

# Malt Modification and Its Effects on the Contributions of Barley Genotype to Beer Flavor

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## ABSTRACT

J. Am. Soc. Brew. Chem. 75(4):354-362, 2017

Based on prior research that showed significant genetic differences between barley genotypes for beer sensory descriptors, the effects of degree of malt modification on these descriptors were assessed in two experiments. The first experiment involved sensory assessment of nano-beers made from micromalts of Golden Promise, Full Pint, 34 doubled haploid progeny, and the check CDC Copeland. Average degree of modification was assessed by sampling grain from each of the 37 genotypes stored for three postharvest intervals prior to malting and brewing. The second experiment involved sensory assessment of pilot beers made from intentionally under-, properly, and overmodified pilot malts of two barley varieties: Full Pint and CDC Copeland. In both experiments, genotypes were the principal sources of significant variation in sensory descriptors. Degree of modification and genotype  $\times$  modification interactions were also significant for some descriptors. Based on the results of this study, the genetic characterization of and selection for barley contributions to beer flavor are warranted, even with undermodified malts. The contribution of barley variety to beer flavor will likely be modest compared with the flavors developed during the malting process and the flavors contributed by hops and yeast. However, in certain beer styles, the contributions of barley genotype may be worth the attention of maltsters, brewers, and consumers.

Keywords: Barley, Malt, Beer, Flavor, Modification, Sensory

Malted barley is a key ingredient in most beers because it provides an optimized source of nutrition for yeast and a suite of color and flavor properties. However, the variety of barley used to produce the malt rarely appears on a beer label. At most, the types and sources of malt may be featured. This lack of barley exposure can be attributed to the same barley variety being used to produce a range of malts and the major contributions of hops and yeast on beer flavor. Despite the general lack of barley visibility to the beer consumer, potential barley varieties undergo extensive and rigorous assessment for agronomic and malting quality attributes prior to their release. In the United States, the American Malting Barley Association (AMBA) ([www.ambainc.org](http://www.ambainc.org)) ensures recommended varieties meet quality specifications established by AMBA members. Comparable agencies are found in other countries where

malting barley is an important crop. Maltsters and brewers ultimately decide which of the recommended varieties will enter their value chains, and these decisions are made based on a host of considerations, including a spectrum of malting quality parameters. These parameters, in turn, are a function of barley variety genetics, the production environment, and the malting regime. Rarely do these considerations include the positive contributions of the barley variety per se to beer flavor. Notable exceptions are the varieties Maris Otter and Golden Promise, both released in the 1960s. Even though agronomic performance and malting quality—in terms of specifications and yield—of these varieties are not at the same level as those of more recent varieties, they continue to be grown, malted, and used owing to real or perceived contributions to the flavor of beers and/or spirits made from them.

The genetic and biochemical basis of the flavor contributions of these “heirlooms” and other varieties is a new area of research with work currently in progress. In a prior report, Herb et al. (5) addressed whether 1) barley genotypes can make differential contributions to beer sensory attributes and 2) production environment can affect flavor, and they concluded that the observed differential contributions to flavor are most likely related to differential performance during malting. Dissecting the relationships of malting quality parameters and contributions to beer flavor is a challenge for a number of reasons. First, a malting protocol for a contemporary variety may not be suitable for Golden Promise, Maris Otter, or other “novel” genotypes, and protocols for novel genotypes may not be suitable (or cost effective) for contemporary varieties (3). Second, the interactions of genetics and environment add a layer of complexity to malting quality (10). For example, an otherwise excellent malting variety may make poor malt when grown in a particular environment owing to factors ranging from the obvious (e.g., excessive nitrogen fertilization, preharvest sprouting, and/or *Fusarium* infection) to the less obvious (e.g., water sensitivity or induced dormancy). Ideally, assessment of flavor contributions in a range of barley genotypes would be based on removal of the confounding effects of malting protocol and environment. There are, however, challenges to achieving this ideal.

Growing all genotypes in the same environment is feasible, and input management can help to minimize interactions. However, tailoring malt quality protocols to achieve the same or similar quality parameters for each genotype is not feasible for a large sample size of genotypes. Instead, in research malting, large numbers of genotypes are typically malted using a single protocol in an automated micromalting system. The resulting malts are analyzed following American Society of Brewing Chemists (ASBC) procedures for  $\alpha$ -amylase,  $\beta$ -glucan, diastatic power, free amino nitrogen (FAN), Kolbach index, malt color, malt extract, and vis-

\*The e-Xtra logo stands for “electronic extra” and indicates that a file containing 15 supplementary tables and one supplementary figure appears online.

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cosity. These traits, their interrelationships, their biochemistry, and their genetics are defined in a number of texts (2) and reviews (4). Of direct relevance to this research is the term “modification,” which Briggs (2) defined as “an imprecise term that signifies all the desirable changes that occur when grain is converted into malt.” Despite the lack of precision, the term is widely used in malting and brewing. Proper modification is a signature of well-made malt. Undermodified and overmodified malts are undesirable. Specific malting quality parameters, such as FAN, can impact the contributions that yeast makes to flavor.

Building on the work of Herb et al. (5), this study explored the effects of degree of malt modification on beer sensory descriptors in two experiments. The first experiment involved sensory assessment of nano-beers made from micromalts of Golden Promise, Full Pint, 34 doubled haploid progeny, and check CDC Copeland. Grain from each of the 37 genotypes was stored for three postharvest intervals prior to malting and brewing. The second experiment involved sensory assessment of pilot beers made from intentionally under-, properly, and overmodified pilot malts of two barley varieties: Full Pint and CDC Copeland.

## EXPERIMENTAL

### Experiment I

Golden Promise, Full Pint, 34 of their doubled haploid progeny, and CDC Copeland were the barley genotypes used for this research. The focus of this report is on grain from one location (Madras, OR, U.S.A.), the procedures/analyses used for storing and micromalting this grain, and the procedures/analyses used for nano-brewing and sensory assessment of the nano-beers made from these micromalts. Detailed descriptions of the full experiment involving the 37 genotypes grown at three locations were given by Herb et al. (5). Briefly, the Madras location is located at 44.63°N, 121.12°W. Because the area receives average annual precipitation of 25.4 cm, barley is grown with supplemental irrigation. The barley genotypes were grown in a two-replicate randomized complete block design. The plot size was 9.2 m<sup>2</sup>. Plots were seeded at a rate of 250 seeds/m<sup>2</sup> using a Plotseed XL grain drill (Wintersteiger AG, Ried im Innkreis, Austria). Plots were harvested at maturity using a Classic plot combine (Wintersteiger AG). Grain samples were cleaned using an SLN3 sample cleaner (Rational Kornservice, Kitzingen, Germany) prior to micromalting. Samples (250 g) of each barley genotype were micromalted using a Joe White Malting unit at Rahr Malting Co. (Shakopee, MN, U.S.A.) at each of three different time points. These three time points (hereafter referred to as storage treatments [ST] with a numerical suffix indicating the order of grain sampling) were as follows: 1 month postharvest (ST-1), 5 months postharvest (ST-2), and 10 months postharvest (ST-3). The three time points were selected to assess the effects of grain storage on malt modification. The complete micromalt quality data are shown in Supplementary Tables I–III, and select data are presented in the body of this report. Malt quality analyses were performed for  $\alpha$ -amylase,  $\beta$ -glucan, diastatic power, color, extract, FAN, soluble protein, total protein, Kolbach index, and viscosity following ASBC procedures.

Malt samples were nano-brewed to a pilsner style with a target specific gravity of 1.0318 and a final volume of 800 mL. One hundred forty-five beers were brewed for this experiment; 111 unreplicated beers were brewed from the 34 doubled haploid progeny, the two parents, and the CDC Copeland check from each of the three storage treatments. In addition, malt produced from grain of each of the parents (2014 harvest, Corvallis, OR, U.S.A.) was used to brew replicated beers of Golden Promise and Full Pint (11 beers each). Twelve replicates of the control beer (Rahr Pils) were also brewed. Rahr Pils (brewed at the Rahr Malting Co. Technical Center) is a light ale made with pilsner base malt and

crystal malt and hopped with Amarillo and Cascade hops. All beers were brewed over a 12 day period. Complete brewing protocols were described in detail in Herb et al. (5).

Assessments of the nano-beers were based on a ballot consisting of 17 robust flavor descriptors as reported in Herb et al. (5). The sensory assessment of the nano-beers used a comparison-to-reference descriptive analysis, in which each sample was compared with an industry reference beer (Miller High Life). Each flavor descriptor was ranked based on the magnitude of difference from Miller High Life using a 0–8 scale, where 0–3 is less than the reference, 4 is no difference, and 5–8 is greater than the reference. Owing to the large number of beers involved in this experiment, an augmented design was used in which reference beers (Golden Promise, Full Pint, Miller High Life, and Rahr Pils) were replicated and beers from the 37 genotypes (Golden Promise, Full Pint, the 34 doubled haploid progeny, and CDC Copeland) were not replicated. The replicated beers provide the variance estimates used to calculate best linear unbiased estimators for the unreplicated samples, as described in the next section. In each of nine sessions, 15 beers were tasted. In the 10th and final session, 16 beers were tasted.

Statistical analyses were conducted using JMP Pro statistical software (version 12, SAS Institute, Cary, NC, U.S.A.). A mixed linear model approach was used to analyze the sensory data. First, analyses of variance (ANOVAs) were performed for the sensory descriptors of replicated sensory checks. Student's *t* test was used to calculate *F*-protected least significant differences (LSDs) for mean separation, and the Bonferroni correction was applied to adjust the critical *P* values to reduce the incidence of false positives. The variance from the replicated beers within each session was used to adjust the unreplicated beers for the calculation of best linear unbiased predictors (BLUPs) using restricted maximum likelihood. Panelists and checks were considered fixed effects. Sessions and unreplicated beers nested within sessions were considered random effects. Principal component (PC) analysis was used to examine the relationships between the sensory descriptors and genotypes based on the correlation matrix formed from the BLUPs (within locations) and sensory checks (across locations). PC analysis was performed using nonrotated correlation matrices.

### Experiment II

Full Pint and CDC Copeland were used for this experiment. Full Pint was selected because it is a parent of the Oregon Promise population and was included in experiment I. CDC Copeland was used as a check in experiment I and is an industry malting quality standard. The grain for both varieties was produced in the same experiment under dryland conditions and harvested in 2015 at Lebanon, Oregon, U.S.A., located at 44.56°N, 122.89°W. The grain of each genotype had the same grain protein (11%) and kernel plumpness (>95% on a 2.4 mm screen).

Grain samples were pilot-malted at the Canadian Malting Barley Technical Center (CMBTC), Winnipeg, Manitoba, Canada. Each genotype (5 kg) was malted in each of three batches to generate malts with different degrees of modification (modification treatment 1 [MT-1] = undermodified, MT-2 = well modified, and MT-3 = overmodified). The malting conditions are described in detail in Supplementary Table IV. Identical malt quality analyses were performed by the CMBTC as in experiment I with the inclusion of water sensitivity, dormancy/germination capacity and germination energy, friability, and fine-coarse difference. All analyses followed the ASBC procedures (1).

The six malt samples were each pilot-brewed to an all-malt ale specification using 1.5 kg of malt and a water/malt ratio of 3.75:1 during mashing. The mash profile was as follows: 1) 48°C for 30 min, 2) raise 1.5°C/min to 65°C for 30 min, and 3) raise 1.5°C/min to 77°C for 1 min. The mash was transferred for lautering

then sat for 5 min, allowing the grain bed to settle before vorlaufing for 1 min until the wort ran clear. Lautering rakes ran at slow speeds while sparging 6 L of water to wash out residual sugars. The wort was boiled for 60 min, adjusted to 1.048 specific gravity with a water addition, chilled to 20°C, pitched with Lallemand Nottingham Ale yeast, and fermented at 14°C until fully attenuated. The beer was lagered at 1°C for 7 days and then bottled using a counterpressure bottle filler. The bottles were then packaged and used on-site for sensory analysis or shipped to cooperating breweries (Deschutes Brewery and New Glarus Brewing Co.) for sensory analysis.

The bottled beers were evaluated for flavor at the CMBTC (Winnipeg Canada), Deschutes Brewery (Bend, OR, U.S.A.), and New Glarus Brewing Co. (New Glarus, WI, U.S.A.). Each sensory assessment utilized a trained panel varying in size and tasting unit. At the CMBTC, 12 panelists tasted 60 mL beer samples; Deschutes Brewery used six panelists tasting 120 mL beer samples; and New Glarus had three panelists tasting 240 mL beer samples. The sensory assessment of the six beer samples used a comparison-to-reference descriptive analysis, in which each sam-

ple was compared with an industry reference beer (Budweiser) to describe flavors at each of the modification levels. The ballots consisted of the same 17 robust flavor descriptors as experiment I, plus chocolate-coffee, dimethyl sulfide, and diacetyl (Supplementary Fig. 1). All beer samples were tasted in replication during a single session at each location.

Statistical analyses were conducted using JMP Pro 12 software. The ANOVA of the sensory data was performed using a mixed linear model. Student's *t* test was used to calculate LSD values for *F*-protected mean separation. PC analysis was used to assess the distribution of variation among malting quality and sensory traits.

## RESULTS

### Beer Sensory Traits

**Experiment I.** Key results from the ANOVA of the 37 genotypes for the 10 most important flavor descriptors identified by PC analysis (see the PC analysis of malting and sensory traits section below) are shown in Table I. Data for sensory traits are presented separately for storage treatment main effects, genotype

**TABLE I**  
Key Terms in the ANOVAs of Beer Sensory Descriptor Data<sup>a</sup>

Source	Body	Chemical	Color	Floral	Fruit	Honey	Malt	Sweet	Toasted	Toffee
Experiment I										
Storage treatment (ST)		*		*						
Genotype (G)	***	***	***	***	***	***	***	***	***	***
ST × G		*	***	**	*			*		
Experiment II										
Modification treatment (MT)	*		**				*			
G	*	**	***	**	*				*	**
MT × G				**	*					**

<sup>a</sup> Experiment I: data from 37 barley genotypes (Golden Promise, Full Pint, 34 Golden Promise × Full Pint progeny, and CDC Copeland) sampled at three post-harvest storage intervals. Experiment II: data from CDC Copeland and Full Pint beers brewed from undermodified, modified, and overmodified malts. \*, \*\*, and \*\*\* indicate significant *P* value from 0.05 to 0.01, from 0.01 to 0.001, and <0.001, respectively.

**TABLE II**  
Grain Storage Duration Treatment Means for Malting Quality and Beer Sensory Traits for 37 Barley Genotypes<sup>a</sup>

Storage treatment	Malting quality traits								Beer sensory traits	
	AA	BG	DP	FAN	KI	MC	ME	VC	Chemical	Floral
ST-1	46.3	537	134	133	33.3	1.3	75	1.7	4.4	4.5
ST-2	46.1	644	129	139	31.5	1.6	75.7	1.8	4.7	4.3
ST-3	56.4	185	146	157	35.9	1.7	76	1.5	5.0	3.9
LSD (0.05)	...	...	...	...	...	...	...	...	0.4	0.4

<sup>a</sup> Genotypes: Golden Promise, Full Pint, their 34 doubled haploid progeny, and CDC Copeland. Values shown are for beer sensory traits that had significant storage treatment effects in the ANOVA (Table I). Storage treatments: ST-1, 2, and 3 = 1, 5, and 10 months of postharvest grain storage, respectively. Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power ( $^{\circ}$ ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU).

**TABLE III**  
Grain Storage Duration Treatment Values for Malting Quality Traits and Adjusted Means for Beer Sensory Traits for 37 Barley Genotypes<sup>a</sup>

Genotype	Malting quality traits								Beer sensory traits				
	AA	BG	DP	FAN	KI	MC	ME	VC	Body	Honey	Malt	Toasted	Toffee
Golden Promise	42.5	384	151	147	34	1.5	74	1.6	2.9	4.2	3.8	4.1	3.4
Full Pint	75.2	278	172	198	40	1.5	78	1.6	3.3	4.7	5.2	4.7	4.8
CDC Copeland	62.6	232	146	182	37	1.6	78	1.6	3.4	4.3	3.8	3.7	3.9
LSD (0.05)	...	...	...	...	...	...	...	...	0.4	0.5	0.7	0.6	0.6
DH mean	50.0	442	138	146	34	1.5	76	1.7	2.8	4.2	4.1	3.9	3.9
DH minimum	33.5	107	89	98	27	1.3	73	1.5	2.0	3.1	2.4	2.6	2.3
DH maximum	75.1	1220	208	214	42	1.8	78	2.2	3.7	5.0	5.6	5.3	5.5

<sup>a</sup> Genotypes: Golden Promise, Full Pint, their 34 doubled haploid progeny, and CDC Copeland. Values shown are for beer sensory traits that showed significant genotype effects but no storage treatment × genotype interaction in the combined ANOVA (Table I). Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power ( $^{\circ}$ ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU). DH = doubled haploid.

main effects, and storage treatment  $\times$  genotype interactions in Tables II, III, and IV. Malting quality data are also presented in these tables; however, because these malting quality data were not replicated, they are addressed separately in the next section. Chemical and floral were the only descriptors significantly affected by the length of grain storage prior to malting and brewing; both traits were also significant for genotype and for the genotypic interaction with storage treatment. Averaged over genotypes, chemical increased with storage duration and floral decreased, most notably from ST-1 to ST-2 (Tables II and IV). Only the genotype main effect was significant for body, honey, malt, toasted, and toffee (Table III). Focusing on the parents and check, Full Pint was significantly higher than the other two varieties for malt and toffee and higher than the other two for honey and toasted, with the differences at, or slightly below, the significance threshold. Golden Promise was significantly lower in body than CDC Copeland and Full Pint. Progeny maximum and minimum values for all descriptors were usually beyond the high and low parent values. There were significant storage treatment  $\times$  genotype interactions for chemical, color, floral, fruit, and sweet (Table IV). Color increased with storage duration and was significantly higher in ST-3 compared with ST-1 and ST-2 for Golden Promise, Full Pint, and CDC Copeland. Fruit was significantly higher in ST-1 than in ST-3 for Golden Promise and CDC Copeland; there was no significant difference between storage treatments for Full Pint. Sweet was significantly higher in ST-3 compared with ST-1 and ST-2 for Golden Promise, Full Pint, and CDC Copeland. Progeny maximum and minimum values for all descriptors were usually beyond the high and low parent values.

**Experiment II.** The complete sensory data sets for each brewery are provided in Supplementary Tables V–VII. In the combined ANOVA (across breweries) of the sensory descriptor data, brewery and panelist terms were the most significant effects and the largest sources of variation (Supplementary Table VIII). Therefore, separate ANOVAs of sensory descriptor data were performed for each brewery dataset. In these analyses, the CMBTC ANOVA had the greatest number of significant terms for the largest num-

ber of sensory descriptors. Therefore, the CMBTC data are the focus of this narrative, with key results highlighted from the sensory panels at Deschutes Brewery and New Glarus. Data for sensory traits are presented separately for malt treatment main effects, genotype main effects, and malt treatment  $\times$  genotype interactions in Tables V, VI, and VII. Body, color, and malt were the descriptors with significant malt treatment main effects; body and color were also significant for genotype (Table I). As shown in Table V, body increased with malt modification from MT-1 to MT-2 and then decreased from MT-2 to MT-3; color increased with malt modification from MT-1 to MT-3; and malt was significantly higher in MT-3 than MT-1. The genotype term was significant for body, chemical, color, floral, fruit, toasted, and toffee; floral, fruit, and toffee also showed significant modification treatment  $\times$  genotype interaction. Differences between varieties, averaged over malt treatments, did not exceed the *F*-protected LSD values for body, chemical, color, or toasted (Table VI). Body, color, honey, and toffee were also significant in the Deschutes Brewery dataset. Considering the descriptors showing significant malt treatment  $\times$  genotype interaction (Table VII), Full Pint was significantly higher for floral and fruit in MT-2 and CDC Copeland was significantly higher in MT-3; there was no difference in floral between the two varieties in MT-1. Full Pint was significantly higher for toffee in all three malt treatments. Although not included in the 11 flavor descriptors identified by PC analysis, significant variety effects were detected for astringency, grain, and vegetable in the New Glarus Brewing ANOVA (Supplementary Table X) and for vegetable in the Deschutes Brewery dataset (Supplementary Table IX); significant treatment and variety effects were both detected for astringency, diacetyl, cereal, grain, and vegetable in the CMBTC dataset (Supplementary Table XI).

### Malting Quality Traits

**Experiment I.** Malts made from ST-1 grain had the highest  $\beta$ -glucan and viscosity levels, and values for these traits reached their lowest levels at ST-3, whereas  $\alpha$ -amylase, diastatic power, FAN, malt extract, and Kolbach index increased from ST-1 to

TABLE IV  
Grain Storage Duration Treatment Values for Malting Quality and Adjusted Means for Beer Sensory Traits for 37 Barley Genotypes<sup>a</sup>

Storage treatment	Genotype	Malting quality traits								Beer sensory traits				
		AA	BG	DP	FAN	KI	MC	ME	VC	Chemical	Color	Floral	Fruit	Sweet
ST-1	Golden Promise	43.6	491	162	122	32.4	1.3	74.7	1.6	4.0	2.6	4.7	5.2	3.6
	Full Pint	78.3	402	170	175	41.0	1.2	77.3	1.7	4.1	2.7	3.7	3.6	4.3
	CDC Copeland	60.5	253	138	179	36.2	1.2	77.7	1.6	4.5	2.9	4.0	4.9	2.7
	LSD (0.05)	...	...	...	...	...	...	...	...	0.4	0.5	0.4	0.6	0.6
	DH mean	46.3	644	134	133	33.3	1.3	75.0	1.7	4.4	2.8	4.3	4.2	4.2
	DH minimum	29.8	114	90	90	27.3	1.0	72.5	1.5	3.5	1.7	3.4	2.8	2.3
ST-2	Golden Promise	72.5	1,244	213	205	40.8	1.8	77.9	2.2	5.4	4.2	4.7	5.2	4.8
	Full Pint	34.7	488	136	176	35.3	1.6	73.0	1.7	4.3	2.7	4.5	5.0	3.8
	CDC Copeland	77.4	292	162	173	38.9	1.6	78.1	1.7	4.0	2.9	3.4	3.4	4.3
	LSD (0.05)	61.9	377	140	134	31.8	1.9	78.5	1.7	4.3	3.0	3.7	4.6	3.3
	DH mean	...	...	...	...	...	...	...	...	0.4	0.7	0.6	0.6	0.6
	DH minimum	46.1	537	129	139	31.5	1.6	75.7	1.8	4.3	2.6	4.0	4.5	4.1
ST-3	Golden Promise	28.9	156	88	92	23.5	1.4	73.0	1.5	3.5	1.0	2.0	3.0	2.3
	Full Pint	77.9	1,812	198	211	42.0	1.8	78.1	2.8	5.0	4.6	5.2	5.3	5.4
	CDC Copeland	49.2	174	156	142	34.3	1.6	75.4	1.5	4.3	4.5	4.2	4.3	4.3
	LSD (0.05)	69.8	141	184	247	40.3	1.6	77.9	1.5	4.5	4.6	3.8	3.2	5.1
	DH mean	65.4	65	160	233	42.4	1.6	76.2	1.5	4.8	4.1	4.0	4.3	4.4
	DH minimum	...	...	...	...	...	...	...	...	0.4	0.5	0.5	0.6	0.6
ST-3	DH mean	56.4	185	146	157	35.9	1.7	76.0	1.5	4.5	2.6	4.2	4.1	4.3
	DH minimum	41.7	51	88	111	29.3	1.5	74.0	1.4	3.8	2.3	3.0	2.7	2.7
	DH maximum	73.8	605	212	226	44.4	1.9	78.8	1.7	5.5	5.2	5.0	5.2	5.7

<sup>a</sup> Genotypes: Golden Promise, Full Pint, their 34 doubled haploid progeny, and CDC Copeland. Values shown are for traits that showed significant storage treatment  $\times$  genotype interaction in the combined ANOVA (Table I). Storage treatments: ST-1, 2, and 3 = 1, 5, and 10 months of postharvest grain storage, respectively. Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power ( $^{\circ}$ ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU). DH = doubled haploid.

ST-3 (Table II). Compared with AMBA criteria, most of the micromalts, even at ST-3, would be described as undermodified. Chit counts for all genotypes were >96% for all storage treatments, ruling out any obvious differences in dormancy between genotypes. Water sensitivity was not measured.

**Experiment II.** The three malts made from each variety differed in degree of modification (Table V). Based on the CMBTC guidelines, both CDC Copeland and Full Pint malts produced in MT-1 were undermodified, as evidenced by low friability, low soluble protein, and high  $\beta$ -glucan. MT-2 malts were better-modified malts based on friability, fine-coarse difference, Kolbach index, and  $\beta$ -glucan values.  $\beta$ -Glucan, however, remained slightly higher than commercial malts. MT-3 produced modified to overmodified malt with the highest values for friability, fine-coarse difference, Kolbach index, and FAN. Full Pint had lower malt extract than CDC Copeland and higher diastatic power and  $\alpha$ -amylase in all malting regimes. Germination and water sensitivity values met AMBA specifications for both varieties (Supplementary Tables XII and XIII). MT-3 produced the most properly modi-

fied malt for Full Pint, whereas MT-2 was most appropriate for CDC Copeland (Table VIII).

### PC Analysis of Malting and Sensory Traits

**Experiment I.** In the PC analysis of malt quality at each of the grain storage intervals, the first two PCs accounted for 64% of the variability (Fig. 1A). PC<sub>1</sub> accounted for 49% of the variation; the major contributors were  $\alpha$ -amylase, diastatic power, FAN, malt extract, and Kolbach index. PC<sub>2</sub> accounted for 15% of the variation, with malt color being the major determinant. The distribution of grain storage treatment values along PC<sub>1</sub> shows that malts made from ST-3 grain were better modified than those made from ST-2 and ST-1 grain. Eight loading coefficients ( $\alpha$ -amylase,  $\beta$ -glucan, diastatic power, FAN, malt extract, malt color, Kolbach index, and viscosity) accounted for most of the variation in malting quality. Data for these eight traits, therefore, are those shown in Tables II, III, and IV.

In the PC analysis of beer sensory traits, the first three PCs accounted for 65% of the variability (Fig. 1B); body, chemical, color, floral, fruit, honey, malt, sweet, toasted, and toffee were the

**TABLE V**  
Modification Treatment Means for Malting Quality and Beer Sensory Traits for CDC Copeland and Full Pint Made from Undermodified, Modified, and Overmodified Malts<sup>a</sup>

Modification treatment	Malting quality traits										Beer sensory traits		
	AA	BG	DP	FAN	FC	FR	KI	MC	ME	VC	Body	Color	Malt
MT-1	70.4	264	107	170	0.9	78.3	42.1	1.8	81.9	1.6	3.3	2.0	4.1
MT-2	73.2	134	114	193	0.6	84.9	45.1	1.8	81.8	1.5	4.2	2.5	4.3
MT-3	75.5	64	118	203	0.7	90.0	47.5	1.9	81.9	1.5	2.9	3.8	4.7
LSD (0.05)	...	...	...	...	...	...	...	...	...	...	0.7	1	0.5

<sup>a</sup> Values are averaged over varieties for malting quality traits and beer sensory traits for which there were significant modification treatment main effects, but no malting treatment  $\times$  genotype interaction, in the ANOVA (Table I). Modification treatments: MT-1, 2, and 3 = malting parameters to produce undermodified, modified, and overmodified malts, respectively. Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power (°ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU).

**TABLE VI**  
Variety Treatment Means for Mating Quality and Beer Sensory Traits for CDC Copeland and Full Pint Made from Undermodified, Modified, and Overmodified Malts<sup>a</sup>

Genotype	Malting quality traits										Beer sensory traits			
	AA	BG	DP	FAN	FC	FR	KI	MC	ME	VC	Body	Chemical	Color	Toasted
CDC Copeland	65	92	87	185	0.7	93	47.8	1.8	82.7	1.5	3.4	4.8	2.7	4.1
Full Pint	81	216	138	192	0.7	76	42	1.8	81.1	1.6	3.5	4.6	2.9	4.4
LSD (0.05)	...	...	...	...	...	...	...	...	...	...	0.2	0.3	0.6	0.4

<sup>a</sup> Values are averaged over modification treatments for malting quality traits and beer sensory traits for which there were significant genotype main effects, but no malting treatment  $\times$  genotype interaction, in the ANOVA (Table I). Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power (°ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU).

**TABLE VII**  
Malt Modification Treatment Values for Malting Quality and Least Square Means for Beer Sensory Traits for CDC Copeland and Full Pint Made from Undermodified, Modified, and Overmodified Malts<sup>a</sup>

Modification treatment	Genotype	Malting quality traits										Beer sensory traits		
		AA	BG	DP	FAN	FC	FR	KI	ME	MC	VC	Floral	Fruit	Toffee
MT-1	CDC Copeland	60.7	160	82	171	0.9	88.3	45.3	82.7	1.77	1.54	4.5	4.0	4.1
	Full Pint	80.1	368	131	169	0.9	68.2	38.9	81.1	1.74	1.62	4.5	4.1	4.2
	LSD (0.05)	...	...	...	...	...	...	...	...	...	...	0.1	0.2	0.2
MT-2	CDC Copeland	65.4	77	88	182	0.6	93.7	47.7	82.5	1.85	1.48	4.0	3.7	4.2
	Full Pint	81.0	191	139	204	0.6	76.1	42.5	81.1	1.83	1.53	4.2	4.0	4.3
	LSD (0.05)	...	...	...	...	...	...	...	...	...	...	0.3	0.3	0.2
MT-3	CDC Copeland	69.1	38	91	202	0.6	97.2	50.5	82.8	1.94	1.45	3.7	3.6	4.2
	Full Pint	81.9	89	144	203	0.7	82.7	44.5	81.0	1.89	1.50	4.1	3.1	4.3
	LSD (0.05)	...	...	...	...	...	...	...	...	...	...	0.5	0.6	0.2

<sup>a</sup> Values shown are for beer sensory traits that showed significant modification treatment  $\times$  genotype interaction in the combined ANOVA (Table I). Modification treatments: MT-1, 2, and 3 = malting parameters to produce undermodified, modified, and overmodified malts, respectively. Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power (°ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU).

principal contributors. PC<sub>1</sub> accounted for 36% of the variation, and the principal contributors were body, honey, malt, sweet, toasted, and toffee. PC<sub>2</sub> accounted for 17% of the variation; predominant contributors were color, fruit, and floral. PC<sub>3</sub> accounted for 12% of the variation, and the primary contributor was chemical. Clustering of flavor descriptors at each grain storage treatment was not as distinct as it was for malting quality traits. However, the distribution of flavor descriptors for genotypes along PC<sub>1</sub> shows that the ST-3 malts were generally higher in honey, malt, sweet, toasted, and toffee flavors than ST-2 or ST-1 malts.

**Experiment II.** PC analysis of malting quality data revealed that the first two PCs accounted for 96% of the variability (Fig. 1C). PC<sub>1</sub> accounted for 64% of the variation; principal contributors were  $\beta$ -glucan, friability, Kolbach index, and viscosity. PC<sub>2</sub> accounted for 31% of the variation, and principal contributors were  $\alpha$ -amylase, diastatic power, and FAN. Fine-coarse difference, malt extract, and malt color were the principal sources of variation in PC<sub>3</sub>. The distribution of treatments along PC<sub>1</sub> indicates that MT-1 (undermodified) was higher in  $\beta$ -glucan and more viscous than MT-3 (overmodified), which had higher Kolbach index and friability values. Clustering of genotypes along PC<sub>2</sub> indicates that Full Pint had higher  $\alpha$ -amylase, diastatic power, and FAN values compared with CDC Copeland, which had higher fine-coarse difference and malt extract.

In the PC analysis of beer sensory traits, the first two PCs accounted for 68% of the variability (Fig. 1D). PC<sub>1</sub> accounted for 42% of the variation; principal contributors were color, floral, fruit, and malt. PC<sub>2</sub> accounted for 26% of the variation; principal contributors were body, chemical, honey, and sweet. Distribution along PC<sub>1</sub> indicates that MT-3 had higher malt and color values than MT-1, which was higher in floral and fruit. Clustering of genotypes along PC<sub>2</sub> indicates that Full Pint had higher values for honey, sweet, toasted, and toffee compared with CDC Copeland, which was higher in chemical.

**TABLE VIII**  
Malting Quality Trait Values and Least Square Means for Beer Sensory Traits for Two Different Malt Treatments for CDC Copeland (MT-2) and Full Pint (MT-3)<sup>a</sup>

Traits	MT-2, CDC Copeland	MT-3, Full Pint	LSD (0.05)
Malting quality traits			
AA	65.4	81.9	
BG	77	89	
DP	88	144	
FAN	182	203	
FC	0.6	0.7	
FR	93.7	82.7	
KI	47.7	44.5	
MC	1.9	1.9	
ME	82.5	81	
VC	1.5	1.5	
Beer sensory traits			
Body	4.3	3.0	0.6
Chemical	4.7	4.7	0.2
Color	2.4	3.7	0.4
Floral	4.0	4.1	0.3
Fruit	3.7	3.1	0.3
Honey	4.2	4.2	0.3
Malty	4.2	4.7	0.4
Sweet	3.7	4.0	0.2
Toasted	4.2	4.4	0.5
Toffee	4.2	4.3	0.3

<sup>a</sup> Modification treatments: MT-2 and 3 = malting parameters to produce modified and overmodified malts, respectively. Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power ( $^{\circ}$ ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU).

### Correlations of Malting Quality and Beer Sensory Traits

Correlations between malting and sensory traits for each treatment in experiments I and II are presented in Supplementary Tables XIV and XV. For ease of exposition, Table IX shows correlations, averaged over modification treatments. Correlations in experiment I were consistently lower than those in experiment II, and there were positive and negative correlations > 0.80 for multiple malting quality/flavor descriptor combinations.

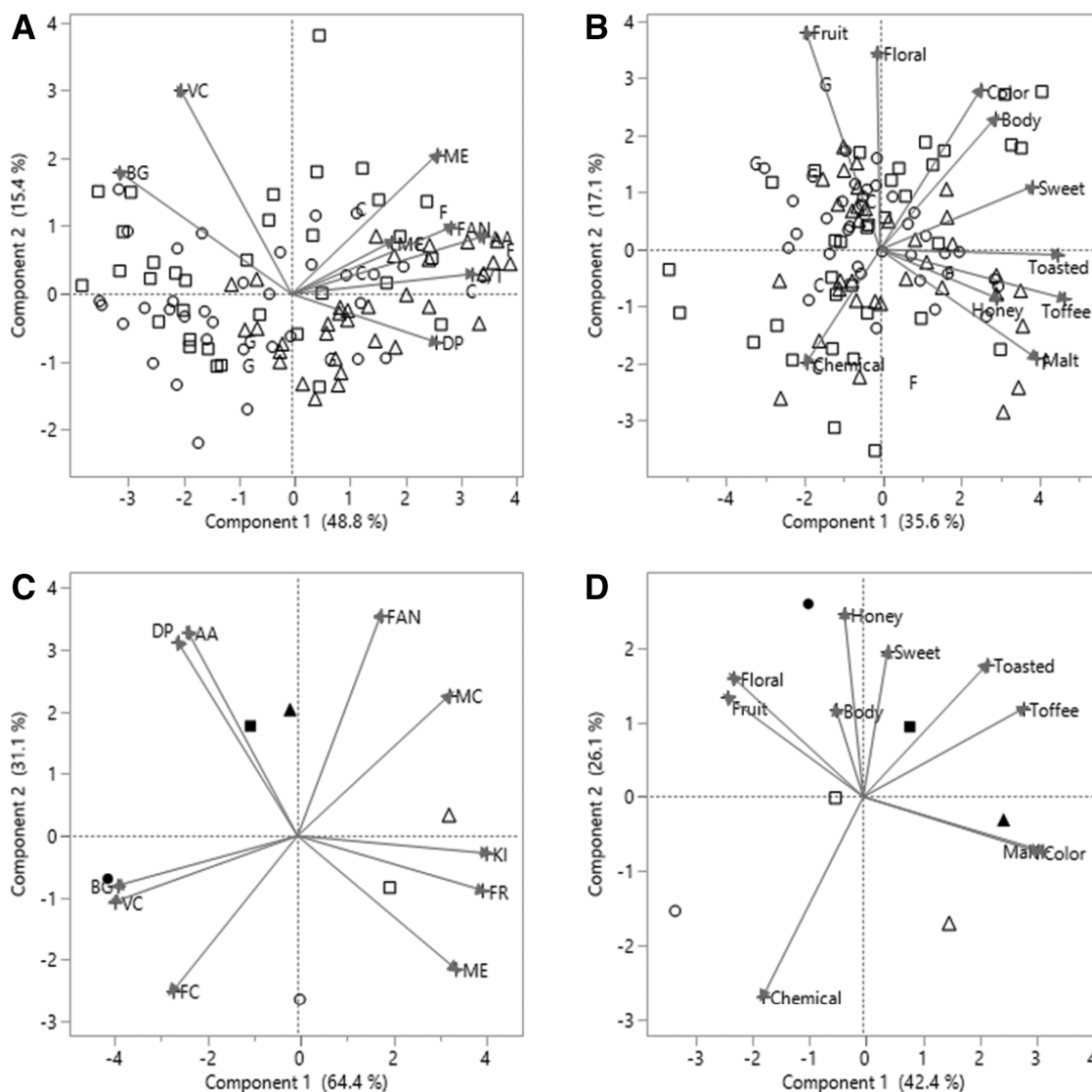
Positive correlations  $\geq 0.30$  for  $\alpha$ -amylase with malt and toasted were observed in both experiments. Kolbach index was negatively correlated with floral in both experiments. The notable change in sign of correlation was for malt extract and toffee (positive in experiment I and negative in experiment II). Overall, the magnitude of correlations between  $\alpha$ -amylase and Kolbach index with sensory descriptors tended to increase with degree of modification.

### DISCUSSION

The rationale behind the current research was the report by Herb et al. (5) that stated that genotype and environment can affect the contributions barley makes to beer flavor. These conclusions were based on nano-beers brewed from micromalts whose malting quality parameters were often outside the range of acceptable standards. For example,  $\beta$ -glucan ranged from a low of 112 ppm to a high of 1,220 ppm, and malt extract ranged from 73% to a high of 78%. Overall, these malts would be described as undermodified. The research described in this report addressed the following question: were observed differences in beer sensory descriptors directly attributable to the degree of malt modification of the genotypes tested? Certain aspects of malt quality, such as Kolbach index and FAN, may have effects on yeast nutrition and therefore flavor.

Prior to discussing the results of experiments I and II and their implications, it is important to address the possibility of an alternative experimental approach: removing the potentially confounding effects of modification on sensory descriptors by optimizing malting protocols for each genotype so that near-equivalent malts are produced. In research involving large numbers of genotypes (i.e., experiment I) the principal constraint is the instrumentation available for micromalting. For example, in an automated Joe White micromalting system (such as that used for the current research) 40 samples, at 250 g each, were malted at the same time using a standard malting protocol. In such a system, the resulting malts from a single batch will inevitably range from undermodified to overmodified. Therefore, the alternative was to assess the possible effects of *average* degree of modification on beer sensory descriptors. Based on the consideration that even in the absence of obvious dormancy or water sensitivity, malt modification can be improved by proper storage of grain (8), grain was stored for three postharvest intervals, malts were made at each interval, and nano-beers were brewed from these malts. In experiment II, assessment of two genotypes allowed for intentional manipulation of the malting protocol to achieve different degrees of modification. However, even with this approach, identical malts were not produced from each of the two varieties at each level of modification. Rather, the overmodified treatment for Full Pint came closest to the properly modified treatment for CDC Copeland.

Both strategies were effective in producing malts that varied in degree of modification, although statistical tests of significance between treatments (grain storage interval or steeping regime) were not possible owing to a lack of replication. Cost and time considerations usually preclude replication of research malts (6). Considering  $\beta$ -glucan level as a key indicator of modification, experiment I values for Golden Promise, Full Pint, and CDC Copeland were 491, 402, and 253 ppm in ST-1 and 174, 141, and 65



**Fig. 1.** Principal component analysis of malting quality and beer sensory traits for experiment I (circle = ST-1; square = ST-2; and triangle = ST-3) and experiment II (Full Pint = solid; CDC Copeland = hollow; circle = undermodified; square = modified; and triangle = overmodified). **A**, Experiment I, malting quality; **B**, experiment I, sensory traits; **C**, experiment II, malting quality; and **D**, experiment II, sensory traits. Abbreviations defined in Table IX.

**TABLE IX**  
**Correlations Among Malting Quality Traits and Beer Sensory Traits Averaged Over Storage Treatments (Experiment I, Italics) and Modification Treatments (Experiment II, Boldface)<sup>a</sup>**

Trait	Body	Chemical	Color	Fruit	Floral	Grass	Honey	Malt	Sweet	Toasted	Toffee
AA	<i>0.16</i>	<i>-0.07</i>	<i>0.21</i>	<i>-0.11</i>	<i>-0.27*</i>	<i>0.01</i>	<i>0.08</i>	<i>0.37*</i>	<i>0.26*</i>	<i>0.32*</i>	<i>0.28*</i>
	<b>0.41</b>	<b>-0.81*</b>	<b>0.43*</b>	<b>0.07</b>	<b>-0.20</b>	<b>0.05</b>	<b>0.38*</b>	<b>0.50*</b>	<b>0.46*</b>	<b>0.87*</b>	<b>0.85*</b>
BG	<i>-0.07</i>	<i>-0.10</i>	<i>0.07</i>	<i>0.07</i>	<i>0.20*</i>	<i>-0.08</i>	<i>0.01</i>	<i>-0.11</i>	<i>-0.11</i>	<i>-0.16</i>	<i>-0.08</i>
	<b>0.05</b>	<b>-0.39*</b>	<b>-0.49*</b>	<b>0.80*</b>	<b>0.67*</b>	<b>0.71*</b>	<b>0.76*</b>	<b>-0.49*</b>	<b>0.54*</b>	<b>0.18</b>	<b>-0.08</b>
DP	<i>-0.03</i>	<i>0.15</i>	<i>0.04</i>	<i>-0.01</i>	<i>-0.11</i>	<i>0.09</i>	<i>-0.04</i>	<i>0.11</i>	<i>0.02</i>	<i>0.11</i>	<i>0.03</i>
	<b>0.32</b>	<b>-0.74*</b>	<b>0.31</b>	<b>0.20</b>	<b>-0.20</b>	<b>0.08</b>	<b>0.30</b>	<b>0.40*</b>	<b>0.40*</b>	<b>0.86*</b>	<b>0.83*</b>
FAN	<i>0.22</i>	<i>0.03</i>	<i>0.07</i>	<i>-0.11</i>	<i>-0.19</i>	<i>0.07</i>	<i>0.09</i>	<i>0.22</i>	<i>0.15</i>	<i>0.16</i>	<i>0.13</i>
	<b>0.04</b>	<b>-0.28</b>	<b>0.80*</b>	<b>-0.71*</b>	<b>-0.60*</b>	<b>0.75*</b>	<b>-0.51*</b>	<b>0.87*</b>	<b>-0.35</b>	<b>0.63*</b>	<b>0.76*</b>
KI	<i>0.17</i>	<i>-0.11</i>	<i>0.21</i>	<i>-0.10</i>	<i>-0.30*</i>	<i>-0.02</i>	<i>-0.02</i>	<i>0.34*</i>	<i>-0.30*</i>	<i>0.29*</i>	<i>0.20</i>
	<b>0.11</b>	<b>0.50*</b>	<b>0.41*</b>	<b>-0.81*</b>	<b>-0.44*</b>	<b>0.52*</b>	<b>-0.62*</b>	<b>0.35</b>	<b>-0.50*</b>	<b>-0.42*</b>	<b>-0.21</b>
MC	<i>0.01</i>	<i>0.01</i>	<i>-0.18</i>	<i>-0.16</i>	<i>-0.07</i>	<i>0.07</i>	<i>0.25*</i>	<i>0.09</i>	<i>0.09</i>	<i>0.03</i>	<i>0.18</i>
	<b>0.20</b>	<b>0.01</b>	<b>0.86**</b>	<b>-0.95*</b>	<b>-0.74*</b>	<b>0.72*</b>	<b>-0.49*</b>	<b>0.85*</b>	<b>-0.27</b>	<b>0.15</b>	<b>0.41*</b>
ME	<i>0.15</i>	<i>-0.08</i>	<i>0.15</i>	<i>-0.28*</i>	<i>-0.24*</i>	<i>0.03</i>	<i>0.13</i>	<i>0.33*</i>	<i>0.25*</i>	<i>0.18</i>	<i>0.30*</i>
	<b>0.33</b>	<b>0.67*</b>	<b>-0.03</b>	<b>-0.49*</b>	<b>0.01</b>	<b>0.15</b>	<b>-0.46*</b>	<b>-0.12</b>	<b>-0.51*</b>	<b>-0.71*</b>	<b>-0.63*</b>
VC	<i>-0.04</i>	<i>-0.04</i>	<i>0.05</i>	<i>-0.03</i>	<i>-0.01</i>	<i>0.05</i>	<i>0.07</i>	<i>-0.02</i>	<i>-0.06</i>	<i>-0.06</i>	<i>0.02</i>
	<b>-0.01</b>	<b>-0.27</b>	<b>-0.57*</b>	<b>0.89*</b>	<b>0.62*</b>	<b>0.65*</b>	<b>0.71*</b>	<b>-0.55*</b>	<b>0.53*</b>	<b>0.10</b>	<b>-0.14</b>

<sup>a</sup> Experiment I = top (italic); experiment II = bottom (bold). Malting quality traits: AA =  $\alpha$ -amylase (20°C DU); BG =  $\beta$ -glucan (ppm); DP = diastatic power ( $^{\circ}$ ASBC); FAN = free amino nitrogen (ppm); KI = Kolbach index (%); MC = malt color (SRM); ME = malt extract (%); and VC = viscosity (CU). Asterisk (\*) indicates significant *P* value at  $\alpha = 0.05$ .

ppm in ST-3. In experiment II, values for Full Pint and CDC Copeland at MT-1 were 160 and 368 ppm; the corresponding values at MT-3 were 38 and 89 ppm.

These differences in modification did not directly lead to significant modification treatment effects in the ANOVA of sensory data (Table I). There were significant treatment effects in both experiments, but different descriptors were significant: chemical and floral in experiment I and body, color, and malt in experiment II. Genotype was consistently the most significant source of variation for most sensory descriptors in both experiments: all descriptors in experiment I and 7/10 descriptors in experiment II. Treatment  $\times$  genotype interactions were significant in both experiments, underscoring that genotypes responded differentially to degree of modification for approximately half of the descriptors. In general, these interactions were owing to changes in the magnitude of response rather than change in rank. Extracting data from specific treatment  $\times$  genotype comparisons (e.g., Full Pint at MT-3 versus CDC Copeland at MT-2), for which degree of modification was most comparable (Table VIII), reveals the fundamental role of genotype in terms of responding to the malting process. Full Pint was notably higher for enzyme-related traits, and CDC Copeland was notably higher for friability and malt extract. There were significant differences between genotypes for sensory descriptors at these two "optimum" malting regimes: CDC Copeland was higher for body and fruit, whereas Full Pint was higher for color and malt.

Relationships between malting quality and beer sensory attributes, as explored by PC analysis (Fig. 1), support the association of degree of modification with specific sensory descriptors in both experiments but do not identify specific causal relationships. In general, increasing modification led to an increase in favorable malt parameters (e.g., lower  $\beta$ -glucan and higher malt extract) and an overall reduction in fruit and floral and an increase in malt, toasted, and related flavors. However, genotypes did not all respond equally, providing further evidence for the importance of genotype and the importance of treatment  $\times$  genotype interaction. The correlations between malting quality and beer sensory traits (Table IX) were affected by sample size: values were always lower for experiment I, which had a larger number of genotypes showing greater genetic variation. The very high correlations observed in experiment II could be useful for developing and testing hypotheses regarding levels of specific malting quality parameters that lead to differential levels of Maillard reaction products and thus differences in sensory attributes. For example, do the higher levels of  $\alpha$ -amylase and diastatic power drive the malt and toasted flavors in Full Pint? Is higher malt extract in CDC Copeland responsible for lower levels of malt and toasted flavors? Further research is necessary to determine if the correlations are causal in these two varieties and if they are of predictive utility in other genotypes.

The two experimental approaches to assessing the effect of modification on beer sensory descriptors, in addition to providing insights into associations between the two classes of traits, have implications for sample size in research malting and brewing. The magnitude of differences in sensory descriptor ratings between genotypes was greater in experiment I, in which micromalts and nano-brews were used. Sample size is of key importance in variety development, genetic analysis, and commercial acceptance. In breeding and genetics, the smaller the biological sample (e.g., grain), the lower the cost, and the sooner the data can be used to make selection decisions. For example, Schmitt and Budde (9) developed methods for malting and assessing malt quality using 2 g samples of grain that would be a tremendous asset to breeding programs needing to evaluate hundreds of potential selections and to geneticists seeking to increase the power of correctly estimating genetic effects, genome locations, and interactions (6). On the

other hand, commercial acceptance of barley varieties (e.g., AMBA Plant Scale approval) is based on malting and brewing large biological samples (e.g., hundreds of metric tons) of one or a few test genotypes. Maximizing the predictive power of research malting and brewing is an evolving process and one that will be particularly important for future research on the contributions of a greater range of barley genotypes to beer flavor. Full Pint, Golden Promise, their progeny, and CDC Copeland represent a small sample of the total genetic diversity in barley. A next step is to develop a standardized methodology for assessing contributions to beer flavor in large numbers of barley samples and to apply this methodology to the abundant genetic resources available. For example, the USDA World Core consists of 33,176 accessions (7).

## CONCLUSIONS

In summary, the results of these two experiments, and those of Herb et al. (5), show that barley varieties can contribute to differences in beer flavor and that these differences are not simply owing to degree of modification of the malts used to produce test beers. Based on the results of this study and the inevitable challenges of malt-based research, genetic characterization of, and selection for, barley contributions to beer flavor may be possible even with undermodified malts. Beer sensory profiling of a limited number of genotypes, in contrast, will be most relevant when properly modified malts are made for each variety. In the final analysis, the contributions of barley variety to beer flavor—be it a contemporary variety, an heirloom variety, or an exotic land race—will likely be modest compared with the effects achieved by manipulating Maillard reactions in malting (2), using liberal amounts of hops with intense aromas, and brewing with different yeasts. Nonetheless, in certain beer styles and for some maltsters, brewers, and consumers, the barley contributions to beer flavor will be worth pursuing.

## ACKNOWLEDGMENTS

We thank Seth Klann (Klann Family Farm) for the field experiment facilities at Madras and Patricia Aron, John Andrews, Paul Kramer, and Xiang Yin (Rahr Malting Co.) for critical review of the manuscript. This research was supported and funded by the Flavor 7-pack of breweries (John Mallett, Bells Brewing; Veronica Vega, Deschutes Brewery; Matthew Brynildson, Firestone-Walker Brewing Co.; Dan Carey, New Glarus Brewing Co.; Mike Gilford and Vinnie Cilurzo, Russian River Brewing Co.; Tom Nielsen, Sierra Nevada Brewing Co.; and Damian McConn, Summit Brewing Co.), the Brewers' Association, the Gatsby Charitable Foundation, and the Spanish Ministry of Economy and Competitiveness (project AGL2015-69435-C3).

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