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Impact of harvest maturity on the aroma characteristics and chemistry of Cascade hops used for dry-hopping

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ABSTRACT

The impact of ripening on the dry-hop aroma potential and chemical development of Cascade hops is not well understood. Therefore, 5–6 weekly hop samples were collected over the 2014, 2015 and 2016 harvests. Concentrations of humulones did not change as a function of harvest date, while total hop essential oil content displayed significant positive trends. Concentrations of thiol precursors decreased over harvest while concentrations of free thiols increased. These weekly samples were used to dry-hop an unhopped base beer. Overall hop aroma intensity and citrus quality attributed to beer during dry-hopping increased as a function of harvest date. These results suggest that for brewers to maximize the efficiency of hop usage, early harvested Cascades might be better for bittering, while, later harvested Cascades might be better for dry-hopping or aroma additions because they attributed more intense citrusy aromas to beer and had higher concentrations of free thiols and terpene alcohols.

1. Introduction

The chemical constituents extracted from hops (*Humulus lupulus* L.) during the brewing process impart aroma and flavor to beer, as well as increase microbial and flavor stability. Therefore, the chemical composition of hops and the factors that drive the changes in hop chemical composition during hop production are important considerations for brewers and hop growers.

Historically, the main consideration around hop quality for brewers has been focused on the bittering potential of hops, which is mainly driven by the concentration of humulones (α-acids) contained in the soft resins of hops (Verzele & De Keukeleire, 1991). While humulones are not directly responsible for leading to beer bitterness, they are isomerized to isohumulones (the main drivers of bitterness in beer) when hops are added to the kettle during wort boiling. A number of studies have shown that pre- and post-harvest factors as well as on the bine ripening time can influence the concentrations of humulones in hops (Bailey et al., 2009; Howard & Tatchell, 1956; Matsui, Inui, Oka, & Fukui, 2016; Probasco & Murphey, 1996; Sharp, Townsend, Qian, & Shellhammer, 2014).

In contrast to traditional kettle hopping, brewers wishing to increase hop aroma without adding hop bitterness are turning to dry-hopping, a brewing practice generally recognized as a cold extraction of hops in fermented or partially fermented beer (Schönberger & Kostelecky, 2011). Recently, Hahn, Lafontaine, Pereira, and Shellhammer (2018) observed that in dry-hopped and hop-forward

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beers the oxidized forms of humulones, humulinones, were also main contributors to beer bitterness. Thus, the concentration of humulinones in hops, which is primarily driven by post-harvest processing or storage conditions (Maye, Smith, & Leker, 2016), should also be considered as a driver of hop quality from a bitterness potential perspective.

In general, the main objective of late/whirlpool- and dry-hopping is to add intense hop aroma to beer without imparting much bitterness. The current thought within the brewing community is that a hop's total essential oils content is a predictor of its aroma intensity and quality. Several studies have observed that both pre- and post-harvest factors as well as on the bine ripening time can influence the total essential oil concentration in hops (Bailey et al., 2009; Howard & Slater, 1958; Matsui et al., 2016; Probasco & Murphey, 1996; Sharp et al., 2014). However, Vollmer and Shellhammer (2016) observed that total essential oil is not a great predictor of hop aroma potential during dry-hopping and suggested that the composition of that essential oil might be more important. Further, work has shown that geraniol is a key component for Cascade's aroma (Lafontaine, Pereira, Vollmer, & Shellhammer, unpublished data). Therefore, the harvest factors influencing the composition of hop essential oil may be the main drivers of hop aroma quality.

The composition of hop essential oil is estimated to be made up of over 1000 compounds (Schönberger & Kostelecky, 2011). The volatiles that compose hop essential oil, which have been shown to be important for beer flavor, can be split into three general groups: hydrocarbons (monoterpenes), oxygenated compounds (terpene alcohols), and sulfur-containing compounds (Rettberg, Biendl, & Garbe, 2018; Schönberger & Kostelecky, 2011). Historically, much of the focus on the harvest factors that influence hop oil composition has been on the development of mono- and sesqui- terpenes (mainly β-myrcene, α-humulene, and β-caryophyllene) because they can comprise up to 80% of the essential oil of certain varieties. The work by Wang et al. (2008) provides great insight into the early developmental biosynthetic and enzymatic pathways that drive terpene synthesis in hops (although only 4 weeks after onset of flowering). However, the work by Wang et al. does not investigate the development of other essential aroma analytes important for beer flavor and is limited in describing hops harvested later in the commercial harvest window. Recent studies by Bailey et al. (2009), (on Hallertauer Mittelfrüh), Sharp et al. (2014) (on Cascade and Willamette), and Matsui et al. (2016) (on Saaz) have shown that on the bine ripening time has a significant impact on the monoterpene and terpene alcohol development of hops as well as their potential to influence beer aroma. However, aside from the work performed by Matsui et al. (2016) these studies were limited only to one or two harvest years and do not consider the impact of harvest maturity on sulfur containing compounds.

Due to their extremely low concentrations in beer (ng/L) and in hops (ng/g), the complexity in measuring sulfur containing compounds has limited much of the work performed on these analytes until relatively recently. Numerous studies have identified the presence of thiol precursors and free polyfunctional thiols, mainly 4-methyl-4-mercaptopentan-2-one (4MMP), 3-mercaptohexyl acetate (3MHA), and 3-mercapto-1-hexanol (3MH) in hops and beer (Gros, Nizet, & Collin, 2011; Kishimoto, Kobayashi, Yako, Iida, & Wanikawa, 2008; Reglitz & Steinhaus, 2017; Roland et al., 2016; Takoi et al., 2009). The impact of these compounds on beer flavor and aroma is dependent on their concentrations in beer. Generally, it has been determined that these compounds attribute distinct aromas to beer such as black currant, tropical fruit, and/or catty qualities. Most of the studies that have identified these compounds in hops and beer have largely focused on the impact of hop variety, and there has been very little work done to investigate the impact of hop harvest factors on the concentrations of sulfur-containing components. Kishimoto et al. (2008) showed that 4MMP concentrations in hop varieties (i.e. Perle and Nugget) grown with copper-containing fungicides in Germany had reduced concentrations as compared to the same varieties grown without those fungicides in the U.S. Kammhuber, Hundhammer, and Weihrauch (2017) (on Cascade, Mandarina Bavaria, Hallertau Blanc, Huell Melon and Polaris) identified some sulfur analytes (dimethyl disulfide, *S*-methylthioisovalerate, and *S*-methylthiohexanoate) that might be responsible for the onion garlic note (largely perceived as negative on hop quality) in late harvested hops. However, this study did not consider the impact of harvest maturity and of these analytes on beer aroma.

Recently, Roland, Delpech, and Dagan (2017) suggested that the thiol potency of hops (free thiols *vs* thiol precursor concentrations) might dictate when/how a brewer should add hops into the brewing process to maximize their value and achieve consistent beer flavor. In Sauvignon Blanc wine grapes, thiol precursors (cysteinylated (3-*S*-cysteinylhexan-1-ol (Cys3MH) and 4-*S*-cysteinyl-4-methylpentan-2-one (Cys4MMP)) and glutathionylated precursors (3-*S*-glutathionylhexan-1-ol (G3MH) and 4-*S*-glutathionyl-4-methylpentan-2-one (G4MMP)) have been shown to be impacted by harvest maturity and ripening (Kobayashi et al., 2011; Roland, Vialaret, Razungles, Rigou, & Schneider, 2010). Therefore, this study serves as the first examination into how the thiol precursor and free thiol concentrations are impacted by the harvest maturity of hops.

The goals of this study were to quantify how hop chemical composition (humulones, monoterpenes, sesquiterpenes, terpene alcohols, free thiols, and thiol precursors) changes throughout a commercial harvest window, using hops sampled from the same plot over three harvest years; to evaluate how hop maturity impacts the quality and intensity of aroma that is attributed to beer during dry-hopping; and to consider the role of polyfunctional thiols (4MMP, 3MH and 3MHA) in beer flavor.

2. Materials and methods

2.1. Experimental design

Cascade hops were harvested during the commercial harvest at five to six weekly time points over the 2014 (5 treatments), 2015 (5 treatments), and 2016 (6 treatments) harvests (Table 1). In total, 16 dry-hopped beers were prepared by statically dry-hopping an unhopped beer with ground, whole cone hops shortly (5–8 months) after harvest.

Table 1
Basic hop quality harvest data.

Harvest date	Dry matter (%)	Humulones (%)	Lupulones (%)	H.S.I.[$]	Total essential oil (mL/100 g)
8/14/2014	20.4	5.00	8.30	0.212	0.70
8/21/2014	22.1	4.90	8.50	0.253	1.00
8/27/2014	24.0	5.20	8.20	0.219	1.20
9/12/2014	24.7	4.40	6.85	0.226	2.00
9/22/2014	28.8	5.00	6.00	0.216	1.75
Pearson's r	**0.955**	−0.310	**−0.964**	−0.211	**0.925**
8/11/2015	20.9	4.60	7.30	0.216	0.47
8/18/2015	22.5	5.12	7.62	0.219	1.03
8/25/2015	25.0	5.79	8.00	0.239	1.53
9/2/2015	26.5	5.16	7.56	0.236	1.48
9/9/2015	28.7	4.81	6.82	0.208	2.59
Pearson's r	**0.996**	0.144	−0.381	0.006	**0.946**
8/23/2016	24.9	5.06	5.81	0.256	0.76
8/29/2016	25.9	5.26	6.31	0.261	0.86
9/5/2016	25.7	5.45	7.11	0.277	1.07
9/12/2016	26.5	5.02	6.15	0.286	0.92
9/20/2016	27.4	5.12	6.27	0.284	1.29
9/28/2016	27.0	5.48	6.72	0.289	2.52
Pearson's *r*	**0.914**	0.342	0.391	**0.925**	**0.832**

[*]Pearson's *r* calculated between the harvest date and the given hop quality measurements. Values in bold are significant (p-value < 0.05).

[$] H.S.I. – hop storage index.

Descriptive sensory analysis was used to scale the aroma intensity and quality of these dry-hopped beers as well as the three "unhopped" beer bases used. The impact of harvest maturity on the chemicals that drive the quality and intensity of hop flavor and aroma in beer were investigated. Both non-volatile (humulones and thiol precursor Cys3MH, Cys4MMP, G3MH, G4MMP) and volatile chemical analyses (monoterpenes, sesquiterpenes, and thiols) were performed on the hop samples used for dry-hopping. The impact of hop polyfunctional thiols on beer thiol concentrations was evaluated by measuring the concentrations of thiols in the dry-hopped beers made with the 2014 and 2016 samples.

2.2. Hop collection

A unique harvest maturity sampling protocol was performed at a commercial hop farm in Yakima, WA and yielded 5–6 weekly time points for each of the 2014, 2015 and 2016 commercial harvest windows (Table 1). The whole cone Cascade samples from this farm were collected from a small area (42 hills, 98 strings, covering a two-row section) within a commercial field. Although there were significant differences in the climate between the harvest years, in general, harvest started when dry matter content was ~20%. However, processing constraints limited the ability to pick early harvest samples in 2016 and harvest collection was started at ~24% dry matter content. Typically, Cascades are harvested commercially from 24 to 26% dry matter content. The soil type in this field was Ashue loam with a 0–2% slope and plant spacing of 1.1 × 4.3 m. In 2014 and 2015, 63.5 kg of nitrogen was applied through irrigation using 32–0–0, and in 2016, 56.7 kg of nitrogen was applied through irrigation using 12–3–3–3.8 (sulfur). A border row around this small area was used to protect from wind and other elements. During harvest, ~15 strings were randomly harvested from 15 different hills to ensure that within a weekly sample two strings were never harvested from one hill. At the time of harvest these small samples were kilned to approximately 10% moisture on a pilot-scale electric dryer (62.8 °C), packaged, and shipped to Oregon State University. Upon arrival at Oregon State University, all hops samples were repackaged in high-barrier foil pouches, purged with nitrogen, vacuum sealed, and stored cold (−20 °C) until dry-hopping and chemical analysis.

2.3. Unhopped beer production

To evaluate the dry-hop aroma of the different hop samples, an unhopped beer was prepared by commercial breweries in Portland (Craft Brew Alliance) for the 2014 harvest samples and Bridgeport Brewing for the 2015 and 2016 harvest samples. 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). The starting extract concentrations to evaluate the 2014, 2015, and 2016 harvest samples were 10.9°P, 11.3°P, and 11.1°P, respectively. Fermentation was carried out with Wyeast 1056 ale yeast at 16.7–18.9 °C for the 2014 harvest samples, Wyeast 1728 at 19.4–20 °C was used for the 2015 harvest samples and BridgePort Brewing Company's house yeast strain at 19.4–20 °C was used for the 2016 harvest samples. Following fermentation and clarification, iso-alpha acids (IsoHop, John I Haas, Yakima, WA) were added at a target concentration of 18 mg/L. This resulted in ~40 hL of a 15.4 BU, 4.5% ABV "unhopped" base beer for the 2014 harvest samples, ~55 hL of a 19.8 BU, 4.75% ABV "unhopped" base beer for the 2015 harvest samples and ~52 hL of a 19.0 BU, 4.37% ABV "unhopped" base beer for the 2016 harvest samples. Beer was carbonated and packaged into 60-L stainless kegs, shipped to Oregon State University and held at 2 °C until dry-hopping.

2.4. Dry-hopping protocol and hop preparation

The dry-hopping process established by Vollmer and Shellhammer (2016) has been shown to be reproducible on a 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 for approximately 24 h to 15 °C. For each treatment, 40 L of warmed beer was transferred into two modified 60-L stainless kegs with a 10.2-cm stainless steel opening fitted with a standard Sankey D-system coupler and modified spear (Sabco, Toledo, OH). The hop treatments were dry-hopped at 386 g hop/hL unhopped beer. The whole cone hops were coarsely ground into a hop grist, which was divided up by mass into two mesh bags (EcoBag, Ossining, NY). 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 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. After purging, 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 the dry-hopping events ranged from 13.3 to 15 °C. Dry-hopping was stopped after 24 h because prior work by Wolfe, Qian, and Shellhammer (2012) showed that the extraction of key hop volatiles occurred within 24 h during dry-hopping. 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) (Vollmer & Shellhammer, 2016). Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyzer (Hach, Loveland, CO). Bright beer was not collected until DO was below 110 μg/L. After DO was within specification, bright, filtered beer was collected in a closed 19.6-L stainless steel keg with sufficient back-pressure to reduce foaming. Between each filter run, filter pads were exchanged to prevent carry-over. Filtered beer was stored at 2 °C and under CO_2 overpressure (83 kPa) until sensory evaluation.

2.5. Sensory: descriptive analysis

To evaluate the sensory qualities of the 2014, 2015, and 2016 harvest samples, 3 descriptive analysis panels were used to quantify perceived hop intensity and quality of the dry-hopped beers. The general approach used trained panelists, who were selected based on previous experience and relevance, to scale only the orthonasal aroma of the beer treatments. Intensive training sessions on commercial samples (Sup. Table 1) and a random set of blind coded dry-hop treatments for each of the harvest years were completed in advance of data collection to develop a relevant lexicon of sensory attributes, establish a scale that best explained the differences in the samples, and to train panelists to use external reference samples as anchors for the most salient attributes. During each session, the panelists had access to external reference samples that 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. The external references and attributes used to evaluate the different harvest samples are outlined in Supplementary Table 1. Due to the seasonal nature of commercial beer production and panel feedback, the same commercial beers and rankings were unable to be used throughout the entire three years of the study. This change in references could have impacted how the panelists were assessing the beers on a year to year basis but is not expected to have had a major impact on the results observed. Panelists were given ~60 mL of dry-hopped beer in a 300-mL glass covered with a plastic lid. For the 2014 Cascade harvest samples beer was packaged and served from bottles that had been warmed to room temperature for 35–45 min. For the rest of the harvest samples beer was served from two 8-head draft systems (Micro Matic, Northridge, CA) into pitchers at ~1 °C and at 82.7 kPa. Beer was poured into sample glasses ~1 h before the start of testing and allowed to warm to room temperature. For

the 2014 Cascade harvest samples panelist responses were collected on paper ballots. For the other harvest samples panelist responses were collected on Chromebook tablets using Qualtrics (Provo, UT). For each of these sessions, Qualtrics was also used to randomly assign the serving order of samples for each panelist. More in-depth details of each descriptive analysis panel, including the differences in how the descriptive analysis panels were carried over the different harvest years, can be found in the Supplementary information.

2.6. Beer and hop chemical analysis

Concurrent with the hop sampling for the dry-hopping, approximately 150 g of the homogenized hop grist were taken for chemical analysis. Beer was stored (< 4 months) in bottles in 2014 and in 19.6-L kegs in 2015 and 2016 at ~1 °C until analysis.

2.7. Non-volatile hop analysis

During harvest, % dry matter of the hop cones was determined by drying ~100 g at 56 °C for 12–14 h. % dry matter was determined by the following formula: (dry cone weight/green cone weight)*100 = dry matter. The total concentration of humulones and lupulones as well as hop storage index (H.S.I.) were determined by ASBC – 6A α-and β-Acids in Hops by Spectrophotometry (ASBC Methods of Analysis). Briefly, 5 g of ground hops were extracted in 100 mL of toluene for 30 min. This extract was then centrifuged and 5 mL of the clarified toluene extract were added to 100 mL of alkaline methanol. The absorbance of this solution was then determined at 275, 325, and 355 nm. H.S.I. is a measure of hop oxidation (or % humulones lost) and is the ratio of the absorbance maximum of hop oxidation products (275 nm) to the absorbance maximum of humulones (325 nm).

2.8. Hop essential oil analysis-reagents and standards

β-Myrcene, β-pinene, linalool, geraniol, citral, limonene, geranyl acetate, α-pinene, nerol, isobutyl isobutyrate, methyl heptanoate, β-caryophyllene, α-humulene, β-farnesene, and caryophyllene oxide were obtained from Sigma-Aldrich (St. Louis, MO). 2-Octanol was obtained through Alfa Aesar (Haverhill, MA). Hexanes purchased from J.T. Baker (Center Valley, PA) were redistilled to remove impurities before analysis.

2.9. Hop essential oil analysis

At the time of dry-hopping, hydrodistillation was performed to determine the total oil content of the homogenized hop grist using ASBC Hops-13 (ASBC Methods of Analysis). In brief, ~105 g of coarsely ground hops were boiled in 3 L of distilled water for 3 h. Post-distillation, the total oil content was recorded and the 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.

In 2014, hop oil compositional analysis was performed under modified conditions from ASBC Hops-17 (ASBC Methods of Analysis). In 2015 and 2016 hop oil compositional analysis was performed using previously published methodology (Lafontaine & Shellhammer, 2018; Sharp, Qian, Shellhammer, & Shellhammer, 2017) using a HP 6890 gas chromatograph with an Agilent 5972a mass spectrometer (GC–MS) under modified conditions from ASBC Hops-17. In brief, a 1% 2-octanol (8190 ppm) 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 (1:20). The analytical column was a 30 m × 250 μm × 0.25 μm Zebron ZB-1 MS (Phenomenex, Torrance, CA) 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 set up to detect ions with a mass-to-charge ratio (*m/z*) of 30–350. Four-point calibration curves (50, 100, 400, and 800 ppm) were created for all target analytes. For high concentration target analytes (β-myrcene, α-humulene, β-caryophyllene, and β-farnesene) three additional calibration points were added (1000, 5000, and 9000 ppm). Target analytes were quantified using the following ions for each analyte: *m/z* 41 (geranial), *m/z* 45 (2-octanol), *m/z* 69 (β-farnesene, geraniol, nerol, neral, and geranyl acetate), *m/z* 71 (isobutyl isobutyrate and linalool), *m/z* 74 (methyl heptanoate), *m/z* 79 (caryophyllene oxide) and *m/z* 93 (α-pinene, β-pinene, 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. A total ion chromatogram is provided in the Supplementary information (Sup. Fig. 1).

2.10. Free thiol and thiol precursor analysis in hops and beers-reagents and standards

The following reagents and standards were used for free thiol and thiol precursor measurements performed by Nyseos in hops and beers. All analytical solvents were purchased from Biosolve (Dieuze, France) and analytical reagents were purchased from Merck (Saint Quentin Fallavier, France). 3MH and 3MHA were purchased from Merck (Saint Quentin Fallavier, France), whereas the other analytical standards were synthesized by Nyseos according to previously published methods (Fedrizzi, Pardon, Sefton, Elsey, & Jeffery, 2009; Roland, Schneider, Razungles, Le Guernevé, & Cavelier, 2010). Briefly, thiol precursors were synthesized by a Michael addition of glutathione or Boc-Cys-OH on either hexenal or mesityl oxide to afford the corresponding glutathionylated and cysteinylated precursors of 3MH and 4MMP, respectively. For the labeled analytical standards, hexyn-1-ol was deuterated using Lindlar's catalyst then oxidized under mild conditions with manganese dioxide to afford hexenal-d_2 (Roland, Schneider, Le Guernevé, Razungles, & Cavelier, 2010), that was directly used to synthesize G3MH-d_2 and Cys3MH-d_2. The labeled mesityl oxide was purchased from Merck (Saint Quentin Fallavier, France) and used to synthesize G4MMP-d_6 and Cys4MMP-d_6.

The following reagents and standards were used for free thiol measurements in beer performed by Asahi Brewing Company Ltd. (Moriya, Ibaraki Prefecture, Japan). 4MMP, 3MH, and 3MHA were purchased from Penta Manufacturing Co. (Livingston, NJ). d10-4-methyl-4-mercapto-2-pentanone (d10-4MMP) was purchased from aromaLAB AG (Freising, Germany). d2-3-mercapto-1-hexanol (d2-3MH) was purchased from NARD Institute, Ltd. (Hyogo, Japan). ETP and tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) solution (1 M, pH 9.0) were purchased from Wako Pure Chemicals (Osaka, Japan). Sodium hydroxide (NaOH) solution (1 N) and ethanol (pesticide residue analysis grade) were purchased from Kanto Kagaku (Tokyo, Japan).

2.11. Free thiol and thiol precursor analysis in hops

Free thiol and thiol precursor analysis was performed by Nyseos (Montpellier, France) on ground hops. Thiol precursors were analyzed in hops by stable isotope dilution assay (SIDA) and nanoLC-MS/MS as previously reported (Roland et al., 2010; Roland et al., 2016). In brief, ground hops (200 mg) were extracted for 1 h at room temperature in an ethanolic mixture (11% ethanol; 4 g/L of tartaric acid; pH = 3.5), centrifuged, and supernatant was spiked with labeled internal standards (G3MH-d_2, Cys3MH-d_2, Cys4MMP-d_6, and G4MMP-d_6) before being analyzed by nanoLC-MS/MS under multiple reaction monitoring mode (MRM). The LOD, LOQ and MS/MS conditions for the methodology

were reported previously (Roland et al., 2016) and the repeatability statistics were reported previously (Roland et al., 2010). The limits of detection (LODs) for free thiols were 0.1–0.5 μg/kg and for thiol precursors were 0.5–19 μg/kg. Chromatograms of the free and thiol precursors are provided in the Supplementary information (Supplementary Figs. 2 and 3).

2.12. Free thiol analysis in beer

Free thiols (4MMP, 3MH, and 3MHA) in the dry-hopped beer made from the 2014 hop samples were measured by Asahi Brewing Company Ltd. (Moriya, Ibaraki Prefecture, Japan) using stir bar sorptive extraction with *in situ* derivatization (der-SBSE) using ethyl propiolate (ETP), followed by thermal desorption and gas chromatography–tandem mass spectrometry (TD–GC–MS/MS) with selected reaction monitoring (SRM) mode using published methodology (Ochiai, Sasamoto, & Kishimoto, 2015). In brief, beer samples were adjusted to pH 9 using NaOH solution (1 M). A total of 10 mL of sample containing 35 mM ethyl propiolate (ETP), internal standards (20 ng/L d10-4MMP and 200 ng/L d2-3MH), and the PDMS stir bar was transferred to 20-mL headspace vials. The vial was sealed with the metal screw cap, and the PDMS stir bar was first stirred at room temperature (25 °C) for 10 min at 500 rpm for the ETP derivatization step (Herbst-Johnstone, Piano, Duhamel, Barker, & Fedrizzi, 2013). After 10 min of stirring, 30% NaCl was added and SBSE was performed for 180 min while stirring at 1500 rpm. The stir bars were thermally desorbed by programming the thermal desorption unit (TDU) from 30 °C (held for 0.5 min) to 200 °C (held for 3 min) at 720 °C/min with 50 mL/min desorption flow. Desorbed compounds were focused at 10 °C on a liner packed with quartz wool in the Peltier-cooled PTV inlet for subsequent TD–GC–QQQ–MS analysis. The column temperature for the DB-Wax was programmed from 100 °C (held for 3 min) to 250 °C (held for 11 min) at 10 °C/min. The QQQ–MS was operated in three acquisition modes: (1) scan mode at a mass range of m/z 29–500, (2) product ion scan mode at a mass range of m/z 29–500, and (3) SRM mode with the selected transitions (precursor to product ion). Transitions of the analytes, LODs, LOQs, and repeatability statistics are listed in (Ochiai et al., 2015). The limits of detection (LODs) ranged from 0.19 to 27 ng/L.

Free thiols (4MMP, 3MH, and 3MHA as well as 3MH disulfides) in the dry-hopped beer made from the 2016 hop samples were measured by Nyseos using published methodology. Both 3MH and 3MHA were analyzed in beer by stable isotope dilution assay and nanoLC-MS/MS as previously described (Fedrizzi et al., 2009; Roland et al., 2016). Briefly, beer sample (1 mL) was spiked with internal standards (3MH-d_2 and 3MHA-d_5) and then derivatized using ammonium bicarbonate buffer (1 M, 300 μL) and *N*-phenylmaleimide solution (25 mM; 120 μL). After quenching with ice acetic acid (200 μL), samples were purified by SPE (Bond Elut Plexa Cartridge, 200 mg), and then analyzed by nanoLC-MS/MS in MRM mode as previously detailed (Fedrizzi et al., 2009; Roland et al., 2016). The limits of detection (LODs) ranged from 0.9 − 2.8 ng/L. For the 3MH disulfides, the reduced form was measured as previously described (Roland et al., 2016). Chromatograms of the free thiols and 3MH disulfides are provided in the Supplementary information (Sup. Fig. 2).

2.13. Statistical analysis

Two-way analysis of variance with a mixed model (including the factors panelist, sample, and replication, as well as corresponding two-way interactions), Pearson correlation analysis, multiple comparison analysis (Tukey's HSD), principal component analysis and graphical construction were carried out using XLSTAT 2017 (Addinsoft, New York, NY). These tests and graphical outputs 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.

3. Results and discussion

3.1. Descriptive analysis: panelist/panel evaluation and the impact of harvest maturity on dry-hop aroma intensity and quality

Following each descriptive analysis panel over the three harvest years, each panelist was evaluated on their performance based upon their ability to discriminate differences between the dry-hop treatments and unhopped control within each harvest year on at least one of the sensory attributes, replicate among all sessions, and lack of interactions. Any panelists that failed these three criteria were removed from further analyses. One panelist was removed from the 2014 panel, one panelist was removed from the 2015 panel and four panelists were removed from the 2016 panel, resulting in descriptive analysis panels that contained 10, 10, and 8 panelists, respectively, over the three harvest years.

Two-way ANOVA using a mixed model was performed on the attributes for each of the remaining panelists (Sup. Table 2). In general, significant sample effects were observed across the attributes and indicated that the panelists were able to detect significant differences between the samples. Significant panelist × sample effects were also observed for most of the attributes (mainly Overall Hop Aroma Intensity (OHAI), Citrus and Herbal/Tea). Significant panelist × sample effects are common in sensory analysis and indicate that there were slight differences in the way the panelists scaled those attributes (Meilgaard, Carr, & Civille, 2007). No significant effect of replication or interactions between panelist and replication or between sample and replication were observed for the OHAI and Citrus attributes. Minor significant interactions were observed, primarily the Sample × Rep effect on the Herbal/Tea attribute for the 2015 panel and the Panelist × Rep effect for the Herbal/Tea and Tropical/Catty attributes for the 2016 panel. These interactions indicate that from one session to another, the panelist scores were not consistent for all the products. With these few exceptions, the panelists could effectively replicate their attribute scaling for most of the qualitative descriptors across all replications for each of the samples. Furthermore, the responses provided for a given panelist did not depend on replication.

The least squared means and results from Tukey's HSD ($p < 0.05$) for the sensory attributes from the descriptive analysis panel on the dry-hop treatments were summarized (Table 2). In general, OHAI and Citrus flavor increased as a function of harvest maturity. This result suggests that that hops picked later in the harvest window attributed significantly more aroma to beer during dry-hopping and that the quality of this aroma was primarily perceived to be citrusy. Similar observations were made by Bailey et al. (2009) in beers that were kettle, whirlpool, and dry-hopped with Hallertauer Mittelfrüh of different harvest maturities from the same location. In that study, beers made with the later picked Hallertauer Mittelfrüh had higher hoppy aroma and flavor intensities. In the study by Bailey et al., the beers made with later picked Hallertauer Mittelfrüh were also rated better for aroma and flavor using a modified German Agricultural Society (DLG) tasting scheme. Matsui et al. (2016) found that beers that were kettle and whirlpool hopped with later harvested Saaz had stronger hoppy aroma intensities. Inui et al. (2016) also found that harvest timing had a significant impact on the floral, citrusy, and fruity aroma characteristics that Saaz hops attributed to hop teas. However, in these studies, hoppy aroma intensity and quality was also dependent on farm location. This indicates that growing conditions and/or post-harvest processing conditions also have a significant impact on hoppy aroma potential.

Sharp et al. (2014) observed that beers that were kettle hopped (at 1.45 g/hl) and whirlpool hopped (at 5.5 g/hL) with Cascade hops harvested at a typical time on a commercial farm had higher overall likings in a consumer panel when compared to beers made with later picked Cascade. The later picked Cascades in this study attributed higher pine,

Table 2
Summary of least squared means for the sensory attributes resulting from descriptive analysis as a function of harvest maturity.

Harvest date	OHAI[a]	Citrus	Herbal	Tropical Fruit	Resinous/Hop Oil	Tropical/Catty
8/14/2014	7.5 b	3.0 a	3.6 ab	3.4 a	3.2 b	–
8/21/2014	7.9 ab	3.4 a	3.6	4.2 a	3.4 b	–
8/27/2014	8.1 ab	3.7 a	2.8 b	3.8 a	4.2 ab	–
9/12/2014	8.7 ab	3.6 a	4.1 a	4.9 a	3.6 b	–
9/22/2014	8.9 a	3.7 a	4.4 a	3.8 a	4.9 a	–
Pearson's r	**0.990**	0.758	0.678	0.421	0.711	
Harvest date	**OHAI**	**Citrus**	**Herbal/Tea**			
8/11/2015	6.7 b	4.3 b	5.0 a	–	–	–
8/18/2015	7.2 b	4.2 b	5.3 a	–	–	–
8/25/2015	8.5 a	5.8 a	6.0 a	–	–	–
9/2/2015	7.6 ab	5.4 a	5.1 a	–	–	–
9/9/2015	8.3 a	6.4 a	5.6 a	–	–	–
Pearson's r	0.744	**0.888**	0.368			
Harvest date	**OHAI**	**Citrus**	**Herbal/Tea**	**Tropical/Fruity**	**Pine/Resinous/Dank**	**Tropical/Catty**
8/23/2016	4.3 d	2.1 e	2.3 d	1.5 d	1.2 d	0.5 c
8/29/2016	4.6 d	2.7 de	2.8 bc	1.7 d	1.5 cd	1.1 b
9/5/2016	6.8 b	4.3 b	3.5 ab	2.5 b	2.5 b	1.1 b
9/12/2016	6.0 bc	3.5 bc	3.2 bc	2.3 bc	2.0 bc	1.3 b
9/20/2016	5.7 c	3.4 cd	3.1 bc	1.9 cd	1.6 cd	0.9 bc
9/28/2016	9.4 a	6.1 a	4.0 a	3.2 a	3.6 a	2.3 a
Pearson's r	**0.818**	**0.817**	**0.828**	0.766	0.726	0.766

Letters indicate statistically significant groupings (Tukey's HSD tests p-value < 0.05) (–) did not measure.
*Pearson's r calculated between the harvest date and the given sensory attribute. Values in bold are significant (p-value < 0.05).
[a] Overall hop aroma intensity.

melon, and floral notes. It is important to note that this study did not utilize dry-hopping, and for reasons that will become apparent later in the discussion, early, or typical harvested Cascade may be better suited for kettle and whirlpool hopping than dry-hopping.

3.2. Influence of harvest maturity on concentrations of humulones and lupulones, total essential oil and % dry matter

Most commercial hop farmers use percent dry matter as an indicator of when to harvest. In general, commercial farms in the USA typically start to harvest Cascade at ~24–25% dry matter. Over the three harvest years, dry matter increased as a function of harvest maturity as expected (Table 1). Similar observations were made by Probasco and Murphey (1996), albeit in different hop varieties (Mt. Hood, Nugget, Galena, and Willamette). This increase in dry matter has a direct impact on a farmer's yield. Therefore, from a grower's perspective, it is key to maximize dry matter without sacrificing cone quality, structure, and/or the pickability of hops. It has been estimated that for every 1% increase in dry matter the increase in yield will be ~90 lb/acre on a variety averaging 2000 lb/acre (or ~100.8 kg/ha on a variety averaging 2240 kg/ha) (Probasco & Murphey, 1996).

When considering the development of the non-volatile fraction and the analytes that impact the bittering potential of hops during harvest, in general, concentrations humulones and lupulones as well as H.S.I. (Table 1) were not dependent on the date harvested throughout the commercial harvest window. The concentration of these compounds plateaued prior to the harvest window and stayed roughly constant throughout harvest. Similar findings were made by Sharp et al. (2014) (in Cascade) and have been seen in other varieties as well (Howard & Tatchell, 1956; Matsui et al., 2016). Other factors, such as the year-to-year growing conditions, are considered to have a larger impact on the concentrations of humulones as compared to on the bine ripening. Also, the optimal harvest timing window for the concentrations of humulones is varietal specific and some hop varieties have been shown to increase concentrations of humulones over harvest with on the bine ripening (Bailey et al., 2009; Probasco & Murphey, 1996).

Historically, total essential oil content has been viewed as an indicator of hop aroma potential in beer. During cone production, the development of total essential oil is delayed in relation to the production of hop acids and has been shown to increase over the commercial harvest window for a number of varieties (Howard & Slater, 1958; Howard & Tatchell, 1956; Matsui et al., 2016; Sharp et al., 2014). Differences from this trend are expected to be a result of the post-harvest processing (for instance kilning parameters) along with storage conditions.

Over the three harvest years, total essential oil content significantly increased as a function of harvest date (Table 1). Recently, Vollmer and Shellhammer (2016) showed that total essential oil content is not an effective predictor of hop aroma potential and suggested that individual components of hop essential oil might yield a better predictor of hop aroma potential. Therefore, consideration of the development of the different hop volatiles throughout the harvest window is important. Although OHAI and Citrus quality increased as total essential oil content increased over the three harvest years (Fig. 1), it is likely that the compositional development of this oil is leading to the sensorial changes observed among the samples to a greater degree than total oil content.

3.3. Effect of harvest maturity on the composition of hop essential oil

Although there are some slight differences between the three harvest years, in general, 11 of the 16 hop volatiles (isobutyl isobutyrate, α-pinene, β-pinene, β-myrcene, methyl heptanoate, limonene, linalool, neral, geraniol, β-caryophyllene, and α-humulene) increased with on the bine ripening time over harvest (Sup. Table 3). Similar to other studies (Howard & Slater, 1958; Sharp et al., 2014), the major hydrocarbon fraction (β-myrcene, β-caryophyllene, and α-humulene) significantly increased over harvest. Although these compounds make up a significant portion of hop essential oil (> 50%), their physicochemical properties make them unlikely contributors to beer flavor (Rettberg et al., 2018). Although these analytes are easy to measure and may help distinguish between varieties (Probasco & Murphey, 1996), they are of little importance to predicting beer flavor during dry-hopping.

There is evidence that suggests monoterpene alcohols play a

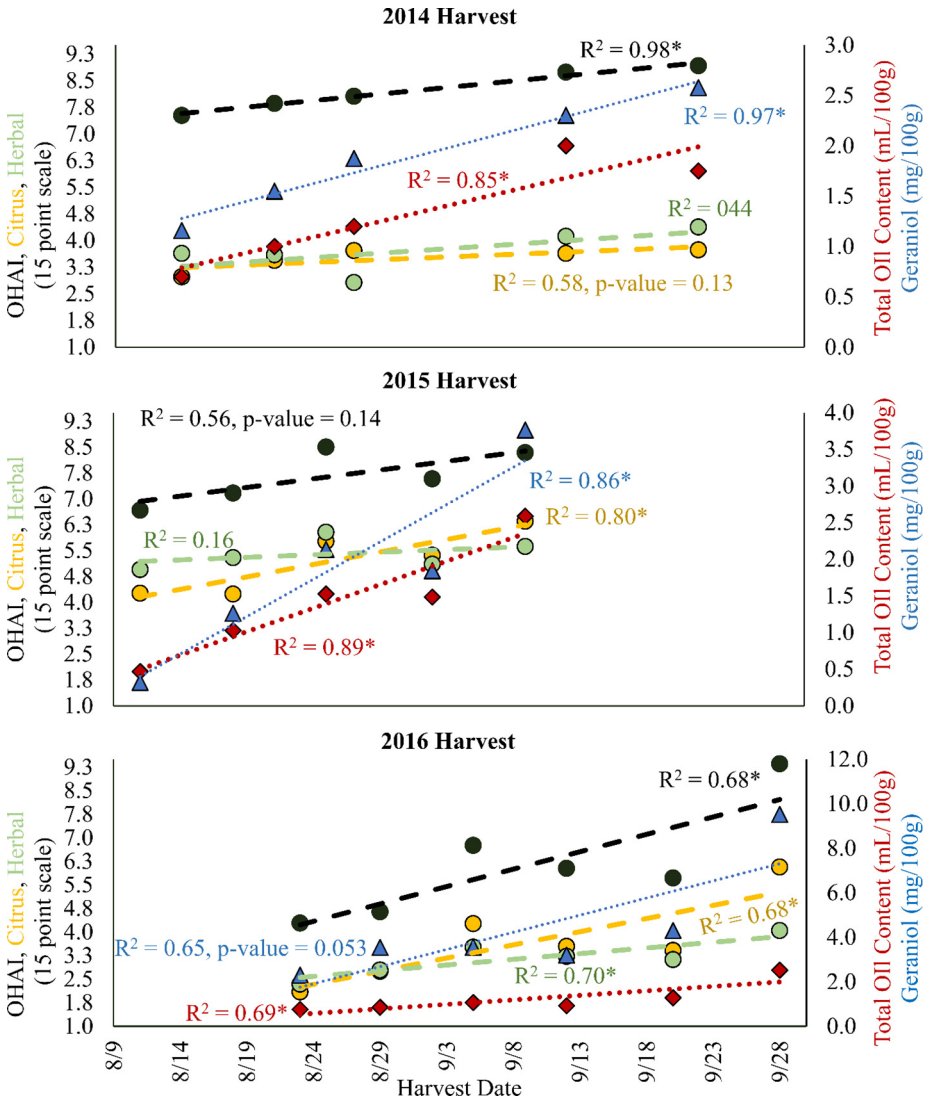

Fig. 1. The impact of harvest maturity on Cascade quality as described by total oil content (mL/100 g) (◆), geraniol (mg/100 g hop) (blue triangle) as well as dry-hop aroma intensity (●) and dry-hop aroma quality (Citrus (●) and Herbal (●)) over the 2014 (n = 5), 2015 (n = 5), and 2016 (n = 6) harvests. *Pearson correlation coefficient significantly different from 0, p-value < 0.05. OHAI = Overall Hop Aroma Intensity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significant role in hoppy beer flavor (Inui, Tsuchiya, Ishimaru, Oka, & Komura, 2013; Kishimoto, Wanikawa, Kono, & Shibata, 2006; Lafontaine & Shellhammer, 2018; Lafontaine, Vollmer, & Shellhammer, 2018; Takoi et al., 2010). Over the three harvest years, the monoterpene alcohols, geraniol and linalool, were found to significantly increase with on the bine ripening time during harvest. Similarly, Sharp et al. (2014) observed that linalool concentrations increased between early, typical, and late harvest Cascades, but that geraniol concentrations peaked in typical harvested Cascades. When considering other hop varieties, Bailey et al. (2009) (on Willamette) and Matsui et al. (2016) (on Saaz) found that linalool and geraniol concentrations significantly increased with on the bine ripening time. Again, although there were some differences between the harvest years, OHAI and Citrus quality were also positively correlated to geraniol concentrations (Fig. 1). These observations highlight the importance of harvest maturity in producing the highest quality Cascades for dry-hopping. Yet harvest maturity/timing must be balanced against the risk of hop cones shattering during harvest and/or post-harvest processing. There is a limit to how late a farmer can pick hops without suffering a dramatic decrease in harvest yield and/or lupulin loss (Sharp et al., 2014).

3.4. Impact of harvest maturity on thiol precursor and free thiol concentrations

The influential role that polyfunctional thiols (3MH, 3MHA, and 4MMP) have on beer aroma has been highlighted in several papers (Gros et al., 2011; Kishimoto et al., 2008; Kishimoto, Morimoto, Kobayashi, Yako, & Wanikawa, 2008; Reglitz & Steinhaus, 2017; Roland et al., 2016; Takoi et al., 2009). These thiols can exist in hops as thiol precursors and as free thiols (Roland et al., 2017; Roland et al., 2016). The form thiols exist in hops may influence how a brewer uses those hops during the brewing process to maximize their potential. Hops with higher thiol precursor concentrations are recommend for use in the kettle and whirlpool, as thiols can be liberated from these precursors during fermentation via yeast β-lyase activity. Hops with higher free thiol concentrations should be used for dry-hopping as they contain greater quantities of free volatiles that can be extracted during this cold extraction process.

Unfortunately, there is no published data on how these analytes develop in hops (or more broadly in flowers) during harvest and how this might impact beer aroma. In general, over the three harvest years it was observed that free thiol concentrations (mainly 3MH) significantly increased with harvest date (Sup. Table 4) and were significantly positively correlated to OHAI and Citrus quality for each of the three harvest years. Notably, 3MHA was not detected in the ground hop material, which is supported by (Kishimoto et al., 2008), but was quantified in distilled hop oil (data not shown). It should be noted that the extraction of hop essential oil *via* hydrodistillation has been shown to lead to artifact formation in the compositional analysis of essential oils (Rettberg, Thörner, & Garbe, 2012). In comparison, thiol precursors

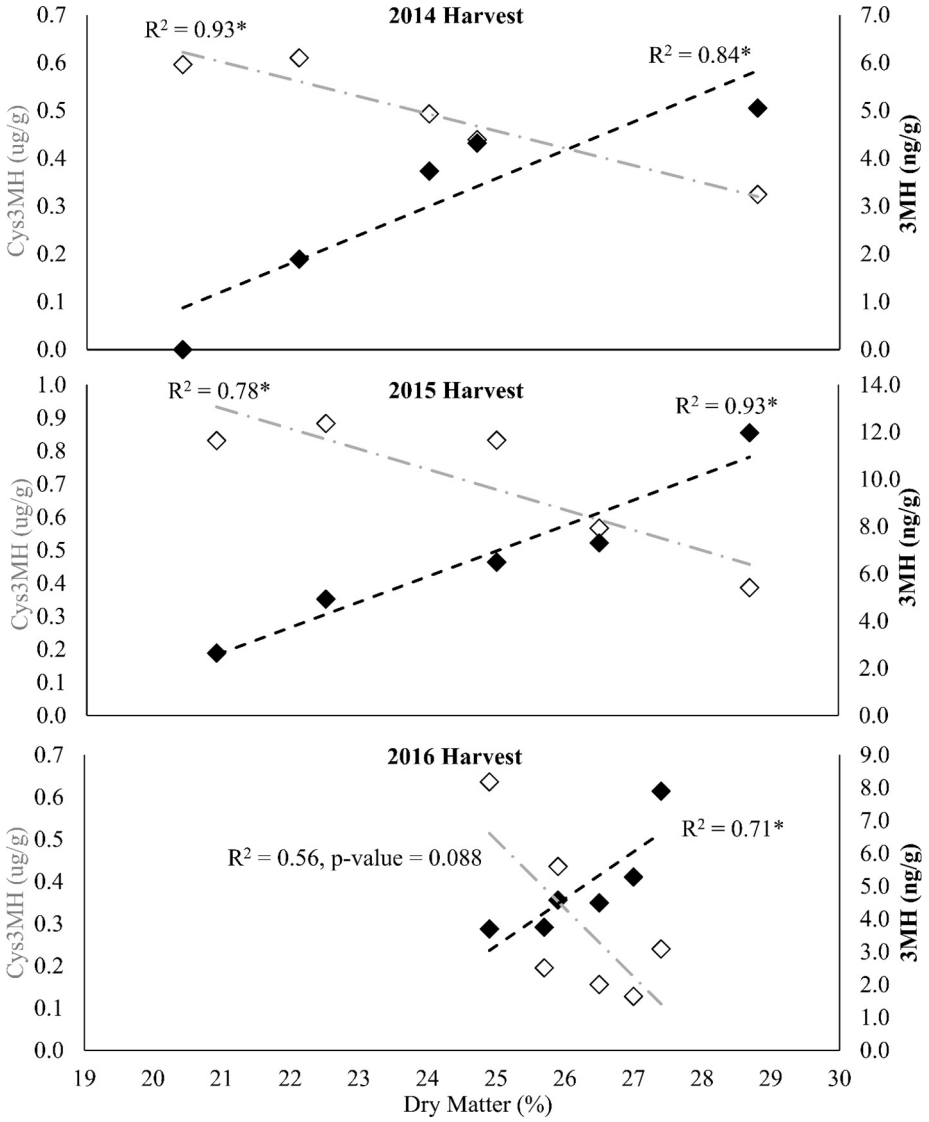

Fig. 2. Dry matter (%) *vs* Cys3MH (μg/g) (◊) and 3MH (ng/g) (♦) concentrations. *Pearson correlation coefficient significantly different from 0, *p*-value < 0.05.

(mainly Cys3MH) significantly decreased as a function of harvest date (Sup. Table 5). In general, the concentrations of thiol precursors were also negatively correlated to OHAI and Citrus quality over the three harvest years. Although the concentrations of free thiols and thiol precursors differed between harvest years, it is clear, at least on this farm, that early harvest Cascade hops had higher thiol precursor concentrations and were better suited for kettle and/or whirlpool additions, while later harvested Cascade hops were higher in free thiol concentrations and might be better suited for dry-hopping additions.

Interestingly, the development of these analytes during on bine maturation in hops was opposite that found for Sauvignon Blanc grapes, where the concentrations of thiol precursors reached a maximum later in the harvest window (Kobayashi et al., 2011; Roland et al., 2010). This suggests that the development of these thiol precursors differs between the maturation of flowers and fruits. Interestingly, the concentrations of cysteinylated 3MH were significantly negatively correlated to dry matter in hops, while the concentrations of free 3MH were positively correlated to dry matter (Fig. 2). Although the concentrations of these analytes were different over the three harvest years for similar dry matter content, later harvested Cascades (with > 25–26% dry matter) would be better suited for dry-hopping because there was a higher amount of terpene alcohols and free thiols. Conversely, earlier harvested Cascades (dry matter content 20–24%) contained high thiol precursor concentrations and should be added to the kettle or whirlpool. This is because these additions occur before primary fermentation and would allow yeast β-lyase activity to liberate the free thiol from the precursor during fermentation and lead to increased aroma in beer.

The following biosynthesis for *S*-cysteine conjugates of 3MH and 4MMP was proposed by Wüst in Sauvignon Blanc grapes (Kammhuber et al., 2017). A glutathione-cysteine conjugate of a polyfunctional thiol is created when glutathione transferase reacts with glutathione and an unsaturated α, ß-unsaturated carbonyl compound. The GS-X glutathione conjugate pump then actively transports this glutathione-cysteine conjugate into the cell vacuole, where a peptidase in the vacuole further cleaves the glutathione moiety and yields the specific *S*-cysteine conjugate. In beer the only proposed release of thiols from these cysteine-conjugates is believed to be a result of yeast β-lyase activity during fermentation, although it is possible that enzymes derived from molds grown on the surface of hops could have impacted free thiol and thiol precursor concentrations. Due to the significant rise in concentration of free thiols and decrease in thiol precursor concentrations throughout ripening over the three harvest years, it is more likely that there may be an enzymatic pathway in hops which drives the conversion of 3MH thiol precursors to free 3MH. Identifying this pathway could be a useful tool for hop breeders and may help explain some of the varietal differences observed in the concentrations of these analytes.

In grapes, concentrations of 4 MMP precursors are found to be equally distributed between the berry skin and pulp, while the precursors of 3MH are detected at concentrations 8× higher in the berry skin as compared to in the pulp (Peyrot des Gachons, Tominaga, & Dubourdieu, 2002; Roland et al., 2011). Due to the non-selective thiol analysis approach used in this study (i.e. analysis of ground hop cones), the location of the thiol precursors and free thiols within the hop cone (i.e. lupulin gland, strig, and/or bract) is unclear. However, the location of these analytes within the cone could have an influence on their concentration during the post-harvest processing of whole hops into concentrated lupulin powders, pellets, and extracts. Further examination of the occurrence of these analytes within the hop cone is of importance to the hop processing industry.

3.5. Investigating the influence of harvest maturity on hop quality using a multivariate approach

Principal component analysis (PCA) was performed on the correlation ($n-1$) matrix of the mean sensory scores for the dry-hop treatments, % dry hop matter, total oil content, as well as the concentrations of humulone, lupulone, free thiols, thiol precursors, and essential oil components (Fig. 3). The first three principal components explained 78.8% of the variation within the data set, with PC1 accounting for 39.8% and describing the harvest date, PC2 accounting for 22.4%, and PC3 accounting for 16.6%. Moving from left to right in the biplots across PC1 shows that as hops were harvested later, the beer sensory attributes, dry matter, total oil content, a majority of the hop essential oil volatiles, and the free thiol concentrations increased. Concentrations of thiol precursors followed an opposite trend. There are also trends between the harvest years, with the samples from 2016 occurring at the top of the biplot (PC2 in Fig. 3a), followed by the 2014 samples, then the 2015 samples. It is not surprising that different growing seasons led to Cascade hops that were chemically different and attributed different aroma profiles during dry-hopping. Similar observations were made in (Forster & Gahr, 2014; Van Holle, Van Landschoot, Roldán-Ruiz, Naudts, & De Keukeleire, 2017). Although there were significant differences between the harvest years, harvest maturity and on the bine ripening time had the largest impact on the development of a majority of the hop volatiles as well as the aroma intensity and quality that the hops attributed to beer during dry-hopping.

3.6. Hop thiol concentrations influencing beer thiol concentrations

The concentrations of only 4MMP, 3MHA, and 3MH were

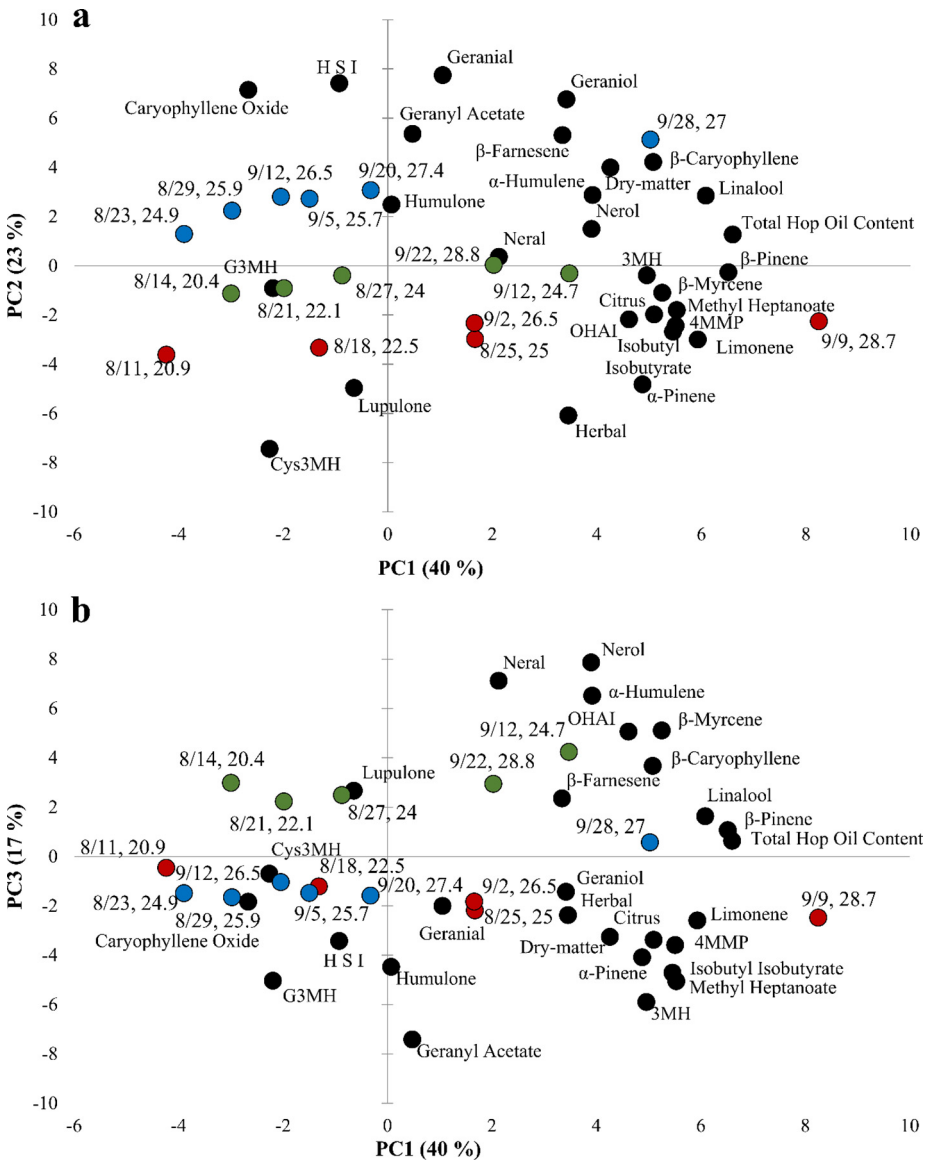

Fig. 3. Principal component analysis of the mean scores of the sensory attributes and hop quality chemical analyses (●) among the (16) dry-hop harvest treatments over the 2014 (●), 2015 (●), and 2016 (●) harvests. (a) Biplot of PC1 *vs.* PC2 explaining 63% of the variation in the data (b) biplot of PC1 *vs.* PC3 displaying an additional 17% of the variation in the data set. The treatment codes represent the (harvest date, dry-matter %). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Impact of harvest maturity on free beer thiol concentrations.

Harvest date	Dry matter (%)	3MH (ng/L)	3MH disulfides (ng/L)	4MMP (ng/L)	3MHA (ng/L)
8/14/2014	20.4	124.6	–	6.7	3.8
8/21/2014	22.1	97.9	–	7.6	3.3
8/27/2014	24	107.9	–	10.4	13.8
9/12/2014	24.7	141.2	–	5.8	9.3
9/22/2014	28.8	108.5	–	6.8	15.3
Pearson's *r* with harvest date		0.187		−0.306	0.755 ($p = 0.14$)
Pearson's *r* with dry matter		−0.069		−0.083	0.857 ($p = 0.06$)
8/23/2016	24.9	72.9	186.4	n.d.	2.1
8/29/2016	25.9	66.5	179.4	n.d.	3.1
9/5/2016	25.7	79.7	119.7	n.d.	3.2
9/12/2016	26.5	83.7	184.5	n.d.	4.8
9/20/2016	27.4	80.3	170.2	n.d.	4.9
9/28/2016	27	74.7	124.2	n.d.	4.8
Pearson's *r* with harvest date		0.447	−0.481		**0.916***
Pearson's *r* with dry matter		0.401	−0.208		**0.952***

* Values in bold are significant (p-value < 0.05); (–) did not measure; (n.d.) not detected.

considered in the dry-hopped beer (Table 3). As discussed previously, there are several studies that highlight the importance of terpene alcohols in hoppy beer flavor (Inui, Tsuchiya, Ishimaru, Oka, & Komura, 2013; Kishimoto et al., 2006; Lafontaine et al., 2018; Takoi et al., 2010). Although no 3MHA was detected in the ground hop material, detectable concentrations of 3MHA were found in the dry-hopped beer. Observations by Kishimoto et al. (2008) were similar and demonstrated that 3MHA increased during fermentation, and they proposed that 3MHA release could be yeast strain dependent and beers with higher 3MHA should have higher aroma. However, in this study, only clarified and fully attenuated beer was dry-hopped at ~386 g hop/hL with the different harvest samples. Therefore, the potential of biotransformation by yeast was not expected and it is possible that something other than yeast, such as hop-derived enzymes, may also drive the conversion of 3MH to 3MHA during dry-hopping. While no strong correlations were found between the beer sensory scores and the concentrations of 3MHA in beer, the threshold for 3MHA (5 ng/L) is 10× lower than that of 3MH (55 ng/L) (Kishimoto et al., 2008), suggesting that concentrations of 3MHA should have a higher impact on beer aroma.

No clear trends were observed between the concentrations of 4MMP or 3MH in beer with harvest date, % dry matter, or the beer sensory attributes. The concentrations of disulfide-bound 3MH was also investigated as a potential aroma reservoir in beer, as it has been highlighted as an important reservoir for wine aroma (Roland et al., 2016). These compounds are an important indicator of the oxidation state for 3MH and it was observed that 60% of the total 3MH was oxidized in the dry-hopped beers (Table 3). Although the impact of 3MH disulfides on overall beer aroma remains unclear, this indicates that even under relatively low dissolved oxygen conditions during dry-hopping (< 110 μg/L), oxidation always occurs and can lead to challenges when trying to identify the impact of polyfunctional thiols on beer flavor. To fully elucidate the impact of polyfunctional thiols on beer flavor, one must evaluate the concentrations of thiol disulfides, thiol precursors and free thiols in relation to each other. This is because there are a number of possible avenues that may influence the impact thiols have on beer flavor such as the direct extraction of free thiols from hops during dry-hopping, the chemical release of free thiols from thiol precursors during dry hopping (i.e. Strecker degradation of dicarbonyls (Tran, Cibaka, & Collin, 2015)), the possible liberation of free thiols from thiol precursors due to residual hop enzymes during dry-hopping, and/ or the oxidation of free thiols into thiol disulfides during dry-hopping and beer storage.

4. Conclusions/industrial considerations

It was observed that overall hop aroma intensity (OHAI) and Citrus quality attributed to beer during dry-hopping increased as a function of harvest date, indicating that later picked Cascades tended to produce dry-hopped beers with higher overall hop aroma intensities that were primarily citrusy in quality. The development of humulones did not change as a function of harvest date. However, total essential oil content displayed a significant positive trend with the harvest date. At an individual component level, a number of different hop volatiles were positively correlated with the harvest date. Most notably geraniol concentrations increased significantly with harvest maturity, and the latest harvested hops had ~2×, ~12×, and ~4× more geraniol than the early harvested samples in 2014, 2015, and 2016, respectively. This study is the first to report how the concentrations of thiol precursors (Cys3MH, G3MH, Cys4MMP, G4MMP) and free thiols (4MMP, 3MHA, and 3MH) in hops are impacted by harvest maturity. Concentrations of thiol precursors decreased over harvest and the concentrations of free thiols increased. Three years of data from this plot indicates that later-picked Cascades had higher total oils, higher geraniol concentrations, lower thiol precursors, higher free thiol concentrations and attributed more intense dry-hop aroma than earlier picked hops.

In general, these results suggest that hops harvested later in the harvest window (dry matter content > 26%) might be better suited for use in dry-hopping because they attribute the highest and most citrusy aroma to beer. This is because later harvested Cascades had the highest concentrations of most of the hop essential oil volatiles and free thiols (mainly 3MH) available to be extracted during dry-hopping. Conversely, early harvested Cascades (dry matter content 20–24%) were higher in thiol precursor concentrations (mainly Cys3MH) and might be better suited for use in the kettle/whirlpool hop additions because bitterness potential has fully developed (i.e. peak humulone concentrations reached) and these additions occur before primary fermentation. Therefore, yeast β-lyase activity may liberate the thiols from these precursors during fermentation to increase the aroma perception of beer.

It is important to note that this study is limited to Cascade hops grown on one farm. There are several studies that have shown that hop quality can vary significantly as a function of harvest timing, harvest location and hop variety (Bailey et al., 2009; Forster & Gahr, 2014; Van Holle et al., 2017). Therefore, future work should explore the impact of harvest maturity for Cascade hops grown on other farms, in addition to examining other hop varieties that have been shown to have high concentrations of thiol precursors, such as Saaz, or free thiols, such as Citra. Investigating the maturity effect on the concentrations of free and bound thiols in these varieties may help identify the genetic pathways that make these varieties unique from a thiol perspective and also prevent off flavors (such as onion garlic notes) from forming due to other sulfur-related analytes in later harvested hops. Future studies

should also investigate the impact of harvest maturity on the development of terpene glycosides, as this may explain some of the increase in geraniol and linalool during harvest.

Due to the nature of commercial hop harvesting, it is possible that the same variety of hop will be picked at different times during the harvest window because of brewer preferences, processing limitations, competing optimal maturity windows with other hop varieties, etc. Understanding how to maximize the brewing potential of hops allows growers to target hop quality based upon how a brewer plans to use hops. However, practical constraints still need to be considered. Hop shattering is a complication that stems from harvesting overly mature hops with high dry matter. While very mature hops might be desirable from a dry-hopping perspective, these mature hops could be a challenge for growers to process. It is up to the hop grower and brewer to set practical and commercially achievable targets for hop quality.

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Conflict of Intrest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.10.148.

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