites, respectively and PC1 scores from sensory analysis. Data is plotted as a biplot for correlation scaled scores (circles colored as per maltster; samples) and loadings (red squares for corr > |0.75| loadings; squares for orthogonal corr < |0.25 loadings; grey circles for metabolites that did not meet the threshold of loading corr) (C) WRC OPLS scores and loadings plot for regression against PC1 scores; (D) NP OPLS scores and loadings plot for regression against PC1 scores, (E) WRC OPLS scores and loadings plot for regression against PC2 scores; (F) NP OPLS scores and loadings plot for regression against PC2 scores. Notations for metabolites displayed as meeting the threshold are in Tables 4 and 5, respectively.

components that explained 74.4% of the variation with a predictive power of $Q^2 = 96.9\%$ (Figure 5F). The metabolites associated with the "DH131144" trend (correlation-scaled PC2 scores) were subject to the thresholds previously mentioned (Figure 5F). A SIMCA 'distance to model' function was applied to characterize the metabolites with the largest contribution to explaining the variation in significantly different sensory traits. The data indicate associations with organic acid esters, fatty acid esters, and benzoic acids, which are known classes of aroma compounds.

The sensory/chemistry which characterizes the "Violetta Trend" demonstrates co-variation of Violetta with traits such as *crisp, overall liking, refreshing, citrus,* and *floral,* but displays a negative association with traits such as *astringent, bitter* (associated with Calypso) (Figure 5A and C). The metabolites that are associated with this trend (correlations greater than 0.5 for Component 1, and less than 0.5 for Component 2) are noted in Table 4 (WRC) and 5 (NP) of sensory/volatiles. Metabolites that were positively correlated with attributes covarying with Violetta included benzenoids (4), fatty acid esters (5), organic acids (7), coumarins (2), ketones (2), and varying other classes. Two of the most correlated metabolites were an hydroxycinnamic acid (putatively identified as chicoric acid, WRC0679), which may impart a woody and nutty flavor (however, there are three other phenylpropanoids that are highly correlated, as well), and isomaltose (WRC0156, fatty acyl glycoside/oligosaccharide), which may contribute to sweetness, isopentyl acetate (WRC0390, banana, fruity). Other fatty acid esters and organic acid esters also had higher rates of correlation and have been associated to not only light, fruity flavors, but also to floral, refreshing flavors. Negative correlations included compounds of many of the same classes, but included many metabolites putatively identified as Maillard Reaction Products (such as WRC08606, ethanoic acid ester; furans, pyrazines, pyrans).

The sensory/chemistry cluster along OPLS Component 1 demonstrates co-variation of Full Pint and traits such as *crisp*, *fruity (tropical)*, and *sour/tart* to a lesser extent, *honey, caramel, toasted, astringent,* and *molasses,* and co-variation of DH131144 with both *fruity (tropical)* and *fruity (non-tropical).* By contrast, they are negatively correlated with *sweet, refreshing,* and *bitter* (Figure 5B and D, Table 5). DH120270 demonstrates co-variation with *bitter* and *thin/watery*. The metabolites that are associated with this trend (correlations greater than 0.5 for Component 1, and less than 0.5 for Component 2) are noted in Table 5 and Figure 5D. Metabolites that were positively correlated with attributes co-varying with DH131144 consisted of fatty acid esters (6) which are known volatiles related to fruity (tropical and non-tropical) attributes, specifically, diethyl maleate (NP477), ethyl hexanoate (NP025), a pentanoic acid ester (NP145), methyl caprylate (NP197), 10-undecenoic acid ester (NP013), and ethyl decanoate (NP021). Other classes which co-vary with DH131144 include benzenoids (benzoic acid esters, 4), organoheterocyclic compounds (potential

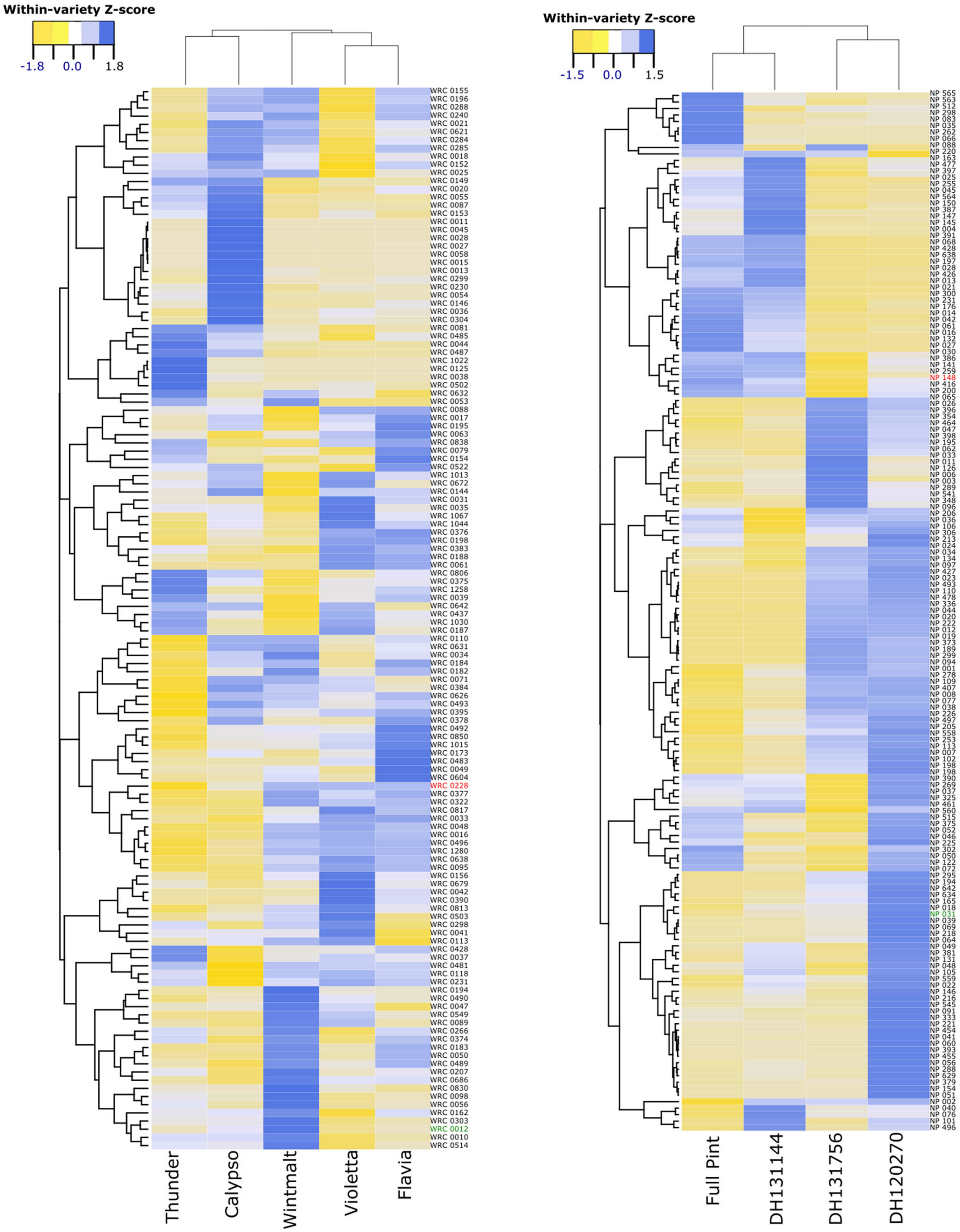

Figure 6. Univariate analysis of volatile metabolite variation among the 9 beers. Prior to heatmapping, volatile metabolite data were normalized within each variety via z-transformation normalized peak area - mean/standard deviation of total peak area of each metabolite).The resulting z-scores were converted into colors and grouped using hierarchical clustering on the Spearman's rank correlation (r^s) between metabolite and sensory trait values. Heat maps with hierarchical clustering were built within for (A) WRC dataset (B) NP dataset. The color in each cell represents the z-transformed abundances of the averaged replicates (n = 2) per beer sample. Z-transformation was based on the mean abundance and standard deviation of the metabolite across all samples. Metabolites in heatmaps are cross-referenced in Tables 3 and 4, and Supplemental Tables.

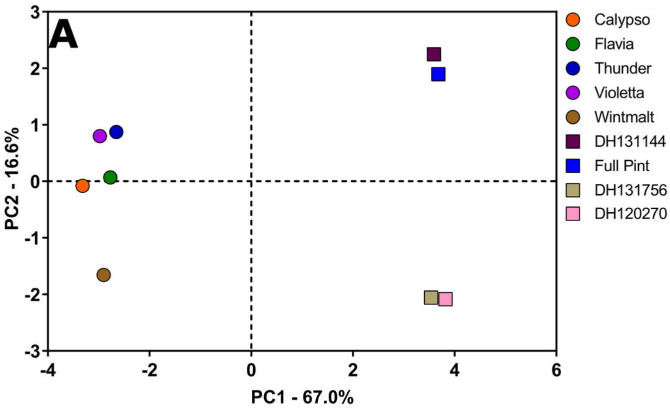

Figure 7. Principal component analysis (PCA) of beer metabolites of the 9 beers from WRC and NP, combined, performed on the annotated metabolites for those datasets. PCA scores plots were produced based on analysis of 290 metabolites.

MRPs, 9), and others. The heterocycles of note include 5-methylquinoxaline (NP150), known to contribute to Maillard-related attributes (coffee, roasted), and a thiophene (NP564), which can be attributed to garlic or onion flavors or aromas. Full Pint had a similar profile, with many similar co-varying metabolites. Three metabolites of note include: one fatty acid ester, ethyl hexanoate-like (NP027), known to contribute many tropical and non-tropical attributes, some of which were found in DH131144, octyl benzoate, a benzoic acid ester (NP035), which can contribute lemon balm, and 2,6-dimethylbenzenethiol (NP565), a thiophene, which can contribute Maillard-type attributes, such as meaty, roasted, and sulfur. DH131756, which contained the most abundant metabolite profile, co-varied with the consumer panel sensory attributes *sweet, refreshing,* and *molasses.* Metabolites which contributed to this are heterocyclic compounds (9), fatty acid esters (9), organic acid esters (4), benzenoids (2), and others. Fatty acid esters of note were ethyl-9-decenoate (NP006), decyl propionate (NP047), and methyl caprylate-like (NP026, NP019) that all are known to contribute to sweeter, more complex, fruity attributes. Vanillylmandelic acid, a benzenoid (phenol, NP011) can contribute to sweet and vanilla attributes; ethyl lactate, an organic acid ester, can contribute to butterscotch, fruity, and tart flavors. DH120270 had a unique profile, co-varying with *light, thin/watery, floral, citrus,* and *bitter* sensory attributes. Metabolite classes included heterocyclic compounds (15), fatty acid esters and terpenoids (11), organic acid esters (6), and others. Two heterocyclic compounds of note are 4-methylpyridine (a pyridine, NP629), known for tea and fig properties, and 5-methylquinoxaline, known for roasted properties. There are many metabolites, which are known to have phenolic and bitter sensory properties that may contribute to the co-variation with *bitter,* assessed by the consumer panel and with the *cracker* and *sweet aromatic* assessed by the laboratory panel. Examples of these metabolites include 2-phenyl-2 butenal (NP146), a phenylacetaldehyde known to contribute a bitter, black tea note and 2-methoxy-4-vinylphenol (NP381), recognized for the contribution of clove, smoky, and spicy attributes.

Other trends among chemical classes

The data were evaluated to determine if broad trends of metabolite classes could distinguish each of the beers within the sets: specifically, for lipids (to include fatty acid ester formation), nitrogenous compounds, organic acids, and phenolics. Metabolite abundances were z-transformed to express the data as a profile within a variety, therefore a range in color denotes range in variation of a compound class within a variety, with very blue (high) or very yellow (low) to indicate proportions of a metabolite's contribution to the profile (Figure 6A,B, Supplementary Tables 4,5).

The heatmap for the WRC beers showed Calypso had a unique profile, abundant in alkanes, alkenes, and benzoic acid esters that were not abundant in the other four varieties, also being more abundant in prenol lipids (terpenoids) including linalool (WRC0071), p-methan-1-ol (WRC1030), alpha-cadinol (WRC0284), alpha-cuebene (WRC0196), and geraniol (WRC0182). These metabolites have been associated, in literature, not with *bitter* and *astringent* sensory attributes, as denoted from the sensory panel, but with the *grassy* and *vegetal* (among other attributes noted in the literature, such as floral, citrus, and menthol) noted in the aroma factor analysis from the laboratory panel.[22–24] Calypso was also abundant in a class of organoheterocycles known as "quinolines," which have been shown to be attributed to a tea-like flavor (bitter, astringent) in the literature.[17] Among the five beers, there were no trends among lipids/fatty acid esters, as they were equally distributed. The nitrogenous compounds shared by Wintmalt and Flavia included 42-diethoaminoethanol (WRC0626), pyridine-like compounds (WRC0374, WRC0493, WRC0489), which may contribute to or overpower the other sensory attribute of *citrus* and instead contribute to the *malty* seen in the consumer panel and *breakfast cereal, bready*, and *earthy* attributes from the laboratory panel. Organic acids predominate Violetta, and to a lesser degree, Thunder (Figure 6, Supplementary Table 4). One organic acid ester, triethyl citrate (WRC0375), which is known to contribute to vinous and non-tropical fruity attributes, is seen to covary with Thunder and the *fruity (non-tropical)* sensory attribute from the consumer panel, as well as the *sweet aromatic* attribute from the laboratory panel. The organic acids most unique to Violetta included acetic acid ester (WRC0035), triethyl citrate (WRC0047), ethyl propanoate (WRC0194), isopentyl acetate (WRC0390), 4-isopropylphenylacetic acid (WRC0638), dimethyl malonate (WRC0384), and heptyl-2-methylpropanoate (WRC0188) (Supplementary Table 5). Violetta, Wintmalt, and Flavia displayed negative correlations with the prevalent benzenoid class that was shown to covary with Calypso. This class included 1,2-benzenedicarboxylic acid ester (WRC0153), known to be associated with almond, floral, herbal, green, and more phenolic attributes, 4-hydroxybenzyl alcohol (WRC 0481), and benzaldehyde (WRC1013), associated with more almond, bitter attributes.

The heatmap for the NP beer set displays trends between Full Pint/DH131144, and within certain classes between DH131756/DH120270, although DH120270 again was recognized as having the most unique profile (Figure 6, Supplementary Table 4). The trends between Full Pint and DH131144 include higher abundances of aldehydes and ketones such as 2-nonen-4-one (NP428), 1-hexene (NP255), and 1-pentanol (an alcohol, NP132). Full Pint and DH131144 also shared many abundant fatty acid esters, noted in the previous section. Trends within the organic acid ester class occurred between DH131756 and DH120270, including many -likes of acetic acid, keto acids, and an acetamide of note (NP097), which in literature has been known to contribute a mousy attribute.

Metabolomics: considering both sets of beers

To assess the Next Pint and WRC beers together, PCA and OPLS was performed on all nine beers (Figure 7). Only metabolites that were annotated and shared among all varieties were included in the analysis, abundances were unit variance normalized. Four principal components were able to explain 94.8% of the data. PC1 (68%) and PC2 (16.6%) were able to explain significant variation among these data (Figure 7). The differences may be attributable to "environment" (i.e., two completely different locations, one dryland, the other irrigated); genetic relationships (i.e., Full Pint as a parent of all NP lines and no WRC lines); growth habit (one set winter and the other spring); degree of selection (one set commercially available, the other set comprised of three advanced experimental varieties and the "control"); and/or to the higher abundance of metabolites in the WRC set (Figure 7).

Discussion

Barley, malting quality, and beyond

The barleys used for this research form two distinct groups categorized by three factors that may confound the data: growth habit, commercial status, and production environment. The WRC set is comprised of winter growth habit, commercially available varieties grown under dryland conditions while the NP set is comprised of spring growth habit experimental selections and a "check", grown under irrigated conditions. Although the two sets were treated identically through brewing, beer and malt hot steep sensory, and beer metabolomics, these treatments occurred at different time points. Therefore, it is necessary to discuss the results of each set separately. However, there are commonalities between sets that merit some further discussion and integration, both *inter se* and with prior research.

The first commonality is genetic relatedness. Violetta, a member of the WRC set, is also a parent of two members of the NP set (DH131756 and DH131144). Violetta is the female parent in one cross and the male parent in the other, which could have some bearing on the flavor differences between the two sister lines: in Angiosperms, organelles show maternal inheritance: therefore, the chloroplast and/or mitochondrial genomes these two selections could be genetically different and those polymorphisms could lead to flavor differences. However, most phenotypes of commercial importance in barley studied to date (e.g., agronomic and malting quality traits) show nuclear, rather than cytoplasmic, inheritance.[25] In this regard, it is not surprising that these two doubled haploid siblings could have contrasting malting quality and other downstream phenotypes based on contributions from the nuclear genome only.

Exploring pedigree records provides insight to possible genetic contributions to beer flavor and malt quality. Tracing further back in the pedigree chart (Supplementary Figure 1) shows many genotypes in this experiment sharing notable malting varieties, such as Hanna (Czech – Haná) and Spratt, in their pedigrees. Haná originates in Moravia (present day Czech Republic) and was used in the development of Pilsner beer in the 1840s. The spread of Pilsner and Pilsner-style beer in the late 19th century and Haná's reputation for agronomic, malting, and beer quality led it to be used in many breeding programs and it factors in the pedigree of many contemporary malting barleys. Spratt is well known as the parent of iconic British malting variety Spratt-Archer, which was lauded for its vastly improved agronomics and adaptability for the time.[26] Spratt-Archer was widely grown in the middle 20th century and figures into the pedigrees of other iconic varieties such as Maris Otter.[27] Klages is a notable American variety that fits in the pedigrees of six of the experimental genotypes, including all of the NP set. It was the dominant malting variety grown in the Pacific Northwest in the 1970s and 1980s and was the 2-row variety adopted by many early craft brewing companies. Maris Otter, an heirloom variety from the United Kingdom with a reputation for providing a unique flavor profile,[28] is a direct parent of one NP member (DH120270) and also figures in the pedigree of one WRC member (Calypso).[29] Full Pint, the "check" in the NP set and a parent of all three experimental varieties in the NP set, was chosen as a parent of the Oregon Promise due its reputed flavor profile, as described by Bettenhausen et al.[3] and Herb et al.[1,2] Other varieties of note that contribute to the pedigrees in this experiment: European landraces Criewener 403, Pflugs Intensiv, Bavaria, and Danubia (all nine lines); Isaria, Kenia, and Gull (all nine lines); and Puffin and Malta (missing from Full Pint and DH120270).

While pedigree doesn't provide the full picture of the genetic relationships between these nine barleys, it is valuable in showing common and different ancestries that may explain some of the phenotypic flavor contrasts. A systematic investigation of flavors contributed by notable varieties in these pedigrees, coupled with genome profiling, is warranted. In order to increase the efficiency of such an undertaking, DNA fingerprinting of the nine genotypes featured in the current research is underway. This information, coupled with the QTL mapping of flavor that is also underway in the Oregon Promise population, could identify specific alleles associated with specific metabolites. These alleles and metabolites could then be traced back through pedigrees to identify specific genotypes for grain production, malting, and brewing.

Capitalizing on this genetic relatedness to identify the genetic drivers of differences in quality parameters, flavor, and metabolic profiles will be the topic of a future paper - where sample size is larger and complete genotype data are available. At this point, however, specific differences and commonalities between the two sets can be pointed out that relate to variety and therefore impact on one of the questions driving this research: "do barley genotypes contribute to beer flavor?" These differences and commonalities will be highlighted during this Discussion, which will proceed sequentially by feature (e.g., malt analysis, sensory analyses, metabolomics) but progressively integrating results for each trait and its impacts on other traits.

Malting quality specifications are key metrics for barley variety release. Within the WRC set, the lower degree of modification of Wintmalt and higher degree of modification and enzyme-related trait values for Thunder were notable. Both varieties are on the AMBA recommended variety list, which requires thorough vetting for quality and brewery performance. Although every effort was made to produce optimum malts for all varieties, for reasons unknown Wintmalt did not achieve target specifications in this project. Lastly, the NP set had overall higher grain protein, which may have affected downstream flavor, sensory, and metabolite composition. The impact of grain protein on beer quality parameters is known,[30] but the specific impact of protein across different genotypes is outside the scope of this paper. Field sites in this study were managed for supplemental nitrogen per their respective standard operating procedures. Research on field nitrogen applications and impact on grain protein, malt quality, and flavor is ongoing.

Sensory attributes of malt hot steeps and beer, and their relationships

Hot steep malt sensory

Prior to the establishment of the hot steep malt sensory method, Congress worts were used for sensory evaluation of malt samples.[31] Since its development, the hot steep malt sensory evaluation method has piqued the interest of the brewing and malting industries to improve analysis of malt sensory and predict beer sensory for malts of interest.[5,6] It is helpful when only a small quantity of malt is available and is more convenient than making beer. The predictive ability of this method, though much more rapid than brewing, has yet to be fully understood. With the analysis pipeline implemented in this research, we can identify relationships of hot steep malt sensory with other traits. However, determining if relationships are causal and predictive will require further experiments.

Within the WRC set, Thunder and Calypso were standout samples for hot steep malt sensory. The former was higher in *sweet bread* and *sweet aromatic* for both aroma and flavor while the latter was *grassy* and *vegetal* in aroma and *cracker* in flavor. Considering the other varieties in this set, Thunder and Violetta were lower in *grassy* thus separating them from the other samples. DH120270 was a standout sample within the NP set. In both the aroma and flavor evaluations, it was consistently described by panelists as more *grassy* and *earthy* than the other samples. Malt analytics provide clues that Thunder was more modified than Calypso, thus leading to differences in hot steep malt sensory. While it seems likely that the *sweet bread* and *sweet aromatic* descriptors for malt hot steeps are attributable to the higher enzyme profiles of Thunder, DH131144, and DH131756, further research is necessary. The basis of the *grassy* profile for Calypso is not obvious, however in the case of DH120270, it could be ascribed to under-modification. Given this line's Maris Otter heritage, this may be a question for further research. From a plant breeding perspective, the poor modification of DH120270 and its *grassy* and *earthy* profile in the hot steep malt sensory would be grounds for not advancing it on to brewing and beer sensory. In this sense, evaluations using hot steep malt sensory could be a tool in variety selection. In order to assess its value for the malting and brewing industries, the key question remains "is hot steep malt flavor predictive of beer flavor"? The current research provides some insights into this relationship, but further experiments will be required. Within the current experiment, the connection between malt and beer sensory is best explored using the laboratory panel data, given the commonality of protocol and lexicon.

Laboratory beer sensory

The laboratory beer sensory panel had some difficulty matching duplicates within the WRC set to one another, with the exception of Calypso. However, differences in sensory attributes were still perceived among the beer samples. This pattern suggests that stringent selection for commercial potential led to barleys that, despite differences in malt and beer analytics, produced beers that are only subtly different in sensory profiles. The nuanced differences may result from inconsistencies in malt-modification (Table 2).[1] There is evidence to show that undermodified malts may result in higher *grassy* qualities.[3] In the NP set, duplicates were more similarly described for both aroma and flavor, indicating that panelists not only found differences among the beers but that these differences could be identified with consistency. This consistency of difference may be due to the more limited selection and validation for malting and brewing properties of the NP set, as compared to the WRC set. DH120270 duplicates were closely grouped, with consistent *grassy* aroma and *vegetal* flavors. This could be due to the lower malt modification of DH120270, leading to *grassy* and *earthy* flavors,[3] compared to the other NP samples. DH131756, DH131144, and Full Pint had similar malt analytical profiles, which may be one reason why there was less distinction in flavor profiles among the beers made from these malts.

Comparing beer and hot steep malt sensory

While beer samples were all duplicated, only one malt hot steep sample per set was duplicated. Therefore, there was only one measurement of panelist consistency for the malt hot steep evaluations. While mashing and steeping processes mirror one another, it is important to note that mashing takes place at a higher temperature for a longer time than steeping. A commercial mashing operation thus converts more starch to fermentable sugar and reduces proteins to smaller polypeptides. Both of these variables can impact flavor and mouthfeel.[32] It is clear that the differences among beers were more subtle and nuanced than those of the malt hot steeps. For example, once the malt was brewed into beer, the *grassy* characteristic of DH120270 decreased, making it more similar to the profiles of the other NP samples. The standout samples for the malt hot steeps, DH120270 (*grassy*) and Thunder (*sweet aromatic*), were less noticeably different in the beer sensory evaluation. Observing patterns of descriptor usage across the two sensory methods can give us insights into the connection between the two. Both *grassy* and *grainy* were used more in malt hot steep characterization than beer characterization. *Floral* was used only once in the description of malt hot steep aroma but became an important attribute for beer sensory. Similarly, *fruity* was used infrequently to describe malt hot steep samples but very frequently to describe the resulting beers. *Floral* and *fruity* aromas were likely present in beer due to the addition of hops and the production of esters by yeast during fermentation.[33,34] Nonetheless, some attributes were stable across both malt hot steep and beer sensory. For example, Thunder retained its *sweet bread* quality from malt hot steep to beer. Results from this study indicate that hot steep malt sensory profiles are more distinct than those of their resulting beers. It is important to note that beer sensory profiles will also be influenced by fermentation byproducts and interactions with hops. More evidence is needed to make further conclusions about the predictive ability of the hot steep malt method.

Comparing consumer and laboratory beer sensory

Differences in lexicon, panel size, methodology (including panel training), and goals preclude directly comparing the sensory results from laboratory panel and consumer panels. Nevertheless, both panels identified differences in beer flavor within the WRC set; in particular, the consumer panel identified *citrus, floral, hoppy*, and *sweet* as the differentiating attributes within the set. For the laboratory panel, *dough, sweet bread fruity,* and *floral* were key attributes that differentiated the finished beer samples. It is important to note that a set of lexicons were preselected and provided to consumers to describe each beer sample due to panelists lacking specific sensory training. The lexicon provided to consumers had fewer attributes related to the *bread* category, while adding more options that fell under *sweet aromatic* (*caramel, honey*). Beers brewed from Violetta and Calypso – at opposite ends of the overall liking spectrum – had very similar malt and beer analytics, suggesting that these objective measures are not necessarily predictive of hedonic assessment. This finding also indicates that there can be differences in beer flavor, attributable to barley variety, in the relatively small number of commercially available winter two-row malting barley varieties.

In contrast to the WRC set, no significant differences were found in overall liking of NP beers evaluated by the consumer panel. However, both laboratory and consumer panels coincided in differentiating DH120270 from other samples: *lighter* and *thin/watery* by the consumer panel and *grassy* by the laboratory panel. DH120270, therefore, is consistently different from the other selections and the Full Pint check, indicating that this experimental variety could have been eliminated at the malt analysis stage, with no need to go on to the expense of malt and beer sensory. In a commercial application, the lack of significant differences in liking between DH131756 and DH131144 indicates that either of them could potentially be selected to replace Full Pint without an adverse consumer perception of beer flavor. The decision could be based primarily on agronomics and malt analytics. The latter, while not necessarily predictive of beer flavor in this research, can be key in variety approval and malt sales.

Beer metabolomics: connecting chemistry with sensory analysis and analytics

Metabolomics and sensory

Of the WRC beers, Violetta produced the beer with the highest score for *overall liking* in the consumer sensory panel, encompassing previously described desirable traits for a lager – namely *refreshing, crisp, citrus, sweet,* and *light.*[3] This variety had reduced MRPs and a unique profile of fatty acid esters (Figures 3 and 6). Calypso, unique in pedigree, similar to the other varieties in malt and beer analysis, and a standout in hot steep malt sensory and beer sensory, had a unique chemical profile. It also had the lowest *likeability* score of the WRC beers in the consumer sensory panel. Because the PCA revealed separation of the WRC varieties that did not match any of the similarity groupings according to malting quality, beer analytics, or laboratory/consumer sensory, we looked to specific variety: metabolite associations.

The stringent selection applied to varieties during breeding and commercialization – which may not have included consumer sensory assessment – may have led to minor differences in volatile compounds, including an increase in compounds that convey *bitter* or *astringent.* As noted in the results, Calypso was more abundant in prenol lipids (terpenoids) and in a class of organoheterocycles known as "quinolines," which are associated with a tea-like flavor (bitter, astringent).[17]

There were no trends among lipids/fatty acid esters among the five varieties, as the lipid/fatty acid ester class (acetate esters) was generally equally distributed. The medium-chain fatty acid ethyl esters (ethyl hexanoate and ethyl octanoate), however, co-varied with Calypso (Figures 5 and 6).[35,36] The nitrogenous compounds shared by Wintmalt (less modified malt) and Flavia (well-modified malt) included 2-mercapto-2-diethylaminoethanol (WRC0626) and pyridine-like compounds (WRC0374, WRC0493, WRC0489) which may contribute to, or overpower, the sensory attribute of *citrus* and instead contribute to *malty* noted by the consumer panel and the *breakfast cereal, bready*, and *earthy* attributes identified by the laboratory panel. Organic acids predominate in Violetta, and to a lesser degree, Thunder (Figure 6, Table 4). An organic acid ester, triethyl citrate (WRC0375), which is known to contribute to vinous and non-tropical fruity attributes, co-varied with Thunder and the *fruity (non-tropical)* sensory attribute from the consumer panel, as well as the *sweet aromatic* attribute from the laboratory panel. The organic acids most unique to Violetta included acetic acid ester (WRC0035), triethyl citrate (WRC0047), ethyl propanoate (WRC0194), isopentyl acetate (WRC0390), 4-isopropylphenylacetic acid (WRC0638), dimethyl malonate (WRC0384), and heptyl-2-methylpropanoate (WRC0188) (Table 5). Violetta, Wintmalt, and Flavia had negative correlations with the prevalent benzenoid class, which covaried with Calypso. This class included 1,2-benzenedicarboxylic acid ester (WRC0153), known to be associated with almond, floral, herbal, green, and more phenolic attributes; 4-hydroxybenzyl alcohol (WRC0481); and benzaldehyde (WRC1013), which is associated with more almond, bitter attributes.

In the NP set, Full Pint and DH131144 had higher abundances of aldehydes and ketones – such as 2-nonen-4-one (NP428), 1-hexene (NP255), and 1-pentanol (an alcohol, NP132) – and they shared many abundant fatty acid esters. Although Full Pint, DH131144, and DH131756 were similar in sensory attributes, DH131756 and DH120270 shared many -likes of acetic acid, keto acids, and an acetamide of note (NP097) that is noted in literature to contribute a mousy attribute. There are many metabolites that are known to have phenolic and bitter sensory properties that may contribute to the covariation with *bitter* in DH120270, identified by the consumer panel and with the *cracker* and *sweet aromatic* assessed by the laboratory panel. Examples of these metabolites include 2-phenyl-2 butenal (NP146), a phenylacetaldehyde known to contribute a bitter, black tea note and 2-methoxy-4-vinylphenol (NP381), recognized for the contribution of clove, smoky, and spicy attributes.

Given the distinctiveness of the WRC and NP germplasm sets in terms of growth habit, production environment, and commercialization status, the causes of similarities and differences are confounded, but notable. Some of these differences could be attributed to genetic relatedness: e.g., Full Pint is unique to the NP set as a member and as a parent. When DNA fingerprint data are available for the WRC and NP sets, causal effects based on genetic differences may be identifiable. The WRC varieties, as a group, contained fewer organoheterocycles (potential MRPs) than the NP varieties (Figure 3). As discussed in Bettenhausen et al.[3] MRPs play a major role in beer flavor. Two metabolites, furfural and 2-pentylfuran belong to the class of organoheterocycles known as furans, furfural serving as a precursor to 2-pentylfuran, which contributes fruity, grassy flavors (NP148 and WRC0228, Figure 6, denoted in red text). All varieties contained this furan, but normalized abundances differed among all varieties. Lower abundances of MRP in the WRC may be related to the lower grain protein, overall. Since degree of modification involves protein breakdown (through protease activity), incomplete modification would leave these varieties lacking in components for the Maillard Reaction (proteins, saccharides).[37] In the NP set there were fewer instances of phenylpropanoids (a class including cinnamic acid esters and coumarins) and more benzenoids (phenols, benzoic acid esters) than in the WRC set. Phenolic compounds are formed via the shikimate pathway and are known to contribute to more *bitter* and *astringent* attributes, such as those found in DH120270. Fatty acid esters, especially ethyl dodecanoate, (WRC0012 and NP031, denoted in Heat Map (HMap) in green text) were present in DH120270 and Wintmalt. Abundances of ethyl dodecanoate in other varieties were well below the amounts in Wintmalt and DH120270. These two genotypes were also the least modified (Table 2) and differed the most for beer analytics. The development of these fatty acid esters, through esterification of ethanol with fatty acids, is crucial for development of flavors, but the lipids that are present in each variety (type and amount) may play a role in how much of that flavor is developed and at what rate. The presence of these compounds (ethyl octanoate, ethyl-9-decenoate, n-decanoic acid) in conjunction with the low MRP/organoheterocycle profile of WRC suggests not only that these compounds contribute to desirable attributes associated with Violetta, but that they could also contribute to off-flavors during aging.[38–40]

Metabolomics, malting quality, and beer analytics

Wintmalt met the fewest malt quality specifications of the WRC set (Table 2) yet produced an acceptable beer by consumer panel standards and no negative attributes were noted by the laboratory panel. Violetta and Flavia were noted as having more complex flavor profiles; this is potentially due to variable (on the edge of acceptability) S/T, total protein, and FAN levels (Table 2), leaving less for the development of Maillard reactions products (MRPs) to create *roasted* and *caramel* attributes from degraded protein and saccharides.[37,38] The lack of MRP attribute creation leaves more room for lipid conversion into fatty acid esters and therefore the potential for lighter *fruity, floral* attributes to be perceived. Thunder, which had the highest diastatic power and lowest RDF, produced a beer that was perceived as more *crisp* and *dry*, with no residual sweetness and showed covariation with the *caramel, honey, toasted,* and *non-tropical fruity* from the consumer panel and *sweet bread* and *sweet aromatic* from the laboratory panel. The higher FAN in Thunder, as opposed to the level found in Violetta, may be a source of MRPs, and thus be an indicator of potential flavors in beer. The lighter flavors expressed by Violetta may be linked to the greater concentrations of fatty acid esters, which are described as *sweet*, *fruity*, and *floral*.[41] The lower degree of modification of Wintmalt and DH120270 could produce beers with *grassy* attributes due to the presence of acetaldehyde, hexanal, hexanol and general "greenness" of the malt.[41] Furthermore, under-modified malt tends to produce less extract during mashing and therefore lower than target ethanol concentrations after fermentation. The lower level of modification combined with low diastatic power of Wintmalt were likely reasons for it producing the lowest RDF in the study (Tables 2 and 3). Wintmalt and DH120270 also had the haziest wort, which may have been due to either low modification or high molecular weight beta-glucans, these in turn could lead to possible unintentional flavor outcomes. Full Pint and DH131144 were chemically the most similar of the NP varieties despite differences in two malt quality parameters linked to endosperm modification - friability and beta-glucan. The NP set as a group was less friable than the WRC set, averaging 77% versus 96% (Table 2). Nonetheless, there were no significant differences in the brewhouse yield between the two sets of malt ($t = 0.494$, $p = 0.318$ for one-sided t-test).

Conclusions

This study contributed to the body of knowledge by examining the effects of more and different barley genotypes on beer flavor. The current results support our previous findings that barley genotype does lead to differences in flavor profiles of lager beer. Two sets of barley germplasm (1) commercially available winter barleys and (2) Full Pint and three advanced progeny breeding lines were found to have distinct, subtle differences that contributed to nuanced flavor profiles of both malt hot steeps and finished lager beer. Variations between and among barley germplasm sets were greatest for malt analytics, and this variation declined for beer analytics and then again for sensory profiling. Consumer and laboratory panels detected differences in sensory attributes of beer and malt hot steeps, but the basis of these differences was not always obvious. It is important to emphasize, in this context, that the descriptors and preferences reported are applicable only to these research beers and should not be taken as representative of the specific barley varieties and/or selections and their production environments.

Nonetheless, the research findings support the value of sensory assessments of pilot and commercial-scale beers of potential and new varieties. While common practice in the final stages of the variety recommendation and/or adoption processes, brewing and sensory assessment may also have value earlier in the variety development pipeline. Sensory assessments can continue to play an important role for defect elimination and can be expanded to include discovery of new flavor opportunities. In the case of the WRC set, a variety with acceptable malt and beer analytics was not favored by the sensory panels while a variety with less favorable malt and beer analytics was acceptable. In the case of the NP set, one potential variety could be eliminated based on flavor as well as on poor malting and brewing quality attributes. The remaining two selections were not appreciably different in sensory profile from the reference variety, which simplifies the variety selection process to decisions based on agronomics, malting quality, and/or beer quality.

All measures and procedures used in this research have value in guiding decisions regarding variety selection, but none were directly predictive of another. For example, malt analytics can guide maltster decisions on what barley varieties are likely to produce consistent malt using existing malting protocols in order to meet brewers' expectations. Additionally, while exploring the ability of hot steep malts as an economical and efficient predictive tool for beer flavor profiles, there were some attributes that were stable across both beer and hot steep malt sensory analysis. Hot steep malt sensory profiles were found to be more distinct than those of their resulting beers. The current research provides some insights into this relationship, but other experiments are justified in order to define the basis of this relationship: the hot steep malt sensory may provide a useful common language for maltsters and brewers. Moreover, metabolomics can provide insights into the chemical basis of specific sensory descriptors and consumer preference. Distinct metabolomic profiles were detected within and between germplasm sets that were attributable to variety. Covariation of metabolomic profiles and sensory attributes was identified in both panels. These observations lead to the conclusion that the variable metabolites observed among the two sets of barley germplasms are a direct result of genetic differences that lead to differential responses within the malting and brewing processes. When metabolites are connected to genes, barley breeders will have additional targets for selection in order to meet target, or novel, beer flavor profiles. Until then, the new knowledge generated by this research can be capitalized upon by extending it to additional barley genotypes, different malts of the same varieties, and different beer styles.

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