JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS, 2018
VOL. 76, NO. 3, 199–208
https://doi.org/10.1080/03610470.2018.1487747

Sensory Directed Mixture Study of Beers Dry-Hopped with Cascade, Centennial, and Chinook

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ABSTRACT

American craft beer style and flavor is often driven by the unique qualities of American hops. Cascade, Chinook, and Centennial hops are used prominently for dry-hopping singly and/or in blends to impart an intense hoppy aroma to beer. A sensory directed dry-hopping mixture study was performed to understand the contribution that each of these hops make to beer aroma. Utilizing a 4th degree simplex-lattice mixture-design, sixteen beers were prepared (including an "unhopped" control) by dry-hopping a common "unhopped" base beer with different blends of ground whole cone hops made from the three hop cultivars. The treatments were evaluated by trained panelists using descriptive analysis, where the response variables used by the panel encompassed the sensory attributes that described the unique aromatic features of these three hops, (i.e., citrus, tropical/fruity, tropical/catty, and herbal). Using these outputs, the sensory contributions of each individual cultivar, as well as mixtures of the cultivars, were examined on a per attribute basis. These results can be used to select combinations or blends of the three hops for use during dry-hopping that provide similar or dissimilar overall aroma intensity and quality in dry-hopped beer.

KEYWORDS

Dry-hopping; flavor matching; hop blending; mixtures

Introduction

The sensory perception of beer is based on a number of factors, which make predicting the aroma and flavor of beer complex. Synergistic, antagonistic, and masking effects have been shown to impact the sensory perception of mixtures of volatile components important for beer aroma.[1] It has been observed that the coexistence of the hop volatiles linalool, geraniol, and β-citronellol can increase the sensory perception of citrus character in model solutions[2] and hopped beer.[3] Controlling hop aroma in beer requires an understanding of the important hop-derived components that are transferred from the hops into beer and how these components interact with one another to impact sensory perception.

Many craft brewers use dry-hopping as a technique to create an intense hop aroma in finished beer.[4] Cascade, Chinook, and Centennial are American hop varieties that are ubiquitously used, singly or in blends, for dry-hopping.[5] In-depth flavor analysis of beer dry-hopped with each of these hop varieties has shown that each of these hop varieties has unique aroma compounds (i.e. character impact compounds [CICs]) that are important for the aroma profile of each of these hops.[6, 7] Although a number of the CICs were unique to each hop cultivar, some of the CICs were important for all three cultivars, albeit to differing degrees. Most likely these compounds occur in different concentrations in finished dry-hop beer due to the amount of these compounds in the hop material (intra and inter cultivar differences) and the amount of hop material added. Recently Takoi et al.[3] observed that using blends of hops during dry-hopping could promote synergy among hop aroma compounds and maximize the sensory perception of certain beer attributes, such as tropical and citrus character.

A sensory-directed mixture study was performed to understand the contribution that Cascade, Chinook, and Centennial make to dry-hopped beer aroma both singly and in combination. The two objectives of this study were (1) to utilize a 4th degree simplex-lattice mixture-design[8, 9] to combine these three hop varieties in different proportions for dry-hopping and to evaluate the qualitative changes in the resultant beers using descriptive analysis with trained panelists and (2) to understand how these combinations drive particular sensory characteristics in dry-hopped beer.

Experimental

Experimental design

In total, 19 beers (15 blends, 3 internal process replicates, and an "unhopped" control) were prepared using a 4th degree simplex-lattice mixture-design to create blends that varied in the amount of whole cone Chinook, Centennial, and Cascade hops. An "unhopped" pale ale was dry-hopped with these blends at a rate of 3.86 g/L (1 lb/US barrel) (Table 1). The internal process replicates were prepared by performing the dry-hopping procedure twice for each of the

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Table 1. The fourth degree simplex-lattice mixture-design of dry-hop treatments from blends of ground whole cone Chinook, Centennial, and Cascade that were used to dry-hop an "unhopped" pale base beer at a rate of 3.86 g/L (1 lb/US barrel).

Dry-hop blending treatments	% Cascade	% Chinook	% Centennial
1-rep 1	100	0	0
1-rep 2	100	0	0
2	75	25	0
3	75	0	25
4	50	50	0
5	50	25	25
6	50	0	50
7	25	75	0
8	25	50	25
9	25	25	50
10	25	0	75
11-rep 1	0	100	0
11-rep 2	0	100	0
12	0	75	25
13	0	50	50
14	0	25	75
15-rep 1	0	0	100
15-rep 2	0	0	100
16	0	0	0

100% (single) cultivar treatments. Discrimination testing was used to evaluate these internal process replicates to ensure the dry-hopping process was reproducible. Descriptive sensory analysis was then used to scale the aroma intensity and quality of the 16 unique treatments. Volatile chemical analyses were performed on the treatment beers to confirm the analytes that may be important for describing the dry-hop aroma these cultivars transmit to beer.

Hop collection

4.5 kg minibales from single lots of whole cone Cascade, Chinook, and Centennial hops were collected after harvest in 2015 courtesy of Crosby hop farms (Woodburn, OR, U.S.A.). Upon arrival at Oregon State University, the hops were repackaged in high barrier foil pouches, purged of air using nitrogen, sealed and stored cold (−20 °C) until dry-hopping and chemical analysis. The total essential oil and compositional analysis of these hops at the time of dry-hopping are shown in Table 2.

"Unhopped" beer production

"Unhopped" beer was prepared on a commercial scale by a regional brewery (BridgePort Brewery, Portland, OR, U.S.A.). Wort was prepared using a single temperature infusion mash of 86% pale two row, 13.5% Caramel 10°L and 0.5% Caramel 120°L malt (Great Western, Vancouver, WA, U.S.A.) to a starting concentration of 10.7°P. Fermentation was carried out using Bridgeport Brewing company's house ale strain at 19.4–20 °C. Following fermentation, a kieselguhr filter was used to clarify the green beer and remove yeast. Post filtration, iso-humulones (IsoHop, John I Haas, Yakima, WA, U.S.A.) were added at concentration of 18 mg/L.

This resulted in ∼46 hL of a 19.7 BU, 4.38% ABV "unhopped" base beer. Beer was carbonated and packaged into 60-L stainless kegs, shipped to Oregon State University, and held at 2 °C until dry-hopping.

Dry-hopping protocol and hop preparation

The 19 treatments were prepared in a randomized order using a dry-hopping process established previously by Vollmer and Shellhammer.[10] In brief, 24 h prior to hop addition the "unhopped" beer was removed from the cooler at 4 °C and allowed to warm to approximately 15 °C. For each treatment, 40 L of warmed beer was transferred aseptically into two modified 60 L stainless steel beer kegs each with a 4″ stainless steel opening fitted with a standard Sankey D-system coupler and modified spear (Sabco, Toledo, OH, U.S.A.). To prepare the hop blends, the whole cone hops were ground into a hop grist, which was divided up by mass into two mesh bags (EcoBag, Ossining, NY, U.S.A.). These bags were stored inside high barrier pouches flushed with N_2 until the dry-hopping event. For each dry-hop treatment, the two kegs filled with 40 L beer were temporarily de-pressurized and opened under a stream of low pressure CO_2. Simultaneously, the high barrier pouch was opened and the mesh bag containing ground hop grist was added to the beer. After the addition, the headspace was flushed with CO_2 and purged.

After 24 h of dry-hopping the beer was filtered to stop the dry-hopping process. The average temperature of the dry-hopping events ranged from 13.3 to 15 °C (56–59 °F). Dry-hopping was stopped after 24 h because prior work by Wolfe et al.[11, 12] showed that the extraction of key hop volatiles occured within 24 h during dry-hopping. The two kegs were blended via a three-way fitting and filtered using a plate and frame filter containing impregnated cellulose pads (HS2000, Pall Corporation, Port Washington, NY, U.S.A.).[13] Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyzer (Hach, Loveland, CO, U.S.A.). Bright beer was not collected until the DO was below 110 µg/L After the DO was within specification, filtered beer was collected in a closed 1/6 bbl stainless steel keg with sufficient backpressure to reduce foaming. Between each filter run, filter pads were exchanged to prevent carry-over. Directly after filtration, the DO of the bright beer was measured and recorded. Filtered beer was stored at 2 °C and under CO_2 overpressure (76–83 kPa) until sensory evaluation. To minimize artifacts from packaging in glass bottles, such as DO pick up and potential aroma scalping via crown liner material,[14, 15] all beer for this experiment was kept in the 19.6 L (1/6 US bbl) kegs at ∼1 °C. To perform sensory and analytical analysis beer was served directly from these kegs using two 8-head draft systems (Micro Matic, Northridge, CA, U.S.A.).

Sensory: Discrimination testing of internal process replicates

Discrimination testing was performed on the internal process replicates for each of the 100% (single) cultivar treatments to examine dry-hopping process variation within treatments. The replicates were evaluated by a panel of 40 craft beer drinkers (23 males and 17 females, 21–66 years of age). Panelists were presented with four triangle tests, the first of which was a warm up. Within each triangle test, there

Table 2. Summary of average mean scores for the sensory attributes resulting from descriptive analysis on the dry-hop blending treatments sorted by increasing overall hop aroma intensity.[a]

	Target Analytes	β-Myrcene	β-Caryophyllene	α-Humulene	β-Farnesene	Terpinen-4-ol	α-Terpineol	Linalool	Nerol	Geraniol	Geranial	Methyl Geranate	Geraniol Acetate	Caryophyllene Oxide	Sum of Analytes Measured
Hop Variety	Total Oil[b] (ml/100 g)	Hop volatile analysis (mg/100g)[c,d]													
Cascade	1.0	209.7	98.7	262.1	100.2	n.d.	n.d.	3.7	1.0	4.5	0.4	9.6	26.6	9.5	726.0
Chinook	1.8	366.9	245.0	552.4	n.d.	n.d.	n.d.	6.1	3.6	65.5	1.1	30.8	0.5	3.5	1275.4
Centennial	1.9	665.2	182.4	347.2	n.d.	n.d.	n.d.	15.2	5.2	125.3	3.2	101.2	1.0	6.2	1452.1
Dry-hop treatment	Blend Code	Beer volatile analysis (μg/L)[e,f]													
16	0:0:0	0.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	n.d.	n.d.	2.2
11-rep 1	0:100:0	4.3	n.d.	0.1	0.3	4.2	12.0	43.2	3.6	44.8	1.6	4.3	1.0	3.1	122.5
13	0:50:50	54.8	n.d.	0.6	0.9	8.5	25.7	85.6	7.1	82.6	2.3	30.7	1.7	3.1	303.6
10	25:0:75	18.0	n.d.	0.4	0.5	3.9	15.7	40.2	3.5	36.6	1.0	19.0	1.8	1.3	141.9
1-rep 1	100:0:0	2.0	n.d.	n.d.	n.d.	3.3	8.4	23.9	1.8	18.6	0.5	n.d.	1.2	1.2	60.9
4	50:50:0	57.1	0.4	1.0	1.2	6.0	26.6	82.1	5.5	66.4	2.5	10.3	4.8	4.5	268.4
5	50:25:25	227.6	0.5	4.3	2.5	7.8	37.3	56.0	6.4	84.6	1.9	39.1	6.4	3.6	478.0
12	0:75:25	58.6	n.d.	0.8	0.6	7.5	24.8	82.6	6.1	78.3	2.3	28.4	2.2	3.9	296.1
7	25:75:0	33.5	0.1	0.6	0.6	5.7	23.4	66.9	4.0	67.4	2.0	11.3	3.1	4.8	223.4
3	75:0:25	57.2	0.4	0.8	0.7	7.0	32.3	82.9	5.0	56.4	1.7	25.9	5.8	2.4	278.5
2	75:25:0	36.6	0.3	0.8	0.9	5.2	27.0	54.7	3.1	45.8	1.0	6.0	4.3	2.9	188.6
8	25:50:25	35.4	n.d.	0.4	0.5	3.8	12.1	49.8	3.8	46.1	1.7	17.1	2.2	2.6	175.5
14	0:25:75	57.6	n.d.	0.4	1.0	11.7	43.8	132.7	11.2	127.6	2.4	48.4	2.2	3.5	442.5
15-rep 2	0:0:100	11.5	n.d.	0.4	0.7	5.4	17.9	86.8	8.5	117.2	1.8	40.5	1.3	2.0	294.0
6	50:0:50	290.2	1.0	5.1	1.7	14.1	44.6	139.4	11.3	144.0	2.9	74.3	8.0	3.0	739.6
9	25:25:50	371.5	2.7	8.0	2.4	16.3	41.1	157.8	12.5	181.4	6.0	88.1	6.4	4.6	898.8

[a]The treatment blending codes are represented as %Cascade:%Chinook:%Centennial.
[b]Measured using ASBC MOA Hops-13.[16]
[c]Analyzed using undermodified GC/MS conditions based on ASBC MOA Hops-17 [16]. Analytes are reported in mg/100 g hops.
[d]Average of 3 instrumental runs.
[e]Analyzed using undermodified GC/MS conditions based on published methodology [16, 17]. Analytes are reported in μg/L and are blank corrected.
[f]Average of 2 instrumental runs.
n.d., not detected.

Table 3. Reference standards with intensity scores used in descriptive analysis panels.

Attributes	Unhopped Control	100% Chinook	100% Centennial	100% Cascade	Sierra Nevada Pale Ale	Ballast Point Pineapple Sculpin	10-Barrel Joe IPA
OHAI	0	6	9	8	7	10–11	14–15
Citrus	0	2	7	8	6	6	5–6
Herbal/Tea	0	3	4–5	6	5	2	1
Tropical/Catty	0	4–5	2–3	3	3	4	9–10
Tropical/ Fruity	0–1	2–3	5–6	3	4	7–8	4–5
Pine/ Resinous/ Dank	0	1	2	2	2	4	4

OHAI, Overall Hop Aroma Intensity.

were three samples; two of the samples were the same and one of the samples was different. Based only on the orthonasal aroma of the sample, the panelists were instructed to select the odd sample for each of the four triangle tests. For each of the three sets of duplicates, the design of the triangle test ensured an equal frequency of appearance of each duplicate as the "odd" sample. The serving order within each triangle test was also randomized. The dry-hopped beer was dispensed from the keg into a pitcher, which was used to pour ~60 mL of beer into 300-mL sample glasses coded with a 3-digit random number. After the beer was poured the glass was covered with a plastic lid and the beer was allowed to warm to room temperature before sensory analysis. Each station was used ~2 times over the course of 2 h.

Sensory: Descriptive analysis

Sixteen trained panelists were used to scale only the orthonasal aroma of the treatments and they were selected based on previous experience and relevance (12 males and 4 females; 21–66 years of age). Five training sessions were completed in advance of data collection. During these sessions panelists were trained using external reference samples and the actual experimental treatments to develop a relevant lexicon of sensory attributes and a scale that best explained the differences in the samples. Based on discussion from these training sessions and prior results,[13] the final ballot included the attributes: *Overall Hop Aroma Intensity (OHAI), Citrus, Tropical/Catty*, *Tropical/Fruity*, *Pine/ Resinous/Dank*, and *Herbal/Tea* to be evaluated on a 0–15 point scale. During each session, the panelists had access to seven external reference samples, four of which were treatment beers (unhopped control, 100% Centennial, 100% Chinook and 100% Cascade) and three of which were commercial, hoppy beers (Sierra Nevada Pale Ale, 10 Barrel Joe IPA and Ballast Point Pineapple Sculpin). These five beers had sensory descriptors with intensity scores assigned by consensus during training, and their purpose was to serve as anchors for the 0–15 point intensity scale (Table 3).

Over the course of eight sessions, the 16 panelists evaluated all of the samples four times. The presentation order throughout the study was randomized and blocked by replication and panelist, and two sessions were needed per replication to evaluate all the samples (two sessions of eight samples). An efficient resolvable incomplete block design was used to create a presentation order for the samples within each of the four replications (SAS, Cary, NC, U.S.A.). Panelists were given ~60 mL of dry-hopped beer in a 300-mL glass covered with a plastic lid. Beer was served from two eight-head draft systems (Micro Matic, Northridge, CA, U.S.A.) into pitchers at ~1 °C and at 83 kPa. Beer was poured into sample glasses ~1 h before the start of testing and allowed to warm to room temperature. Panelist responses were collected on Chromebook tablets using Qualtrics (Provo, UT, U.S.A.). For each session, Qualtrics was also used to randomly assign the serving order of samples for each panelist.

Volatile analysis reagents and standards

β-myrcene, linalool, geraniol, citral, methyl geranate, geranyl acetate, 4-octanol, terpinen-4-ol, α-terpineol, nerol, β-caryophyllene, α-humulene, and β-farnesene were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). The 2-octanol was obtained from Alfa Asear (Haverhill, MA, U.S.A.). Hexanes were purchased from J.T. Baker (Center Valley, PA, U.S.A.) and were redistilled to remove impurities prior to analysis. Sodium chloride was purchased from EMD Millipore (Billerisa, MA, U.S.A.).

Hop volatile analysis

Hydrodistillation was performed to determine the total oil content of the homogenized hop grist using ASBC Hops-13.[16] Post-distillation, hop oil was collected in 2.5-mL amber vials with foil-lined closures. After filling with oil, the amber vials were flushed with N_2. Hop oil was stored at −20 °C until compositional analysis.

Hop oil compositional analysis was performed using a HP 6890 gas chromatograph with an Agilent 5972a mass spectrometer (GC-MS) under modified conditions from ASBC Hops-17.[16] In brief, a 1% 2-octanol (8190 mg/L) solution was prepared in reagent grade hexane. Hop oils were diluted to 10% with the 1% 2-octanol/hexane solution in a crimped glass vials. A 1-µL aliquot of the diluted hop oil was directly injected into the injection port held at 200 °C and operating in split mode (1:50) using the septum purge option. The analytical column was a 30 m × 250 µm × 0.25 µm Zebron ZB-1 MS (Phenomenex, Torrance, CA, U.S.A.) and ultra-pure helium was used as the carrier gas (a constant flow rate, 1.4 mL/min). The following temperature program was used: 50 °C hold for 1 min, 50–180 °C (2 °C/min) hold for 10 min, 180–200 °C (3 °C/min), and 250 °C hold for 5 min. The auxiliary line and mass spectrometer were operated at 280 and ~180 °C, respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and in full scan mode set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. Four-point calibration

Table 4. Triangle test results of dry-hopping process replicates.[a]

Triangle tests	Number of correct responses	Z value	P value
100% Centennial (15-rep 1 vs. 15-rep 2)	13	−0.28	0.39
100% Cascade (1-rep 1 vs. 1-rep 2)	18	1.40	0.08
100% Chinook (11-rep 1 vs. 11-rep2)	16	0.73	0.23

[a] The panel was comprised of 40 hoppy beer consumers; 17 females and 23 males with ages 21–66.

curves (50, 100, 400, and 800 mg/L) were created for all target analytes. For high concentration target analytes (β-myrcene, α-humulene β-caryophyllene, β-farnesene) three additional calibration points were added (1000, 5000, and 9000 mg/L). Target analytes were quantified using the following ions for each analyte: m/z 41 (geranial), m/z 45 (2-octanol), m/z 59 (α-terpineol), m/z 69 (β-farnesene, geraniol, nerol, methyl geranate, and geranyl acetate), m/z 71 (terpinen-4-ol and linalool), and m/z 93 (β-Myrcene, β-caryophyllene, and α-humulene). The target analyte concentrations in hop oil were then standardized on a per-mass basis using the total oil content determined during hydrodistillation.

Beer volatile analysis

Headspace-Solid Phase Micro Extraction (HS-SPME) was performed on the dry-hop treatments using a 1 cm 24-gauge divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) Stableflex fiber with 30/50 µm coating thickness (Supelco, Bellefonte, PA, U.S.A.).[16, 17] An 8 mL sample of each was placed into a 20-mL screw top amber vial with 3 g sodium chloride. The compound 4-octanol (911 µg/L) was used as an internal standard and added to each vial. A MultiPurpose auto sampler (MPS2; Gerstel, Mülheim, Germany) was used for pre-incubation, stirring, extraction, and injection. Samples were preincubated for 15 min at 30 °C and adsorbed by piercing the vial septa and exposing the fiber to the headspace for 45 min with agitation. After adsorption, the fiber was desorbed into the GC sample inlet (splitless mode, 250 °C) for 10 min. The analytical column was a 30 m × 250 µm × 0.25 µm Zebron ZB-1 MS (Phenomenex, Torrance, CA, U.S.A.) and ultrapure helium was used as the carrier gas (at constant pressure, 73 kPa). The following temperature program was used: 50 °C hold for 1 min, 50–250 °C (5 °C/min) hold for 11 min and 250 °C hold for 5 min. The auxiliary line and mass spectrometer were operated at 280 and 180 °C respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and in full scan mode set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. Three point calibration curves (40,100, and 200 µg/L) were created for all target analytes. Calibration curves were made in a model beer solution (5% v/v ethanol) and were prepared using the methodology previously described. Target analytes were quantified using the following ions for each analyte: m/z 55 (4-octanol), m/z 59 (α-terpineol), m/z 69 (β-farnesene, geraniol, nerol, methyl geranate, geranial, and geranyl acetate), m/z 71 (terpinen-4-ol and linalool), and m/z 93 (β-myrcene, β-caryophyllene, and α-humulene).

Statistical analysis

The Z tests on proportions were used to evaluate the sensory discrimination tests on the internal process replicates. Two-way analysis of variance with a mixed model (including the factors panelist, sample, and replication as well as corresponding two-way interactions), multiple comparison analysis (Fisher's LSD), hierarchical cluster analysis, principle component analysis (PCA), and graphical constructions were carried out using XLstat 2017 (Addinsoft, New York, NY, U.S.A.). These tests and graphs were used to gauge the panel and panelist effectiveness in generating descriptive data, evaluate the significant differences in aroma quality and intensity among the dry-hopping treatments, and assess the associations between the chemical and sensory data collected. When performing statistical analysis on the data generated from the beer volatile analysis, all nondetected values were treated as zero values.

Results and discussion

Discrimination testing: Evaluating internal process replicates

Discrimination testing on the internal process replicates for each of 100% cultivar treatments yielded no significant differences (Table 4). This indicated that any process variation during dry-hopping had a negligible impact on the dry-hop aroma within the same treatment and therefore any differences observed among the treatments were not due to processing variation. For descriptive analysis testing, only one of the replicates for each of the 100% cultivar treatments was evaluated and it was randomly selected.

Descriptive analysis: panelist/panel evaluation

Each panelist was evaluated on their performance to discriminate differences among the treatments on at least one of the sensory attributes, their ability to replicate among all sessions, and their lack of interactions. Any panelists that failed these three criteria were removed from further analyses. Seven of the 16 original panelists were removed from the data set. The resultant data set included 36 observations per attribute, per sample.

Two-way ANOVA with a mixed model was performed on the attributes using the remaining 9 panelists (Table 5). Significant ($P < 0.05$) sample effects were observed across the attributes. Significant ($P < 0.05$) panelist effects were observed for all of the attributes. Panelist effects are expected in descriptive analysis because panelists tend to express their perceptions on the same sample using different parts of the scale. Overall it is the relative differences in

Table 5. Mixed model analysis of variance of the sensory attributes.

			OHAI		Citrus		Tropical/Catty		Tropical/ Fruity		Pine/ Resinous/ Dank		Herbal/Tea	
Source	Type	DF	*F*	*P* value[a]	*F*	*P* value[a]	*F*	*P* value[a]	*F*	*P* value[a]	*F*	*P* value[a]	*F*	*P* value[a]
Sample	Fixed	15	13.7	**<0.0001**	10.4	**<0.0001**	3.7	**<0.0001**	6.1	**<0.0001**	3.4	**<0.0001**	4.0	**<0.0001**
Panelist	Random	8	7.3	**<0.0001**	17.4	**<0.0001**	12.2	**<0.0001**	6.5	**<0.0001**	26.2	**<0.0001**	7.4	**<0.0001**
Rep	Fixed	3	1.6	0.2180	0.5	0.6970	1.7	0.1940	0.8	0.5130	0.2	0.8720	0.3	0.7970
Sample*Panelist	Random	120	1.4	**0.0140**	1.2	0.0830	1.2	0.1070	1.3	**0.0410**	1.2	0.1340	1.3	**0.0310**
Sample*Rep	Fixed	45	1.2	0.2080	1.0	0.4190	0.9	0.6380	1.0	0.5290	0.9	0.5950	0.8	0.8110
Panelist*Rep	Random	24	1.3	0.1430	1.4	0.1160	0.8	0.7590	0.8	0.7070	1.5	0.0770	2.2	**0.0010**
Error		360												

[a] Values in **bold** indicate *p*-value <0.05. DF, degrees of freedom.

their ratings, and not the absolute value of their ratings, that is important.[18] Significant panelist × sample effects were observed for OHAI, Herbal/Tea and Tropical/Fruity. Significant panelist × sample effects are common in sensory analysis and indicate that there were slight differences in the way the panelists scaled these attributes.[18] With the exception of a significant ($P < 0.05$) panelist × replication effect observed for Herbal/Tea, no significant effects of replication or interactions between panelist and replication or between sample and replication were observed. This indicates the panelists could effectively replicate their attribute scaling for the samples across the four replications and that the ratings provided for the attributes for a given panelist did not depend on replication.

The mean values for the sensory attributes and results of the Fisher's LSD tests on these attributes were summarized and sorted by OHAI (Table 6). Fisher's LSD tests were chosen as the mean comparisons technique instead of a more conservative method, such as Tukey's HSD tests, to highlight the potential differences that exist between the dry-hop aroma profiles of the treatments. The greatest amount of variation was found in the OHAI and Citrus attributes and the least in the Tropical/Fruit and Herbal/Tea attributes. For all of the attributes, the "unhopped" blank was not grouped with any of the dry-hop blending treatments. Interestingly, the panelists perceived the 50:0:50 and 25:25:50 blends of Cascade, Chinook, and Centennial to be the most intense in OHAI and Citrus. Similarly, Takoi et al.[3] observed that the coexistence monoterpene alcohols (linalool and geraniol) that can occur when dry-hopping with blends of hops in comparison with single-hopped control beers increased average Citrus scores and created drastically different flavor profiles. The coexistence of polyfunctional thiols and monoterpene alcohols has also been shown to increase the aroma perception of blending treatments [3]. Therefore, it is possible that these interactions were responsible for the increased aroma perception of the blended dry-hop treatments.

Multivariate analysis of sensory data

Hierarchical cluster analysis and PCA have been shown to be successful data dimension reduction techniques involving the sensory and chemical analyses of beer[19, 20] and other carbonated beverages.[21, 22] Three clusters were formed when performing agglomerative hierarchical clustering using the Euclidean distance for the dissimilarly scale and Ward's method as the agglomeration method (Figure 1). The 100% Cascade, Chinook, and Centennial dry-hopping treatments were sorted into three different clusters. This suggests that dry-hopping with each of these cultivars individually leads to beers with different hop aroma intensities and qualities. This observation is emphasized if the Ward clusters are overlaid onto a ternary plot (Figure 2). Previous work has shown that each of these cultivars has distinct character impact compounds that define the dry-hop aroma in beer for these cultivars.[7] However, it was also observed that in combination, blends of these three hops may lead to dry-hop aroma profiles that are similar in quality and intensity to the 100% Cascade, Chinook, and Centennial dry-hopping treatments. In general, the cluster in blue was defined by the 100% Chinook treatment, which could also be built from blends of Centennial and Chinook. The cluster in green was defined by the 100% Cascade treatment, along with of blends of Cascade, Centennial, and Chinook. The cluster in red was defined by the 100% Centennial treatment and included blends made with Cascade and Centennial.

PCA was performed on the covariance (n-1) matrix of the mean sensory scores for the dry-hop treatments and the resulting biplots were colored based on the agglomerative hierarchical Ward clusters (Figure 3). Overall, the first three principal components explained 95% of the variation within the data set, with PC1 accounting for ~72% and described variation in OHAI and Citrus qualities and to a lesser degree Tropical/Fruity aroma. PC2 accounted for ~16% and described variation in Tropical/Catty, and PC3 accounted for ~7% and described variation in Herbal/Tea. Each of the of the three Ward clusters highlights the aroma profiles observed for the single-cultivar dry-hopping treatments and the corresponding blending treatments that produce similar dry-hop aroma profiles. The cluster in blue, which included the 100% Chinook treatment, was perceived to be the lowest in overall hop aroma intensity but was highlighted by the Tropical/Catty and Pine/Resinous/Dank attributes. Modest and negative Pearson correlation coefficients were observed between % Chinook and the sensory attributes Citrus ($r = -0.51$, $p = 0.53$), Herbal/Tea ($r = -0.57$, $p = 0.26$) and Tropical/Fruity ($r = -0.47$, $p = 0.76$). This indicates that as the percentage of Chinook increased, the perceived value of these attributes decreased. The cluster in green, which included the 100% Cascade treatment, was perceived to be between Chinook and Centennial in terms of overall hop

Table 6. Summary of mean scores for the sensory attributes of the dry-hop blending treatments sorted by increasing overall hop aroma intensity (OHAI).[a]

Dry-hop treatment	Blend Code	OHAI[b]	Citrus[b]	Herbal/Tea[b]	Tropical/Catty[b]	Tropical/Fruity[b]	Pine/Resinous/Dank[b]
16	0:0:0	2.6 [f]	1.2 [f]	1.3 [c]	1.4 [c]	1.2 [e]	0.8 [d]
11-rep 1	0:100:0	6.4 [e]	3.9 [e]	2.8 [b]	3.5 [ab]	3.3 [d]	2.2 [abc]
13	0:50:50	6.6 [de]	4.2 [de]	2.9 [ab]	3.7 [ab]	3.5 [cd]	2.2 [abc]
10	25:0:75	6.7 [de]	4.1 [e]	2.9 [b]	3.7 [ab]	3.4 [cd]	2.0 [abc]
1-rep 1	100:0:0	6.8 [cde]	4.6 [abcde]	3.4 [ab]	3.1 [ab]	3.8 [bcd]	1.9 [bc]
4	50:50:0	6.9 [cde]	4.2 [de]	3.3 [ab]	3.6 [ab]	3.3 [d]	1.9 [bc]
5	50:25:25	7.0 [bcde]	4.7 [abcde]	3.3 [ab]	3.1 [b]	4.2 [abc]	2.1 [abc]
12	0:75:25	7.0 [bcde]	4.4 [cde]	3.1 [ab]	4.1 [a]	3.2 [d]	2.4 [ab]
7	25:75:0	7.1 [bcde]	4.6 [abcde]	2.8 [b]	3.8 [ab]	4.4 [ab]	2.2 [abc]
3	75:0:25	7.3 [bcd]	4.9 [abcd]	3.4 [ab]	3.8 [ab]	4.3 [abc]	2.5 [ab]
2	75:25:0	7.4 [abcd]	5.1 [abc]	3.2 [ab]	3.8 [ab]	3.8 [bcd]	1.8 [c]
8	25:50:25	7.4 [abcd]	5.0 [abcd]	3.3 [ab]	3.2 [b]	4.2 [abc]	2.1 [abc]
14	0:25:75	7.6 [abc]	4.6 [bcde]	3.2 [ab]	4.0 [ab]	4.5 [ab]	2.2 [abc]
15-rep 2	0:0:100	7.6 [abc]	5.3 [ab]	3.2 [ab]	3.5 [ab]	4.9 [a]	2.1 [abc]
6	50:0:50	7.8 [ab]	5.3 [ab]	3.5 [a]	4.1 [a]	4.5 [ab]	2.5 [a]
9	25:25:50	8.1 [a]	5.4 [a]	3.5 [ab]	4.3 [a]	4.5 [ab]	2.5 [ab]

[a] The treatment blending codes are represented as %Cascade:%Chinook:%Centennial.
[b] Letters in brackets indicate statistically significant groupings within each descriptor (Fisher's LSD tests P value <0.05).

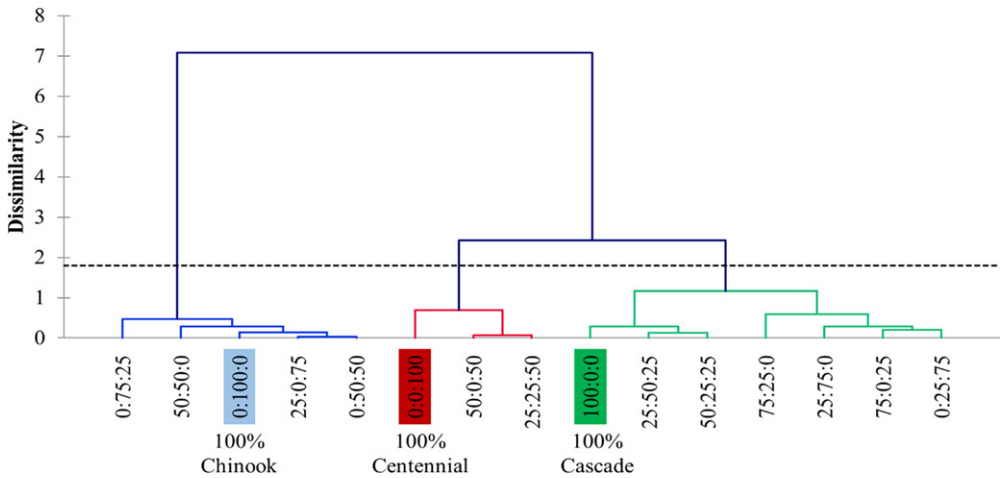

Figure 1. Agglomerative hierarchical clustering based on the sensory attributes using the Euclidean distance for the dissimilarly scale and Ward's method for agglomeration. The dotted line represents the automatic truncation option based on entropy. The treatment blending codes are represented as %Cascade; %Chinook; %Centennial.

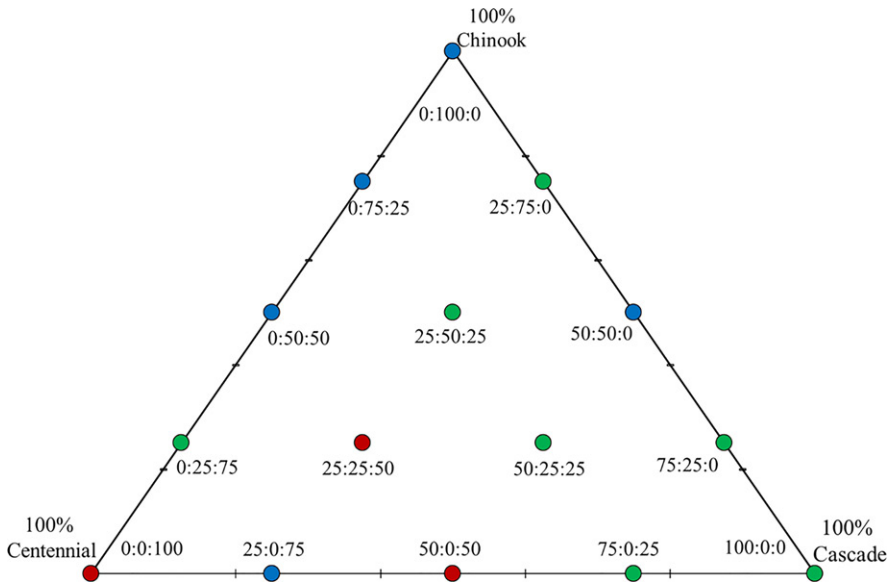

Figure 2. Ternary plot colored by the agglomerative hierarchical Ward clusters (red, blue and green circles). The treatment blending codes are represented as %Cascade; %Chinook; %Centennial.

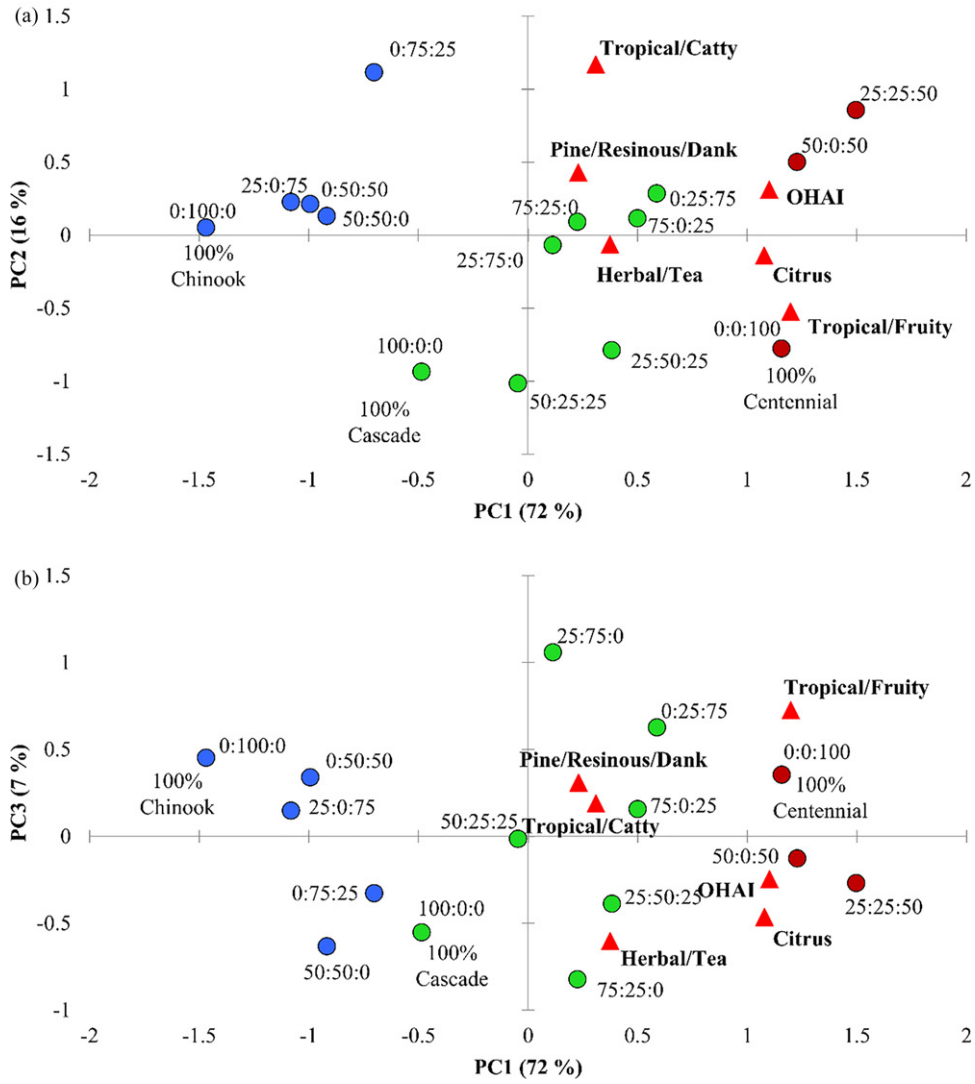

Figure 3. Principle Component Analysis biplots of the mean scores of the sensory attributes (red triangles) among the (16) dry-hop blending treatments colored by the agglomerative hierarchical Ward clusters (red, blue and green circles). (a) biplot of PC1 & PC2 explaining 88% of the variation in the data (b) biplot of PC1 & PC3 displaying an additional 7% of the variation in the data set. The treatment blending codes are represented as %Cascade; %Chinook; %Centennial.

aroma intensity and was primarily defined by the Herbal/Tea and Citrus attributes. As the % Cascade increased, the perceived Herbal/Tea attribute increased significantly ($r = 0.51$, $P = 0.53$). The cluster in red, which included the 100% Centennial treatment, was perceived to be the highest in overall hop aroma intensity and was primarily defined by the Tropical/Fruity and Citrus attributes. As the % Centennial increased, the perceived Tropical/Fruity attribute increased ($r = 0.46$, $P = 0.087$).

Using binary and tertiary blends of the three hops increased the hop aroma intensity above that from a single-hop treatment and this effect was observable in all three treatment clusters in the PCA biplot (Figure 3a). Notice, within each cluster, the single-hop treatment sits in the lower left-hand region and as other hops are blended in, the data cluster moves upward and toward the right, which indicates greater aroma intensity. This was the case even in the most intense variety in the study, Centennial (0:0:100), where blends with Cascade (50:0:50) or Cascade and Chinook (25:25:50) produced more intensely hoppy beers. This indicates that using blends of hops during dry-hopping might increase the aroma potential of hops as compared to using single varieties and could be a way to reduce hopping rates while maintaining high aroma intensities.

Chemical analysis of select hop volatiles in hops with gas chromatography/mass spectrometry (GC/MS) and dry-hop treatments with solid-phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS)

The measured volatile components in the hops used for dry-hopping and the impact the different blending treatments had on selected hop volatiles in beer were examined (Table 2). Hop volatile concentrations and the perceived overall hop aroma intensity were significantly positively correlated ($r = 0.533$–0.744, P values $= <$ 0.001–0.41) for all of the hop volatiles with the exception of caryophyllene oxide. Surprisingly, there appeared to be a synergistic effect of dry-hopping with blends of hops on the concentration of hop volatiles, which was highlighted when comparing the concentrations of the blends to the single cultivar treatments. It is unclear what caused this effect. There was no yeast

present in the dry-hopping treatments; therefore, it is not attributable to yeast biotransformation. However, this does not rule out the possibility of a hop-derived enzyme catalyzed biotransformation.

Significant positive Pearson correlation coefficients were observed between % Centennial and linalool ($r = 0.464$, $P = 0.082$), geraniol ($r = 0.550$, $P = 0.034$), methyl geranate ($r = 0.558$, $P = 0.021$), and nerol ($r = 0.615$, $P = 0.015$), indicating that as the percentage of Centennial increased in the dry-hopping treatments, the concentrations of these hop volatiles increased. Previous work has identified linalool, geraniol, and methyl geranate as character impact compounds for describing Centennial dry-hop beer.[7]

While none of the hop volatiles were individually correlated to the % Cascade, a significant positive correlation was observed between the ratio of linalool/geraniol and % Cascade, suggesting that the amount of Cascade in the dry-hop blends had an impact on this ratio indicating the importance of these analytes for Cascade dry-hop aroma in beer. These hop volatiles were also identified as character impact compounds for Cascade dry-hop beer[7] and highlighted as key drivers of Cascade aroma in numerous other studies[23–26] further highlighting the importance of these analytes in describing Cascade hop aroma and flavor in beer.

Caryophyllene oxide was significantly positively correlated with % Chinook ($r = 0.557$, $P = 0.024$). While the concentration of caryophyllene oxide was the lowest in Chinook, the concentration of β-caryophyllene was the highest of the three hop varieties. Although dissolved oxygen was monitored during processing and was relatively low (<110 µg/L post filtration), it is speculated that β-caryophyllene could potentially oxidize to caryophyllene oxide during dry-hopping and thus might be a marker for oxidation during dry-hopping with Chinook.

A limitation of the analytical analysis in this study is that polyfunctional thiols were not measured or considered. There are a number of studies[3, 27–30] that have emphasized the importance of these analytes in describing hop aroma in beer. In future studies, these analytes should be considered to have a possible impact on dry-hop flavor, especially when dry-hopping with blends of hops.

Conclusions

This study demonstrated that it is possible to achieve similar aroma profiles when dry-hopping beer with varying blends of Cascade, Chinook, and Centennial hops and that some of these blends may achieve an aroma profile similar to a single variety. Using blends of hops during dry-hopping has obvious benefits and promotes both the increase in perceived aroma intensity and quality as well as the increase in hop volatile extraction in dry-hopped beer. By utilizing a blending approach for dry-hopping, the brewer is able to make substitutions when faced with shortages due to cost and/or quality. While only hop aroma was evaluated in this study, dry-hopping can also impact bitterness. Therefore, the humulone content and age of the hops that will be blended for dry-hopping should be considered. These factors have a direct effect on the humulinone concentration of the hops and will subsequently modify the bitterness profile of beer.[31–33]

Acknowledgments

The authors would like to thank the Brewers Association for funding this research, Crosby Hop Farm for the donating hops, and BridgePort Brewing Company for producing the unhopped ale for this project. We wish to acknowledge the diligent work of the Cameron McDaniel, Ian Schacter, and Ryan Howe whose valuable assistance with the dry-hopping treatments helped this project run smoothly. We offer sincere appreciation for Cliff Pereira's expertise in designing the efficient resolvable incomplete block designs used for sensory analysis and would like to acknowledge Daniel Vollmer for helping to provide insight on the hop blending design used for this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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