Research article

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Impact of static dry-hopping rate on the sensory and analytical profiles of beer

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Dry-hopping is a technique that has been used by brewers to increase the hop aroma and flavour of beer for centuries. Throughout the twenty first century, dry-hopping has become an increasingly popular method among craft brewers to impart intense hoppy aroma and flavour to beer. Many US craft brewers use extremely high dry-hop dosing rates of up to 2200 g/hL and this is both unsustainable and potentially wasteful. This study examines the impact of dry-hopping rate on the sensorial and analytical characteristics of dry-hopped beers. An unhopped pale beer was statically dry-hopped with whole cone Cascade from the 2015 harvest over a broad range of dry-hopping rates (200–1600 g/hL) in replicated, pilot scale (80 L) aliquots. Trained panellists using descriptive analysis scaled the overall and qualitative hop aroma intensity of these beers, as well as the unhopped base beer. Instrumental analysis was used to measure the levels of hop volatile and non-volatile extraction between the treatments. The relationship between dry-hopping rate and the sensorial and analytical characteristics of the finished beer was not linear and, based on the extraction efficiencies of select hop volatiles, had an ideal range between 400 and 800 g/hL. © 2018 The Institute of Brewing & Distilling

Keywords: hop aroma; dry-hopping rate; hop extraction efficiency; hop sensory; hop chemistry

Introduction

Dry-hopping has been defined as the cold extraction of non-volatile and volatile chemicals from hops into an alcoholic solution *(1)* and has been a technique used by brewers to increase both the microbial *(2)* and flavour stability *(3)* of beer. As hoppy beer styles have risen in popularity with consumers over the twenty first century *(4)*, craft brewers have turned to dry-hopping as a way to enhance beer aroma and flavour. To achieve intense hop aromas and flavours there are a number of factors that brewers can modify during dry-hopping, such as static vs dynamic extraction *(5)*, the presence/absence of yeast *(6,7)*, different temperatures *(8,9)* and varying hopping amounts *(8)*.

Historically, static dry-hopping of cask beer was performed over a period of weeks *(10)*. However, current industrial static dry-hopping timeframes occur over a few days in large cylindroconical vessels. Using a 2 hL pilot-scale system, Wolfe *(5)* showed that after static dry-hopping (at 386 g hop/hL beer) for 6 h, the majority of selected key hop volatiles were extracted from hops and after 24 h the extraction peaked for these hop volatiles for both static and stirred/dynamic extractions. In the same study, extraction rates of key hop volatiles were found to vary based on the hop format during dynamic and static dry-hopping. Pelletised hops increased the extraction of linalool and geraniol during static dry-hopping by ~20%. Dynamic dry-hopping also promoted the extraction of hop volatiles for both whole cone and pelletised hops and increased the overall aroma intensity of dry-hopped beer. However, the bitterness intensity, bitterness duration and astringency of dynamically dry-hopped beers also increased owing to the elevated extraction of polyphenols and humulinones. Due to these unintended flavour consequences and the ease of implementation, static dry-hopping is often preferred to dynamic dry-hopping in the industry.

In terms of adding flavour to beer, varying the hopping amount is one of the easier levers to change during the brewing process. The underlying assumption is that adding more hop material to beer via dry-hopping will lead to more aroma and flavour. Around the 1890s, dry-hopping rates in the UK ranged from 65 to 274 g/hL *(11)*. In the 1960s, dry-hopping rates for British beers were reported to be ~4.3 g/hL for low gravity draught beer and ~138.6 g/hL for high gravity beers *(10)*. Current industrial hopping rates in the USA on average range from 500 to 800 g/hL; however, it is not difficult to find beers that have been dry-hopped at rates as high as 2200 g/hL. These extreme cases are both unsustainable from an agronomic perspective and potentially wasteful. Although it has been suggested that maximum hop flavour is achieved when dry-hopping with ~500 g/hL *(1)*, there have been few studies that have explored how dry-hopping rate specifically impacts beer aroma/flavour and the extraction of hop constituents.

The goals of this study were to (a) scale the changes in hop aroma intensity and quality for 'unhopped' beer dry-hopped statically with ground whole cone cascade at five different rates, 0, 200, 386, 800 and 1600 g/hL and (b) examine the impact and changes in extraction efficiencies on the non-volatile and volatile constituents over these dry-hopping rates.

Materials and methods

Experimental design

Five beers (including the 'unhopped' control) were prepared by statically dry-hopping an 'unhopped' beer with ground, whole

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cone Cascade hops from a single harvest lot by varying the dry-hopping rate at 200, 386, 800 and 1600 g/hL. Descriptive sensory analysis was used to scale the aroma intensity and quality of these beers. Non-volatile and volatile chemical analyses were performed on the hops used for dry-hopping and on the finished beers to determine the extraction efficiencies of hop derived aroma and flavour compounds into beer.

Hop collection

A 10 lb (4.5 kg) mini-bale from a single lot of whole cone Cascade hops was collected after harvest in 2015 from Crosby Hop Farms (Woodburn, OR, USA). Upon arrival at Oregon State University the whole-cone hops were repackaged in high-barrier foil pouches, purged and sealed with nitrogen, and stored cold (−20°C) until dry-hopping and chemical analysis.

'Unhopped' beer production

'Unhopped' beer was prepared on a commercial scale by a regional brewing operation in Portland, Oregon. The 'unhopped' wort was prepared with 86% pale two-row, 13.5% Caramel 10°L and 0.5% Caramel 120°L malt (Great Western, Vancouver, WA, USA) to a starting concentration of 11.3°P. Fermentation was performed using a Scottish ale yeast (Wyeast 1728) 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, USA) were added at a concentration of 18 mg/L. This resulted in ~55 hL of a 19.8 BU, 4.75% ABV 'unhopped' base beer. The beer was carbonated and packaged into 60 L stainless steel kegs, shipped to Oregon State University and held at 2°C until dry-hopping.

Dry-hopping protocol and hop preparation

The dry-hopping process reported by Vollmer *et al*. *(12)* was used to reduce the variation between treatments on the pilot scale. In brief, 24 h prior to hop addition the 'unhopped' beer was removed from the cooler at 4°C and allowed to warm to ~15°C. For each treatment, 40 L of beer was transferred into two modified 60 L stainless kegs with a 4 inch stainless steel opening fitted with a standard Sankey D-system coupler and modified spear (Sabco, Toledo, OH, USA). To achieve the 200, 386, 800 and 1600 g hop/hL unhopped beer treatment rates, the whole cone hops were ground into a hop grist which was divided by mass into two mesh bags (EcoBag, Ossining, NY, USA). These bags were stored inside high barrier pouches flushed with nitrogen until dry-hopping. For each dry-hop treatment, the two kegs filled with 40 L beer were temporarily de-pressurised and opened under a stream of low-pressure carbon dioxide. Simultaneously, the high-barrier pouch bag 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. The kegs were inverted three times to ensure proper mixing.

After 24 h of dry-hopping the beer was filtered to stop the dry-hopping process. The average temperature of dry-hopping ranged from 13.3 to 15°C. During filtration the two kegs were blended via a three-way fitting prior to entering a plate and frame filter using diatomaceous earth impregnated cellulose pads (HS2000, Pall Corporation, Port Washington, NY, USA) *(13)*. Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyser (Hach, Loveland, CO, USA). Bright beer was not collected until the DO was <110 μg/L. When in specification for DO, bright, filtered beer was collected in a closed 19.6 L 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 was measured and the bright beer tank DO concentration was recorded. Filtered beer was stored at 2°C and under CO_2 overpressure (11–12 psi) until sensory evaluation. To minimise artefacts and scalping in the crown liner owing to packaging in glass bottles *(14,15)*, all beer for this experiment was kept in 19.6 L kegs and served directly from two eight-head draught systems (Micro Matic, Northridge, CA, USA) throughout the sensory and instrumental data collection periods.

Sensory descriptive analysis

Thirteen trained panellists were used to scale orthonasal aroma of the treatments and were selected based on previous experience (11 males and two females; 25–66 years old). Four intensive training sessions were completed in advance of data collection. During these sessions panellists 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* and *Herbal/tea* evaluated on a 0–15 point scale. Previous work in our laboratory used a broader array of descriptors to describe Cascade hop aroma including *Resinous/Hop oil*, *Green* and *Tropical Fruit* plus *OHAI*, *Citrus* and *Herbal/tea*. However, the quality attributes that described the most variation for the Cascade dry-hop aroma were *OHAI*, *Citrus* and *Herbal/tea*. Therefore, these attributes were used to characterise the changes in Cascade hop aroma in the present study. During each session, the panellists had access to five external reference samples, three of which were the experimental treatments (i.e. the unhopped control, plus 386 g/hL and 1600 g/hL dry-hop treatments) and two of which were commercial, hoppy beers (Hop Valley Citrus Mistress and Ballast Point Grapefruit Sculpin). These five beers had sensory descriptors with intensity scores assigned by consensus during training, and their purpose was serve as anchors for the 0–15 point intensity scale (Table 1).

The four dry-hop dosage treatments and the unhopped control were evaluated randomly amongst 28 beers dry-hopped at 386 g/hL with different lots of Cascade as part of a separate study. Over the course of 16 sessions, the 13 panellists evaluated all samples four times. An efficient resolvable incomplete block design was used to create a presentation order for the samples across four replications (SAS, Cary, NC, USA). Four sessions were needed per replication to evaluate all the hopped samples (three sessions of

Table 1. Reference standards with intensity scores used in descriptive analysis panels

Attributes	Unhopped control	386 g/hL	1600 g/hL	Citrus Mistress	Grapefruit Sculpin
OHAI[a]	0	8–9	14–15	7–8	14–15
Citrus	0	7–8	5–6	6–7	13–14
Herbal/tea	0	5–6	12–13	6–7	1–2

[a]OHAI, Overall hop aroma intensity.

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eight samples and one session of nine samples). Panellists 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, USA) into pitchers at ~1.5°C and at 12 psi. Beer was poured into sample glasses ~1 h before the start of testing and allowed to warm to room temperature. Panellist responses were collected on Chromebook tablets using Qualtrics (Provo, UT, USA). For each session, Qualtrics was also used to randomly assign the serving order of samples for each panellist.

Beer and hop analysis

Simultaneous with the hop sampling for the dry-hopping, a ~150 g portion of the homogenised hop grist was taken for chemical analysis. All beer was stored in 19.6 L kegs at ~1°C until analysis.

Non-volatile analysis reagents and standards

Octyl alcohol was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). HPLC-grade methanol was obtained from VWR International, BDH analytical (West Chester, PA, USA). Hydrochloric acid, 2,2,4-trimethylpentane and phosphoric acid were obtained from Avantor performance materials (Center Valley, PA). DCHA-Iso ICS-I3 and international calibration extract ICE-3 standards were obtained from ASBC. Humulinone standards were produced *(16)* and DCHA-humulinones standards were obtained through Robert Smith from S.S. Steiner Inc.

Non-volatile beer and hop analysis

Total humulones, lupulones and hop storage index were measured and calculated using ASBC Hops – 6 *(17)*. The concentrations of hop acids in hops and beer samples were analysed using ASBC methods Hops – 14 and Beer – 23E under modified HPLC conditions *(17)*. The modified HPLC analysis was performed on an Agilent 1200 HPLC. Prior to analysis beer was degassed via filtration through GHP Acrodisc® 13 mm, Pall Corporation (East Hills, NY, USA) syringe filters. Analysis was performed using a 2.6 μm EVO C-18 100 Å 100 × 4.6 mm LC column (Phenomenex, Torrance, CA, USA) held at 40°C. A 7 μL aliquot of each beer sample was injected and the elution was carried out using a flow rate of 1.6 mL/min. The solvent gradient was as follows: 10% solvent A (reagent water)–90% solvent C (90% 75% MeOH, 24% H2O, 1% H_3PO_4) held for 5 min, then changed to 100% solvent D (100% MeOH) over 5 min and held for 2 min, then returned to 10% solvent A–90% solvent C over 2 min, for a total run time of 14 min. Based on absorbance maximum of each hop acid, the absorbance of *iso*-humulones and humulinones were measured at 275 nm and that of humulones was measured at 314 nm *(16)*.

Bitterness units were measured according to ASBC methods of analysis Beer – 23A *(17)*. Spectrophotometric analysis for bitterness units were carried out using a Shimadzu PharmaSpec UV-1700 spectrophotometer, Shimadzu Corporation (Columbia, MD, USA). Residual extract and pH were analysed using an Anton-Paar Alcolyser with supporting pH module (Anton Paar USA, Ashland, VA, USA).

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, USA). 2-Octanol was obtained through Alfa Asear (Haverhill, MA, USA). Hexanes purchased from J.T. Baker (Center Valley, PA, USA) were redistilled to remove impurities before analysis. Sodium chloride was purchased from EMD Millipore (Billerisa, MA, USA).

Hop volatile analysis

Hydrodistillation was performed to determine the total oil content of the homogenised hop grist using ASBC Hops – 13 *(17)*. 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 nitrogen. Hop oil was stored at −20°C until subsequent compositional analysis.

Hop oil compositional analysis was performed using an HP 6890 gas chromatograph with an Agilent 5972a mass spectrometer (GC–MS) under modified conditions from ASBC Hops – 17 *(17)*. 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 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, USA) and ultra-pure helium was used as the carrier gas (a constant flow rate, 1.4 mL/min). The following temperature programme was used: 50°C held for 1 min; 50–180°C (2°C/min), held for 10 min; 180–200°C (3°C/min); and 250°C held 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 mass-to-charge ratios (*m/z*) of 30–350. Four-point calibration 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 standardised on a per-mass basis using the total oil content determined during hydrodistillation.

Beer volatile analysis

Headspace solid phase microextraction was performed on the dry-hop treatments using a 1 cm 24 gauge divinylbenzene–carboxen–polydimethylsiloxane Stableflex fibre with 30/50 μm coating thickness (Supelco, Bellefonte, PA, USA) *(6,18)*. An 9 mL aliquot of each sample was placed into a 20 mL screw-top amber vial with 3 g sodium chloride. 4-Octanol (911 μg/L) was used as an internal standard and added to each vial. A MultiPurpose autosampler (MPS2; Gerstel, Mülheim, Germany) was used for pre-incubation, stirring, extraction and injection. Samples were pre-incubated for 15 min at 30°C and adsorbed by piercing the vial septa and exposing the fibre to the headspace for 45 min with agitation. After adsorption, the fibre 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, USA) and ultra-pure helium was used as the carrier gas (at constant pressure, 11 psi). The following temperature programme was used:

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50°C held for 1 min; 50–250°C (5°C/min); held for 11 min; and 250°C, held 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 above. 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

Two-way analysis of variance with a mixed model (including the factors panellist, sample, and replication as well as corresponding two-way interactions), Pearson correlation analysis, multiple comparison analysis (Tukey's HSD), principle component analysis and graphical construction were carried out using XLstat 2017 (Addinsoft, New York, NY, USA). These tests and graphs were used to gauge the panel and panellist 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 collected chemical and sensory data.

Results and discussion

Descriptive analysis: panellist/panel evaluation

Each panellist 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 panellist that failed these three criteria were removed from further analyses. Three of the 13 original panellists were removed from the dataset. The resultant dataset included 40 observations per attribute, per sample.

Two-way ANOVA with a mixed model was performed on the attributes using the remaining 10 panellist (Table 2). Significant sample effects were observed across the attributes and a significant panellist × sample effect was observed for OHAI. The significant panellist × sample effect is common in sensory analysis and indicates that there were slight differences in the ways the panellist scaled OHAI *(19)*. No significant effect of replication or interactions between panellist and replication or between sample and replication were observed. This indicates the panellists could effectively replicate their attribute scaling for the samples across the four replications and that the ratings provided for the attributes for a given panellist did not depend on replication. Although there are inconsistencies among the group of panellists with scaling OHAI, individually the panellist results demonstrate consistent ratings across the sensory attributes. The least square means and results from Tukey's HSD ($p < 0.05$) for the sensory attributes from the descriptive analysis panel on the dry-hop treatments are summarised in Table 3.

Descriptive analysis: aroma intensity and quality response to hop dosage

It was hypothesised that the greater the concentration of hops used for dry-hopping was, the higher the overall hop aroma intensity would be. Significant ($p < 0.05$) positive Pearson correlation coefficients (r) were observed between dry-hopping rate and the sensory attributes OHAI (0.960) and Herbal/tea (0.994), indicating that as the dry-hop rate increased so did the values of these attributes. However, when examining dry-hop rate vs overall hop aroma intensity, a nonlinear relationship between dry-hopping rate treatments and the sensory attributes was observed (Fig. 1). Although there were five statistically significant groupings for OHAI, indicating that the overall intensity increased over the dry-hopping treatments, the 1600 g/hL appeared to yield diminishing returns. In fact, there was no significant difference in Citrus intensity between the two highest hopping rates. When considering the hop quality sensory attributes over the dry-hopping treatments it can be seen that the aroma quality changed over the treatments. At the low dry-hopping rates the Citrus and

Table 3. Summary of least squared means for the sensory attributes resulting from descriptive analysis

	Dry-hop rate (g/hL)				
Sensory Attributes	0	200	386	800	1600
OHAI	3.0^{e}	6.3^{d}	8.1^{c}	10.4^{b}	12.3^{a}
Herbal/tea	2.5^{d}	4.3^{c}	5.7^{c}	7.4^{b}	10.4^{a}
Citrus	1.9^{c}	4.4^{b}	$5.8^{a,b}$	7.1^{a}	7.0^{a}

Letters indicate statistically significant groupings (Tukey's HSD tests *p*-value < 0.05).

Table 2. Mixed model analysis of variance on the sensory attributes

Source	Type	d.f.	OHAI		Herbal/tea		Citrus	
			F-Statistic	*p*-Value	*F*-Statistic	*p*-value	*F*-Statistic	*p*-Value
Sample	Fixed	4	41.7	< 0.0001	34.1	< 0.0001	28.3	< 0.0001
panellist	Random	9	1.2	0.358	1.5	0.237	1.8	0.138
Rep	Fixed	3	0.4	0.721	0.5	0.690	0.7	0.582
Sample × panellist	Random	36	2.1	0.002	1.4	0.079	1.1	0.295
Sample × Rep	Fixed	12	0.8	0.683	0.5	0.895	0.8	0.630
panellist × Rep	Random	27	0.7	0.835	0.8	0.680	1.3	0.147
Error		108						

Values in bold indicatte *p*-value <0.05.

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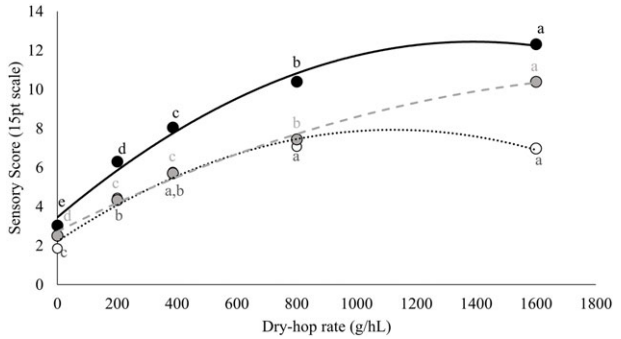

Figure 1. Mean values of overall hop aroma intensity (OHAI; black circles), Citrus (white circles) and Herbal/tea (gray circles) sensory attributes vs dry-hopping rate. Letters associated with the markers in the figure indicate statistically significant groupings (Tukey's HSD tests p-value < 0.05).

Herbal/tea attributes appeared to increase at similar rates, but the high dry-hopping rate (1600 g/hL) overall hop aroma character was predominately Herbal/tea. This is evidence that using very high hopping rates may not necessarily result in amplification of just hop aroma intensity and that the quality of the hop aroma will change as a function of hopping rate.

Steven's power law has been used previously to describe olfaction and the relationship between odourant concentration and aroma intensity *(20)*. The log–log plot of the sensory attributes vs the dry-hopping rate (Fig. 2) shows that both OHAI and the Herbal/tea quality are described by Steven's power law. The exponents n measured for OHAI ($n = 0.35$) and Herbal/tea ($n = 0.30$) are similar to those found in literature for single hop constituents *(21)*. For each of these attributes, n was <1, which indicates that the exponent is compressive and that aroma intensity was increasing slowly as the dry-hop rate increased. The Citrus quality did not follow the Steven's power law and this could be due to suppression of this quality by the Herbal/tea quality or by its reaching a solubility limit.

Hop dosage and hop volatile extraction

The measured volatile components in the hops used for dry-hopping and the impact that dry-hop rate had on selected hop volatiles in beer were examined (Table 4). Significant ($p < 0.05$) positive Pearson correlation coefficients (r) were observed for β-caryophyllene (0.964), α-humulene (0.963), terpinen-4-ol (0.971), α-terpineol (0.973), linalool (0.994), nerol (0.985), geraniol (0.982)

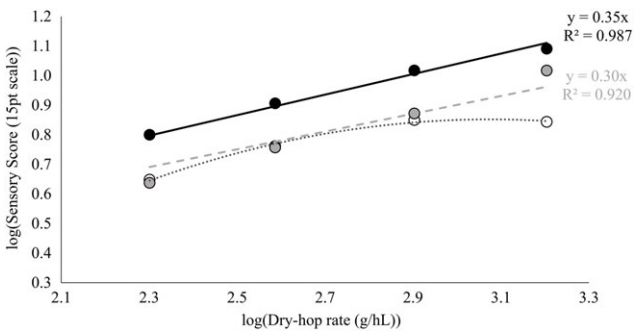

Figure 2. Logarithmic values of the mean values for OHAI (black circles), citrus (white circles) and herbal/tea (gray circles) sensory attributes vs thecorresponding logarithmic dry-hopping rate.

and geranial (0.954), indicating that as the dry-hopping rate increased, so did the values of these analytes in beer (Fig. 3).

Dry hopping at the lowest rate, 200 g/hL, led to the concentrations of the terpene alcohols, linalool, geraniol and nerol being above their reported difference threshold values in beer *(22)*. The extraction rates of these analytes decreased with increased dry-hopping rate (Fig. 3). At 200 g/hL only ~23, ~13 and ~6% of the total amounts of linalool, geraniol and nerol were extracted from the hops into the beer during dry-hopping, while at 1600 g/hL even less (~7, ~3 and ~1%) was extracted for each of these analytes respectively. Wolfe *(5)* observed similar peak extraction rates when statically dry-hopping (at 386 g/hL) with whole cone hops for linalool and geraniol to be ~29 and 70% respectively. Although the contact time was much longer (~4 weeks) and the technique for hop addition during dry-hopping differed significantly from this study, Forster *et al. (23)* reported extraction rates of linalool and geraniol during dry-hopping to be ~100 and 50–100% respectively. It is expected that the static dry-hopping technique used in this study led to the observed reduced extraction rates of terpene alcohols. However, these low extraction rates indicate that hop volatiles may not be fully extracted from hops during static dry-hop events and potentially more aroma can be extracted from hops used for dry-hopping.

Very low extraction rates ($< 1\%$) for β-myrcene, β-caryophyllene, α-humulene and β-farnesene were observed. Similar extraction rates (0.3–2.6%) for these analytes were observed in a number of studies *(23)*. The physical–chemical properties of these analytes make them insoluble in beer and therefore they are not extracted to an appreciable degree during dry-hopping. It is unlikely, unless at extreme dry-hopping rates, that these analytes play much of a role in the sensory perception of dry-hopped beer.

Terpinen-4-ol and α-terpineol were not found to be present above the detection limit in the hop oil or the 'unhopped' beer but were found to be present in the dry-hopped beer. There is evidence that these analytes can appear in beer via degradation *(24,25)* or enzymatic *(7,26)* transformation of other hop volatiles such as linalool. In this study dry-hopping occurred in the absence of yeast; therefore it is likely that these analytes are degradation products.

Methyl geranate and geranyl acetate were both found to be present in the 'unhopped' beer. Although non-significant Pearson correlation coefficients (r) were measured, as dry-hop rate increased the concentrations of methyl geranate ($r = -0.854$, p-value = 0.15) and geranyl acetate ($r = -0.920$, p-value = 0.08) decreased. It has been previously reported by Forster *et al. (27)* that trace levels of geranyl acetate have been observed in dry-hopped beers and that it can be hydrolysed to geraniol. There is evidence that geranyl acetate esterase is commonly present in plant species *(28)* and has been shown to regulate the level of geraniol in lemongrass *(29)*. One explanation for the decrease in geranyl acetate concertation as the dry-hopping rate increases is that hops may contain geranyl acetate esterase that could convert geranyl acetate to geraniol during dry-hopping. There is also evidence that methyl geranate may be converted into geranic acid. Therefore it is possible that the reduction in concentration of these analytes may be a result of hop-derived enzymes extracted from the plant material during dry-hopping.

Hop dosage impact on BU and non-volatile chemistry

The measured non-volatile components in the hop material used for dry-hopping and the impact of dry-hop rate on the non-volatile

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Table 4. 2015 harvest Cascade hop[a] and beer volatile chemistry over the dry-hopping rate treatments

Target analytes	Hop volatile analysis (mg/100 g)[b,c]	Beer volatile analysis (μg/L)[d]				
		Dry-hop rate (g/hL)				
		0[c]	200[e]	386[e]	800[e]	1600[f]
β-myrcene	729.4	0.9	41.1	35.2	56.9	20.5
β-caryophyllene	95.4	n.d.	n.d.	0.2	0.4	2.3
α-humulene	184.8	n.d.	0.6	0.4	0.9	2.8
β-farnesene	47.9	n.d.	0.5	0.5	0.5	2.1
Terpinen-4-ol	n.d.	n.d.	1.8	3.6	3.9	7.0
α-terpineol	n.d.	n.d.	9.0	10.7	11.4	13.7
Linalool	8.4	n.d.	38.3	53.9	71.1	104.3
Nerol	0.7	n.d.	0.8	0.8	1.1	1.6
Geraniol	6.9	n.d.	17.8	21.4	27.8	34.3
Geranial	0.4	n.d.	0.5	0.5	13.1	19.5
Methyl geranate	0.4	1.3	4.7	3.3	4.4	0.7
Geranyl acetate	21.2	n.d.	7.0	5.2	5.9	1.5

[a]Total oil content = 2.0 (mL/100 g). Measured using ASBC MOA Hops – 13 *(17)*.
[b]Analysed using under modified GC/MS conditions based on ASBC MOA Hops – 17 *(17)*. Analytes are reported in mg/100 g hops.
[c]Analysed using under modified GC/MS conditions based on published methodology *(6,18)*. Analytes are reported in μg/L and are blank corrected.
[d]Based on one instrumental run.
[e]Average of four instrumental runs.
[f]Average of two instrumental runs.
n.d., Not detected.

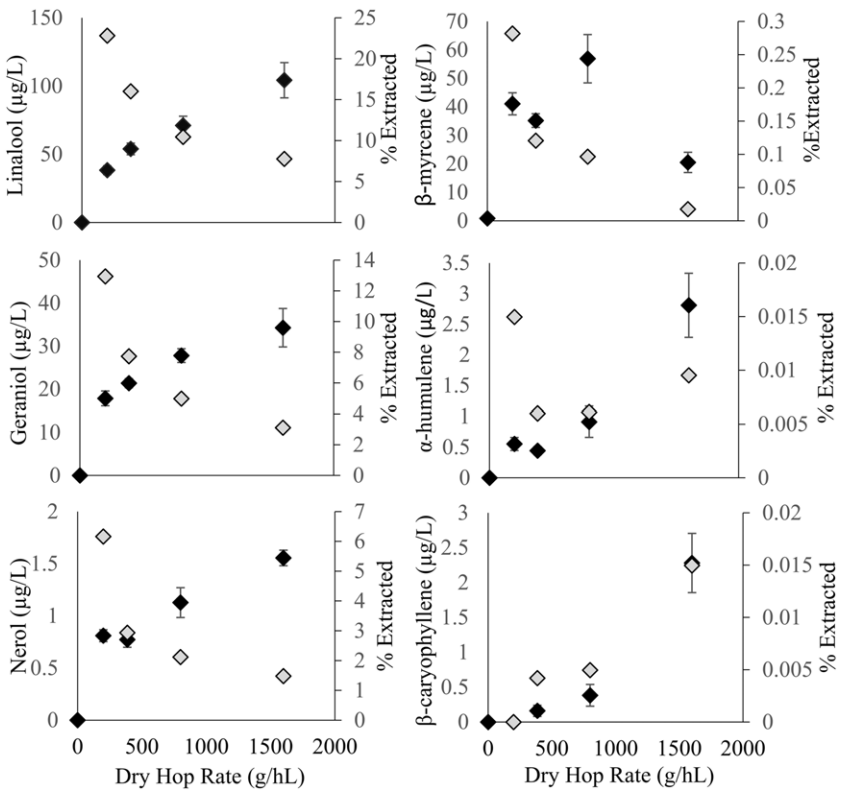

Figure 3. Comparison of mean concentrations (black diamonds, μg/L) and extraction rate from hops into beer (gray diamonds, %) for selected hop volatiles across all dry-hopping rates. Error bars represent one standard error within instrumental replicates (n = 2–4).

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beer profile was also reviewed (Table 5). As the dry-hopping rate increased, a rise in the bitterness units and humulinone concentration was observed (Fig. 4). Extraction of humulinones during dry-hopping has previously been associated with an increase in bitterness units *(30)*. Interestingly, as the dry-hopping rate increased, the extraction rate of humulinones from the hops into the beer decreased: 200 g/hL (113%), 386 g/hL (76%), 800 g/hL (74%), and 1600 g/hL (47%). After five days dry-hopping, Maye *et al. (31)* observed similar extraction rates of humulinones from Centennial hop pellets: 200 g/hL (98%), 386 g/hL (91%) and 800 g/hL (87-88%). The extraction rate of humulone from the hops into beer was very low over the dry-hopping treatments: 386 g/hL (2%), 800 g/hL (1%) and 1600 g/hL (1%). Other studies have also shown that the extraction of α-acids during dry-hopping was low and roughly 4–6% *(23)*. No change was observed in the *iso*-humulone concentration over the dry-hopping treatments. However, at concentrations ≥50 mg/L *iso*-humulone, a decrease in *iso*-humulone concentration should be expected as the dry-hopping rate increases *(31,32)*.

Owing to the amount of hop material used at the high dry-hopping rates and the static dry-hopping technique used in this study, it is likely that the decreased humulinone extraction at the high dry-hopping resulted from the hops not being homogenously dispersed in solution and the increased hop solids load. These factors may have led to poor mass transfer and decreased diffusion rates of the humulinones out of the plant material and into the beer. This phenomenon may also occur in commercial dry-hopping where there are high hop solid concentrations and similar beer-to-hop solid ratios.

It was also observed that the rise in BU across the dry-hopping rates was slightly more than the sum of *iso*-humulone and humulinone concentrations in the dry-hopping treatments. Although polyphenols were not measured in this study, it is expected that the extraction of hop polyphenols at the higher dry-hopping rates may have led to this deviation. Hahn *et al. (33)* recently observed that the perception of bitterness intensity of hoppy beers is associated with primarily humulinone and *iso*-humulone concentration. Therefore, although the sensory bitterness of these dry-hopped beers was not evaluated, it is clear that dry-hopping rate has a direct impact on the concentration of analytes that are important for the perception of bitterness in beer.

Table 5. 2015 harvest Cascade hop[a] and beer non-volatile chemistry over the dry-hopping rates

Target analytes	Hop non-volatile analysis (% *w*/w)	Beer non-volatile analysis (mg/L)[c]				
		Dry-hop rate (g/hL)				
		0	200	386	800	1600
α	5.5[b]	n.d.	n.d.	4.0	5.7	9.0
Humulinone	0.1[b]	n.d.	2.5	3.3	6.5	8.2
iso-α-Acid		16.4	16.7	17.0	16.5	16.4
BU		17.0	19.4	21.0	25.0	26.0
pH[d]		4.11	4.20	4.25	4.33	4.50
Real Extract[d] (w/w%)		3.16	3.19	3.23	3.28	3.51

BU, Bitterness units; n.d., Not detected.
[a]Hop storage index, 0.381. Measured using ASBC MOA Hops – 6 *(17)*.
[b]Measured using modified conditions of ASBC MOA Hops – 14 *(17)*. Analytes are reported as *w*/w %
[c]Measured using modified conditions of ASBC MOA Beer – 23E *(17)*. Analytes are reported as mg/L.
[d]Measured using an Anton Paar Alcolyzer with supporting pH module.

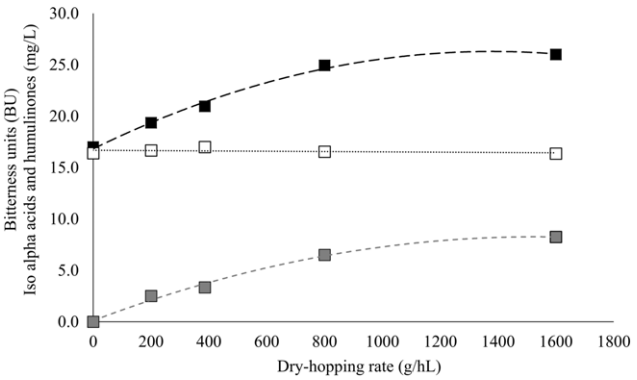

Figure 4. Hopping rate influences final beer bitterness units (BU) (black squares), *iso*-α-acids (mg/L) (white squares) and humulinones (mg/L) (gray squares).

A linear increase in pH (~0.14 pH for every 386 g/hL) was observed over the dry-hopping treatments (Table 5). This has also been reported in the literature and seems independent of both hop variety and beer style. Maye *et al. (31)* observed a similar pH value increase when dry-hopping with both Cascade hop pellets and spent CO_2-extracted hop powder, and has suggested that the increase in the pH value may be a result of the vegetative material. This increase in the pH value may lead to an improved flavour stability of dry-hopped beers by driving the formation of less reactive oxygen radicals *(21,34)* and has been shown to reduce the flavour perception of both *trans*-2-nonenal (cardboard-like aroma) and methional (potato) during aging *(35)*.

Real extract (RE, %*w*/w) was also observed to linearly increase as a function of dry-hopping rate (~0.07 %w/w) for every 386 g/hL (Table 5). It has been shown that there are numerous sugars in hops *(36)* accounting for ~2% w/w of hop cones *(10)* with 0.38–0.55% fructose, 0.32–0.44% glucose and 0.10–0.57% sucrose as well as small amounts of raffinose, stachyose and pentosans *(36)*. This implies that the increase in RE is due to the addition of hops and not a result of the measurement technique. In dry-hopped beers that are bottle conditioned with yeast or bacteria this increase in fermentable sugar from hops should be considered along with the enzymatic/reducing power of hops. These factors may impact secondary fermentation in bottle which could influence diacetyl concentration and package over pressurisation, the former being a quality issue and the latter being a serious consumer safety risk.

Conclusions/industrial considerations

Adding more hops by static dry-hopping does not simply lead to increased aroma intensity but also changes aroma quality in the finished beer. Dry-hopping rates >800 g/hL lead to hop aromas that were more herbal/tea in quality than citrus. To maintain a more balanced hop aroma quality this study suggests using a static dry-hopping rate between 400 and 800 g/hL. Using dry-

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hopping rates >800 g/hL leads to diminishing returns in terms of increasing hop aroma and is an inefficient use of raw material. Although work needs to be done to evaluate what is left in hops post dry-hopping, there is evidence that most of the analytes (humulinones) that impact bitterness perception are extracted from hops during dry-hopping (~75%), but that there are still hop volatiles left in the spent hop material. In addition, there are also a considerable amount of humulone left in the spent dry-hop material. Therefore, this spent dry-hop material could potentially have use elsewhere in the brewing process.

It is expected that the low extraction rates of terpene alcohols observed in this study are a result of the static dry-hopping technique used. These extraction rates may be impacted by tank/dry-hopping dynamics such as tank or extraction environment dimensions as well as hop particle settling velocity and concentration in the dry-hopping vessel. Therefore, it is important to consider the beer-to-solids ratio within the vessel during dry-hopping as this may have an impact on the extraction of analytes that impart hoppy flavour. It is hypothesised that extraction during static dry-hopping may be promoted through multiple static dry-hopping events or gentle agitation. However, agitation has been shown to change the quality of the hop aroma extracted to more of a herbal/grassy character and promote the extraction of polyphenols, which may increase the astringency of beer *(5)*. Ultimately it is up to the brewer to decide which dry-hopping technique promotes the best usage of hops and achieves the desired sensory profile. It is expected that understanding how static dry-hopping rates impact aroma quality and intensity will help promote environmentally and economically sustainable brewing practices.

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