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RESEARCH-ARTICLE

# Aroma Extract Dilution Analysis of Beers Dry-Hopped with Cascade, Chinook, and Centennial

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**ABSTRACT**

Cascade, Chinook, and Centennial hops are used extensively throughout the brewing industry either individually or in various combinations to add hoppy aroma to beer. This high use of hops, particularly via late- or dry-hopping, creates a need to better understand the chemical contribution of these hop varieties during dry-hopping beer in order to predict brewing performance. Solvent-Assisted Flavor Evaporation (SAFE) and Aroma Extract Dilution Analysis (AEDA) was performed on unhopped beer that was dry-hopped individually with each of these varieties as well as the unhopped base. This technique was used to determine the aroma compounds that were the greatest contributors to the dry-hop character of these hops. The analysis of beer prepared with Cascade, Chinook, and Centennial identified 9, 10, and 11 character impact compounds, respectively. Commonalities were observed among the three varieties regarding 2-furanmethanol, linalool, geraniol, cis-geranic acid methyl ester, and n-decanoic acid in dry-hopped beer. Variation between the hop volatiles found to be important for Centennial and Chinook dry-hop aroma was a function of only a few character impact compounds, whereas Cascade was slightly different, anchored heavily by benzenacetaldeyde. The relative similarities and differences that these three hop cultivars attribute to beer during dry-hopping were revealed by comparing which compounds were important for the characteristic aroma profiles of these cultivars in single dry-hop beers.. This knowledge is important for brewers wishing to introduce potential replacement hops and/or reductions for these hop cultivars in the future and guide the direction of future blending studies.

**KEYWORDS**

AEDA; beer aroma analysis; Cascade; Centennial; Chinook; dry-hopped beer

## Introduction

A comprehensive understanding of the drivers of hoppy aroma in beer is far from complete. To accurately describe the aroma of hops in beer, the results must be interpreted in the context of the operation that was used to add the hops to the wort or beer. The primary reservoir of compounds in hops that potentiate hoppy aroma in beer is the essential oil in hops, which contains upward of several hundred compounds that could impact hoppy flavor in beer.[1] While no single compound is solely responsible for hop aroma intensity or character, the list of analytes should be composed of a reasonable number, as it is impractical to measure each and every compound in order to characterize hop aroma in beer. Instead, the list needs to be reduced to a subset of compounds that collectively contribute most significantly to beer flavor, and this subset will most likely be varietal specific. According to Chang,[2] the compound(s) that are responsible for the sensory responses attributed to a particular food/beverage are considered "character impact compounds" (CIC), and the list of CICs in most foods is seldom longer than 10 compounds. When considering beer, the CICs of interest related to hops are often present in trace quantities and are difficult to measure.

Solvent-Assisted Flavor Evaporation (SAFE) was developed by Engel et al.[3] as a way to carefully extract the volatile fraction of foods by using high vacuum and low-temperature conditions. Compared to the traditional Simultaneous Distillation Extraction (SDE) apparatus for flavor extraction originally developed by Likens and Nickerson,[4] the SAFE method reduces the formation of artifacts during the extraction process. Aroma Extract Dilution Analysis (AEDA) is a procedure developed by Grosch[5] to determine the key CICs within a food product. This technique utilizes a sensory-directed approach combining chromatographic separation using traditional gas chromatography and an olfactory port that runs in parallel with the instrument's detector. During the analysis, the detector and the operator experience each chemical simultaneously. This allows the operator to describe the sensory characteristics of an individual compound that is a constituent of the sample. After a series of dilutions of the parent extract, the compounds and/or responses that remain have a greater importance/responsibility for the character of that food/beverage. In combination with SAFE, AEDA is a powerful analytical tool for investigating the CICs in different matrices. There have been a number of attempts to use olfactometry to study hop and beer systems.[6–18] The results of each must be interpreted as a function of hop cultivar, hop usage

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method, biotransformation reactions (if yeast is present), and/or chromatographic method and/or detector type. Few studies have specifically focused on investigating the compounds responsible for Cascade, Centennial, and Chinook dry-hop aroma.

The work presented here discusses the results of a study that employed an AEDA of dry-hopped beers prepared individually with the Cascade, Chinook, and Centennial hops cultivars. The outputs of this study are threefold: (1) characterize the compounds that contribute strongly to beers dry-hopped individually with Cascade, Chinook, and Centennial to understand their similarities and differences; (2) investigate the relevance of the compounds elucidated in the context of prior literature; and (3) guide future dry-hop blending studies to manipulate cultivar-specific flavor contributions in an effort to introduce new cultivar replacements and/or removals.

## Experimental

### Hop material

Cascade, Centennial, and Chinook hops were received in whole cone form shortly after the 2014 harvest. Cascade and Centennial hops were shipped directly to Oregon State (OSU), from farms in Washington (U.S.A.), and were packaged in inert, 4–6 kg foil pouches. Upon arrival at OSU, they were transferred to high barrier foil pouches, purged with nitrogen, sealed, and stored cold (−20 °C) until needed. Whole-cone Chinook samples were provided to OSU from a regional brewery. Upon arrival, these hops were handled and stored in an identical fashion as the Cascade and Centennial hop samples. The hops selected were designated by growers and brewers as high quality representative samples of each variety.

### Beer production

Beer was brewed on a commercial scale and sourced from Portland, OR. Unhopped wort was prepared using 86% pale two row (Rahr, Shakopee, MN), 13.5% caramel 10°L, and 0.5% caramel 120°L malt (Briess, Chilton, WI, U.S.A.) to a starting concentration of 10.9°P. Fermentation was carried out at 18–19°C using American Ale Yeast (1056 Wyeast, Hood River, OR, U.S.A.). The finished beer attenuated to 3.9 residual extract (RE) (%wt/wt) and 4.5% alcohol by volume (ABV). Post clarification, iso-alpha acids (IsoHop®, Barth-Haas Group, Yakima, WA, U.S.A.) were added at a concentration of 18 mg/L resulting in a 15.4 bitterness units (BU) beer. The beer was carbonated (~2.6 vol/vol) and filled into 60-L stainless kegs, shipped to Oregon State University, and held at 4 °C until dry-hopping.

### Dry-hopping process

The dry-hopping procedure developed by Vollmer and Shellhammer[19,20] was used to make the dry-hopped beer for each of the cultivars. In brief, on the day each dry-hopping treatment ocurred, the hop sample was coarsely ground and added to mesh bags (EcoBag, Ossining, NY, U.S.A.). These bags were stored in foil pouches flushed with nitrogen until the dry-hopping event. Hops were dosed at a rate of 3.8 g/L (approx. 1 lb/U.S. beer barrel). To prevent the hop bag from floating inside the dry-hop vessel, a clean stainless steel fitting was added to the mesh bag with the hops for extra mass. Packaged beer was removed from the cooler at 4 °C and transferred into sanitized, stainless steel dry-hop vessels that had been purged with carbon dioxide, and then allowed to warm for approximately 24 h prior to hop addition. Dry-hopping was carried out in modified 60-L stainless beer kegs with a 4" stainless re-sealable opening (Sabco, Toledo, OH, U.S.A.). These vessels were outfitted with a standard Sankey D-system coupler and modified spear. The temperature of the beer for dry-hopping varied between 12.7 and 15.5 °C across all dry-hopping events for the duration of the exposure time.

For each dry-hop event, two kegs each filled with 40 L of beer were temporarily depressurized and opened under a stream of low-pressure $CO_2$. Simultaneously, the foil pouch bags were opened and the mesh bag containing the hop material was added to each of the vessels. Afterward, the headspace of the dry-hop vessel was purged with $CO_2$ and resealed.

After 24 h of dry-hopping, the beer was filtered through diatomaceous earth impregnated cellulose pads (HS2000, Pall Corporation, Port Washington, NY, U.S.A.) to stop the dry-hopping process. Dry-hopping was stopped after 24 h as prior work by Wolfe et al.[21,22] has shown that the extraction of key hop volatiles occurs within 24 h during dry-hopping. The two experimental replications were blended via a three-way fitting prior to entering the plate and frame filter. Dissolved oxygen (DO) was monitored during filtration and filtered beer was not collected until the DO in the beer leaving the filter was less than 100 ppb. After the DO was within specification, the filtered beer was collected in a 60-L stainless steel vessel with sufficient backpressure to prevent foaming. Between each filter run, filter pads were replaced to reduce carry-over between lots of hops. Filtered beer was stored cold (1–2°C) and under pressure at 117–124 kPa.

### Study design

The control (unhopped) and Cascade dry-hopped beers were selected from another ongoing study. For that study, the beers were packaged in amber glass bottles. The treatments for Chinook and Centennial were prepared separately and remained in 60-L stainless kegs and were not packaged in bottles. Each dry-hop treatment was extracted using the SAFE apparatus, concentrated, and analyzed using AEDA. For the analysis, aroma events (related to a compound) pertaining to the control (unhopped) sample were subtracted from all samples.

### Solvent-Assisted Flavor Evaporation (SAFE)

A 350 gram sample of beer was combined with 150 mL of dichloromethane (DCM) that had been re-distilled before the beer extraction to remove impurities. Prior to combining

the beer and the DCM, 5 μL of 2-octanol was added as an internal standard. Beer and solvent were placed in a 500-mL separatory funnel, shaken and then allowed to sit for 2–3 h. The nonpolar DCM phase was removed from the bottom of the funnel and then added to the sample side of the SAFE unit. The distillation flask was kept at 50 °C and under high vacuum (approximately $10^{-4}$ Torr). The DCM/beer extract was introduced into the device via a high vacuum valve. The low pressure of the SAFE apparatus vaporized the extract that then underwent separation in the distillation head. The receiving flask, where the final extract was collected was kept cold with liquid nitrogen (−200 °C). Post extraction, the device and receiving flask were warmed to room temperature to thaw the extract. The final extract was washed with distilled dichloromethane, dried with anhydrous $Na_2SO_4$, and filtered through Whatman #1 paper (G.E. Healthcare, Chicago, IL, U.S.A.). Evaporation of residual solvent was completed using a Kuderna Danish Evaporator Concentrator (Corning Inc., Corning, N.Y., U.S.A.) under atmospheric conditions with the assistance of a stream of inert nitrogen gas to achieve a final volume of 5-mL concentrated flavor extract.

### Aroma Extract Dilution Analysis (AEDA)

AEDA was performed on the SAFE extract by serially diluting the parent extract ($2^n$) with dichloromethane. A 1-μL aliquot of extract at each dilution was introduced using a Gerstel MPS2 autosampler (Lithicum, MD, U.S.A.) operating in liquid injection mode. The extracts were injected into a 7890 A Agilent Gas Chromatograph coupled to a 5975 C Mass Spectral Detector (Agilent Technologies, Santa Clara, CA, U.S.A.). Separation was performed with an Agilent DB-5MS ultra inert column (30 m × 0.32 mm × 1.00 μm) (Agilent Technologies, Santa Clara, CA, U.S.A). The injection was performed in splitless mode with an injector temperature of 260 °C. The carrier gas was hydrogen at a 2.55 mL/min constant flow. The oven temperature program was as follows: 35 °C for 4 min followed by 5 °C/min ramp to 180 °C, 40 °C/min ramp to 220 °C with a hold for 1 min. Mass spectrometer (MS) parameters were as follows: transfer line temp. 280 °C, source temp. 230 °C, quadrapole temp. 150 °C, operation was in the scan mode from 34 to 300 amu in EI mode at 70 eV. The column effluent was split using an open split interface between the MS detector and an aroma port (Microanalytics, Round Rock, TX, U.S.A.). The aroma port was held at 220 °C and swept with humidified nitrogen. Olfactory data was collected using AromaTrax software (Microanalytics, Round Rock, TX, U.S.A.) and a touch screen interface. AEDA was performed by two analysts over 10 days. For each aroma event, the highest aroma dilution value assigned by either analyst was used for each compound.[23] Compound identification was based on a mass fragmentation pattern library match, retention index, chemical standard matching, and/or odor quality. Following an adapted method outlined by Schieberle,[23] the AEDA analysis was completed over the course of a short period of time (9–10 days), during which both evaluators scored the control (no dry-hop), Cascade, Chinook, and Centennial beers by assessing odor qualities at each dilution: 1:1, 1:4, 1:6, 1:64, 1:256, and 1:1024 on the first day and 1:2, 1:8, 1:32, 1:128, and 1:512 on the second day.

### Compound identification and confirmation

Retention indices (RI) were calculated using a standard alkane mixture (carbon numbers 8–19). Aroma events identified during olfactometry analysis were initially matched based on percentage match (based on mass fragmentation data and RI) using the NIST database as an initial screening tool. Every aroma event was summarized by a series of aroma descriptors, as well as by the NIST database. This initial screening provided guidance for a series of validations involving the pure chemical compounds potentially involved in the aroma event. Confirmation of each compound was achieved by injecting the suspected chemical standard diluted in dichloromethane and completed under the same instrumental conditions to match the retention index and the mass spectral information. Ideally, the aroma event and the results from the initial AEDA screening would be congruent with the spectral information and retention time/retention index with the suspected pure chemical standard that lead to a positive ID. It was not possible to identify all the odors and compounds responsible for these odors within each sample at the olfactory port.

### Bench top aroma quality evaluation

A panel of five individuals (one female and four males, ages 27–52) was used to evaluate the aroma quality of analytical grade standards of target compounds identified by AEDA G-CO. In brief, each analytical standard was diluted to 1% in 2 mL of food grade polypropylene glycol. A 100-uL aliquot of this solution was then spiked into an empty 25-mL scintillation vial. The vials were blind coded with three digit random numbers and panelists were instructed to smell the vials. For each standard, panelists recorded two descriptors (if possible) that best described the aroma quality.

### Chemical standards

The 2-octanol was purchased from Spectrum (New Brunswick, NJ, U.S.A.). Standard alkane mixture (carbon numbers 8–19), methyl geranate, furfuryl alcohol (98%), decanoic acid, ethyl butyrate (≥98%), gamma-butyrolactone (≥99%), heptanoic acid (≥99%), isoamyl butyrate (≥98%), octanoic acid (≥98%), 4-hydroxy-2,5-dimethyl-3(2H)-furanone, geranyl acetate (≥97%), linalool (97%), geraniol (98%), phenylacetic acid (99%), phenyl ethyl acetate (99%), and isovaleric acid (99%) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Phenylacetaldehyde (95%) was purchased from Alfa Aesar (Haverhill, MA, U.S.A.). Dichloromethane for the SAFE extractions was purchased from Mallinckrodt (Dublin, Ireland). Dichloromethane for the AEDA dilutions was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

**Table 1.** Aroma Extract Dilution Analysis (AEDA) of Cascade (CAS), Chinook (CHI), and Centennial (CEN) dry-hopped beer.[a]

| DB-5[b] | Compound | Odor Qualities[c] | FD Value[d] | | | Identification Method[e] |
|---|---|---|---|---|---|---|
| | | | CAS | CHI | CEN | ID |
| 896 | Isovaleric acid | cheesy, sweaty, bread-like | | | 128 | MS, RI, CI |
| 860 | 2-Furanmethanol | pineapple, fruity, strawberry | 4 | 512 | 256 | MS, RI, CI |
| 912 | Unknown-1 | malty, bread-like | | | 8 | |
| 927 | Butyrolactone | bready, musty, dog food | | 512 | 64 | MS, RI, CI |
| 1115 | Heptanoic acid | sweaty, cheesy | | 32 | | MS, RI |
| 1059 | Isoamyl butyrate | strawberry, tropical | 256 | | | MS, RI, CI |
| 1060 | Benzeneacetaldehyde | cotton candy, candy | 1024 | | | MS, RI, CI |
| 1082 | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | cotton candy, candy, peach, pineapple | | | 64 | MS, RI, CI |
| 1105 | Linalool | fruity, tropical | 64 | 256 | 512 | MS, RI, CI |
| 1147 | Unknown-2 | fruity | | 32 | | |
| 1201 | Octanoic acid | buttery, toffee | | | 256 | MS, RI, CI |
| 1215 | Unknown-3 | fruity, strawberry | | 32 | | |
| 1261 | Benzeneacetic acid | floral, sweaty | | | 128 | MS, RI, CI |
| 1256 | Geraniol | musty, floral | 8 | 128 | 8 | MS, RI, CI |
| 1268 | Phenyl ethyl acetate | fruity, tropical | 4 | | | MS, RI, CI |
| 1284 | cis-Geranic acid methyl ester | anise, musty, floral, minty | 32 | 512 | 32 | MS, RI, CI |
| 1377 | n-Decanoic acid | anise, musty, floral, minty, sweaty | 16 | 512 | 1024 | MS, RI, CI |
| 1382 | Unknown-4 | musty, unknown | | 128 | | |
| 1387 | Geranyl acetate | sweaty, cheesy, earthy | 128 | | | MS, RI, CI |

[a]Table shows events with a flavor dilution value greater than or equal to 4 (FD >4). All of the events found to be important in the unhopped base beer were removed from the table.
[b]Kovat's Retention Index on a DB-5 column.
[c]Odor Qualities perceived at the olfactory port.
[d]FD Value is the Flavor Dilution Value, which is the dilution factor at which the last perceived stimulus was detected by the operator.
[e]Identification Method: MS, mass spectral identification; RI, retention index confirmation with olfactory event; CI, chemical standard identification using pure analytical standards.

### *Data analysis*

AEDA responses were provided by each operator for all of the samples. The AromaTrax computer software assembled the collected olfactory data and aligned each event with a series of selected descriptors at each FD level for all of the samples. For the assembly of Table 1, events that were found only in the control sample were removed. In some cases, an aroma compound present in the unhopped beer was enhanced by the dry-hop treatment, and when this was the case it was included in Table 1. Statistical analysis was completed using XLstat 2016 (Addinsoft, New York, NY, U.S.A.). Multivariate statistical analysis was carried out to understand the underlying structure of the data set. Practically, this approach helped identify the impact compounds driving the variation among Cascade, Centennial, and Chinook dry-hopped beers. Principle Component Analysis (PCA) utilizing the Pearson (n) correlation matrix was completed on the flavor dilution data contained within Table 1.

## Results and discussion

According to Grosch,[5] the highest dilution at which a compound can be detected at the olfactory port is defined as its flavor dilution (FD) factor (shown in Table 1). The higher the dilution factor, the more persistent the compound is in the dilution sequence and in turn the more important it is in terms of its contribution to the overall flavor of the sample.

### *Cascade*

The global popularity of Cascade has driven numerous bodies of work surrounding its chemistry as a hop plant, as well as its chemistry in beer. Our AEDA output suggests that, 9 compounds contributed with an FD level greater than 4 to the character of Cascade dry-hopped beer. These compounds are 2-furanmethanol, isovaleric acid, butanoic acid 3-methylbutyl ester, benzeneacetaldehyde, linalool, geraniol, phenyl ethyl acetate, cis-geranic acid methyl ester, n-decanoic acid, and geranyl acetate (Table 1). Similar work by Steinhaus et al.[15] on extracts made with diethyl ether from powered hop pellets, indicated that both linalool and geraniol (among others) are important to the flavor of Cascade hops with FD values of 1024 and 128, respectively. The greater FD values found in the Steinhaus study as compared to our work were likely attributable to the fact that their analysis was carried out by examining the aroma compounds found in hops, while in this study we examined hop compounds in beer. It is likely that a number of the compounds that Steinhaus detected and analyzed as important in hops do not transfer into beer. The individual role of linalool as it relates to hop aroma in beer was more recently debated. Both Nielsen[24] and Kaltner et al.[25] identified the importance of linalool in kettle hopped and hop-backed ales. However, Peacock et al.[26] has argued that linalool's importance in kettle hopped beers had been overstated. Early work by Peacock et al.[26] on floral hop aroma in kettle hopped beer using Cascade suggested that linalool, geraniol, and other geranyl esters are involved in the floral component of Cascade hop flavor in beer. According to Kishimoto et al.,[12] in kettle hopped beer, linalool, geraniol, and to a lesser extent, n-decanoic are important in terms of their combined hedonic aroma response measurement (CHARM) values in beer in the context of Cascade hop flavor. Inui et al.[27] also identified geraniol as driving the citrus flavor profile of Cascade in kettle and whirlpool hopped beer. Our results are in agreement with these observations

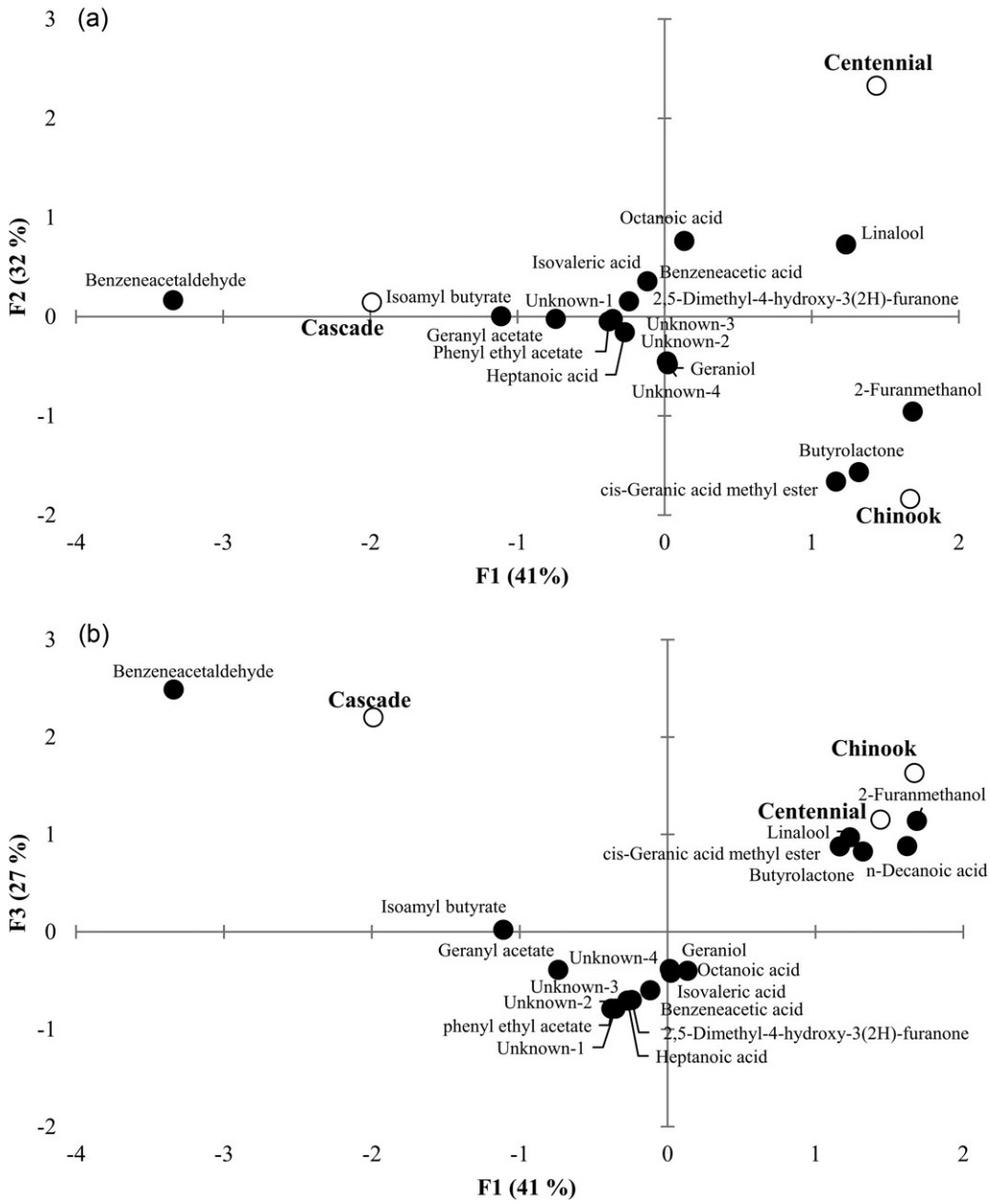


**Figure 1.** Principle Component Analysis of the flavor dilution values (●) among the (3) hop cultivars (○). a) Biplot of PC1 and PC2 explaining 73% of the variation in the data. b) Biplot of PC1 and PC3 displaying an additional 27% of the variation in the data set.

and highlight the importance of linalool, geraniol, and geranyl acetate at FD values of 64 (fruity, tropical), 8 (musty, floral), and 128 (sweaty, cheesy, earthy), respectively, for Cascade dry-hop beer flavor. The fatty acid decanoic acid was also detected at an FD of 16 (anise, musty, floral, minty, sweaty).

## *Centennial*

The analysis of Centennial hops aroma in dry-hopped beer resulted in 11 unique aroma events (Table 1). Of high importance (FD $\geq$64) were the compounds isovaleric acid, 2-furanmethanol, butyrolactone, linalool, octanoic acid, benzeneacetic acid, n-decanoic acid, and 2,5 dimethyl-4-hydroxy-3(2H)-furanone. Compounds such as, cis-geranic acid, unknown-1, as well as geraniol contributed to a lesser extent (FD$\leq$32). These compounds, although not present at higher FD values, still play a role in Centennial hop flavor in dry-hopped beer. Feng[7] in 2014 performed AEDA on distilled and fractionated hop oil and characterized Centennial hops primarily (i.e., FD $>$ 64) by geraniol, isovaleric acid, vanillin, linalool, myrcene, diacetyl, and octanoic acid. Our work is in alignment with the work by Feng[7] with regard to Centennial in beer. The FD values in our work suggest the importance of linalool, geraniol, as well as isovaleric acid in the context of the Centennial aroma in beer. Feng[7] also found isovaleric and octanoic acid in the hop material. These compounds are associated with aromas that are subjectively negative, resembling cheese, sweat, and rancidity. In general, compared to

Table 2. Hop aroma compounds reviewed post-AEDA for possible incongruencies in their aroma description.

| Hop Aroma Compounds | GCO Aroma Descriptors | Bench-Top Aroma Descriptors |
|---|---|---|
| Benzeneacetic acid | floral, sweaty | rose, floral, leafy, vegetal |
| cis-Geranic acid methyl ester | musty, floral, citrus | carrot, green, fruity, melon rind, woody |
| n-Decanoic acid | anise, musty, floral, minty, sweaty | soapy, rancid, fatty acid, earthy musty |
| Butanoic acid, 3-methylbutyl ester | strawberry, tropical | cherry, candy, fruity |
| Benzeneacetaldehyde | cotton candy, candy | roses, fruity, lilac, floral, berry |
| 2-Furanmethanol | pineapple, fruity, strawberry | smoke, paper, medicinal |
| Butyrolactone | cheesy, sweaty, bready | rancid, vomit, unripe melon |
| Heptanoic acid | sweaty, cheesy | musty, stale |
| Geranyl acetate | sweaty, cheesy, earthy | pear, waxy, fruity floral |
| Octanoic acid | butter, toffee | soapy, musty, fatty |
| 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | cotton candy, candy, peach, pineapple | caramel, fruity, toasted marsh mellow |

AEDA, Aroma Extract Dilution Analysis; GCO, gas chromatography olfactometry.

the work by Feng,[7] the values in this study in beer were lower than what was found in hops. The compound 2,5 dimethyl-4-hydroxy-3(2H)-furanone (FD = 64) is characteristic of pineapple aroma as shown by both Rodin et al.[28] and Tokitomo et al.[29] In their work, it was described as burnt pineapple, sweet, and caramel-like, which is in alignment with its aromatic display in the Centennial extract Table 1.

### Chinook

This investigation of character impact compounds in Chinook hops will serve as one of the first in-depth flavor investigations of this hop cultivar. The AEDA output displayed the second greatest amount of impactful aroma events among the three varieties examined in the study. Of high importance (FD ≥128) for beer dry-hopped with Chinook were the compounds 2-furanmethanol, butyrolactone, linalool, geraniol, n-decanoic acid, and an unknown compound (unknown-4) with a musty aroma that still requires identification (Table 1). To a lesser extent, but still important in terms of contribution to flavor and aroma, are the compounds heptanoic acid, unknown-2 (GC-O: fruity), and unknown-3 (GC-O: fruity, strawberry). The investigation into the impact compounds for Chinook will provide guidance and focus within the larger suite of hop aroma analytes to best characterize and measure their aroma quality in dry-hopped beer.

### Commonalities across the three cultivars

Five compounds displayed consistent presence, with FD values greater than four across all three cultivars in dry-hopped beer but were present at different dilution levels. These compounds are 2-furanmethanol, linalool, geraniol, cis-geranic acid, and n-decanoic acid (Table 1). The importance of linalool varied across the three cultivars with the highest FD measured in Centennial, followed by Chinook, and then Cascade. Geraniol was of equal importance to Cascade and Centennial (FD =8) but far greater in terms of contribution to Chinook (FD =128).

### Variation among the three cultivars

Principle Component Analysis was carried out using the AEDA outputs to help visualize the interrelationships as shown in Table 1. The projected space across the three principal components explained 100% of the variation in the data using the Pearson correlation matrix (Figure 1). PC1 is anchored by a number of the analytes in the negative direction with benzeneacetaldehyde, isoamyl butyrate, geranyl acetate, phenyl ethyl acetate, heptanoic acid, as well as unknown 1 and 3 and in the positive direction with linalool and 2-furanemethanol. The Cascade cultivar aligns strongly with PC1 in the negative direction. PC2 in the negative direction is associated with geraniol and unknown-4, while in the positive direction, PC2 is associated with octanoic acid and to a lesser extent benzeneacetic acid. PC3 was not anchored by any particular compounds, but butyrolactone and cis-geranic methyl ester contributed to variation in its positive direction. Centennial and Chinook sit in separate quadrants in the PCA biplot, whereby they have similar PC1 loadings but vary along PC2.

### Character impact compound orthonasal evaluation

The results from the bench top evaluation of selected hop aroma standards are summarized in Table 2. There was agreement between the bench top evaluation of the aroma quality of the standards and the aroma quality used to identify the target compounds via AEDA gas chromatography olfactometry (GCO), with the exception of octanoic acid. The differences in the bench top generated aroma quality and AEDA GCO aroma quality could be attributed to concentration differences between the bench top and G-CO evaluation at the sample port. Often, it is the case that both in its pure form and at an elevated concentration, the sensory qualities of a given compound may be different than that at a much lower concentration or in the presence of other background aromas. The lack of congruency in some, but not all cases, between the perceived GC-O aroma and that perceived during the bench top assessment alludes to this phenomenon.

### Pedigree

Cascade (USDA 56013) was created via a cross in 1956 between [Fuggle x (Serebrianka-Fuggle Seedling)] and an unknown male plant via open pollination.[30] Chinook was created via a cross between Petham Golding × Brewer's Gold.[31] Centennial was created via a cross between (Brewer's Gold[2] × Fuggle-Fuggle Seedling) × (Brewer's

Gold × East Kent Golding—Bavarian Seedling).[32] Given the pedigrees of these cultivars, Centennial (USDA 21507) and Chinook (USDA 21226) share an ancestral relative in Brewer's Gold. This genetic commonality may be one reason for the similarities in their character impact compounds. Individually, Brewer's Gold is typically described as spicy/fruity; this connection with Brewer's Gold may describe why Centennial and Chinook have a higher FD for both linalool and 2-furanmethanol as the aroma event for these compounds both were described by GCO generally as fruity. Centennial and Cascade share an ancestral link in Fuggle. Again, this may be one reason for the similarities in the important of character impact compounds between these two hops.

### *Potential limitations*

Many factors in both the brewing process as well as sample preparation during SAFE extraction could lead to the absence of aroma events in AEDA and subsequent identification. For instance, the hopping rate and hopping method play a considerable role in terms of brewing and beer production and the resultant hoppy aroma in the finished beer. A few of the compounds identified in this study (e.g., furanmethanol,[33] heptanoic acid,[33] octanoic acid,[34] Phenyl ethyl acetate,[35,36] and n-decanoic acid[34]) have been shown to be derived from amino acids and sugars during fermentation or via the Maillard reaction. These compounds, while not found to be important for the aroma in the unhopped base beer, could have had increased importance in the dry-hopped beers due to matrix effects with the hop components.

Considerable variation in chemical composition has also been observed between different lots of the same cultivar of hops due to maturity[37] or growing region[37,39] and these factors need to be considered in future hop flavor and aroma identification studies. Separately, the absence of polyfunctional thiols, compounds associated in other published studies regarding hop flavor, from the list of potential odor impact compounds identified in this study could result in an incomplete picture of the contributors of hop aroma for these varieties. Though preliminary work (data not shown) resulted in the tentative identification of 4-mercapto-4-methylpentan-2-one (4MMP) via RI match of aroma event, it was not positively identified in this study. Dating back to the early reviews on hop oil,[40] as well as more recent work,[1] the role of polyfunctional thiols in hops and their contribution to beer flavor has been well-stated[41,42] and should still be considered as potential drivers of dry-hop aroma.

In regard to sample preparation and handling, the solvent used for extraction could induce radical and/or artifact formation or compounds of importance could have evaporated during sample handling. The AEDA protocol, as well chromatographic parameters, (e.g., column type, length, oven temperature program) and detection methods (i.e., selective detectors, mass spectrometer sensitivity) could have also influenced the output. Therefore, these results must be interpreted in the context of dry-hopping and the chromatographic methodology used to execute this work.

## Conclusion

AEDA showed the importance of 9, 10, and 11 analytes for the Cascade, Chinook, and Centennial aroma in dry-hopped beers, respectively. This work suggests that the aroma of beers dry-hopped with Chinook, Centennial, and Cascade are each defined by characteristic impact compounds that make the dry-hop aroma profile of these hops unique. It is clear also that some of the same hop volatiles found to be important in driving kettle-hop and whirlpool hop flavor also drive dry-hopping flavor. Lafontaine and Shellhammer[43] investigated the sensorial significance of these identified volatiles in single and blended dry-hopped beers made with each of these varieties and verified the importance of a few of the volatiles identified.

The practical outcome of this work involves the similarities in chemical profiles when extracted during dry-hopping and analyzed using AEDA. If the similarities in compounds, not FD levels, are congruent between selected cultivars, there may be potential to reduce the use of one and increase the use of the other to match flavor profiles when dry-hopping with blends of these cultivars.[43] This potential could benefit both large and small brewers who wish to purchase less of one cultivar and/or blend with another in incidences of shortages. If the compounds that characterize the flavor of each are remotely similar, there is the potential for an overlap in their aroma performance characteristics in a dry-hopped beer.

Understanding the key chemical contributors to dry-hop aroma in beer is of value to both the hops and brewing industries since these cultivars are used ubiquitously throughout the brewing industry to add hop aroma to beer. As the availability and cost of certain cultivars changes on a year-to-year basis, both industries benefit from an understanding of what makes hop varieties unique and different when added to beer.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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