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An exploratory study toward describing hop aroma in beer made with American and European Hop Cultivars

Notable differences exist between American and traditional European hops in terms of the types of flavor they contribute to beer. Brewers tend to describe the former as contributing citrusy, fruity and in some instances floral aromas, while the latter are often described as contributing herbal, tobacco, woody, and spicy notes. Single-hop brewing trials were carried out with Cascade, Chinook, Centennial, Citra, Simcoe, East Kent Goldings, Hallertau Mittelfrueh and Saaz to identify differences between the hop-derived volatiles characteristics of American and European hop aroma in beer. The eight resultant beers were evaluated using both sensory and instrumental analyses. The sensory analysis identified Centennial as having the highest piney and green hop aromas, while Citra and Simcoe were characterized as being very fruity, citrusy, and tropical (especially Citra). The Hallertau Mittelfrueh was similar to the East Kent Goldings, and these two were more floral and rose-like than the Saaz sample with more melon than the American cultivars. Volatile analysis of the beer samples was performed using a stir-bar sorptive extraction (SBSE) of the beer samples followed by quantification by gas chromatography mass spectrometry (GC-MS). Principal components analysis of the instrumental data identified distinct differences between the citrusy American cultivars (Centennial, Chinook and Citra) and the non-citrusy European cultivars (East Kent Goldings, Hallertau Mittelfrueh and Saaz). Mapping the sensory data with the instrumental data via Generalized Procrustes Analysis revealed interrelationships between the aromatic descriptors and the individual volatile compounds that were separated by the GC.

Descriptors: SBSE GC-MS, descriptive analysis, hop aroma, principle component analysis, generalized procrustes analysis

1 Introduction

Much of the aroma quality in beer contributed by hops (*Humulus lupulus*) can be attributed to the essential oil fraction produced in glandular trichomes, called lupulin glands, of hops. The composition of the hop essential oil found in the lupulin is extremely complex; over 450 chemical compounds have been identified, and research suggests the total number may exceed 1000 [1]. For current in-depth reviews on the aroma chemistry of essential oil from hops and in beer see *Sharpe and Laws* [2], *Schönberger and Kosteletzky* [3] and *Briggs et. al.* [4]. Indeed, as suggested by the sheer number of possible chemical combinations due to the diversity of hop cultivars, it has been difficult for hop analysts to provide a short list of chemicals that can predict the aroma impact of hops in a finished beer. In addition, low sensory detection thresholds in the parts per trillion range, synergistic effects of compounds [5] and varying brewing techniques for imparting aroma can influence the composition of hop aroma in the finished beer which further confounds the complexity of hop aroma analysis. While it is true that

extrinsic harvest and post-harvest conditions and handling of hops impact hop aroma [6, 7], perhaps the biggest factor affecting hop aroma in beer, all else being equal, is the cultivar(s) used in beer production. Previous work by *Peacock et. al.* [8] and results from an industry survey of brewing professionals (n = 201) conducted in the Oregon State University (OSU) Brewing Laboratory [9] regarding opinions of how specific hop cultivars contribute to the flavor and aroma in beer show a clear distinction between beers made with either American hop cultivars or European hop cultivars. It is the intention of the work presented here to investigate the differences between beers brewed with American and European hops using chemical and sensory analysis with the goal of advancing the understanding of what characterizes American hoppy beer aroma in relation to beers made with traditional European hop cultivars.

2 Materials and methods

2.1 Cultivar selection

Data from an industry survey of 201 brewing professionals' opinions of how specific hop cultivars contribute to the flavor and aroma in beer were used to select specific hop cultivars to include in a study of citrus/fruity aromas in hops [9]. This survey was aimed at understanding brewers' expectations about hop flavor in beer that originates from specific hop cultivars. A clear difference was observed among American and European hop cultivars whereby

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Table 1 F-values from mixed model analysis of variance of descriptive attributes. Bold = significant at $p < 0.05$

Source	Fruit cocktail	Guava	Passion fruit	Papaya	Banana	Melon	Grapefruit	Lemon	Estery	Green apple	Rose	Floral	Green Hop	Piney	Onion/Garlic	Soy Sauce	Buttery	DMS
Products	1.5	4.5	4.8	2.2	2.8	1.0	4.8	1.7	0.7	2.0	1.5	2.3	5.0	5.4	4.3	0.7	8.7	7.3
Assessors	17.2	2.9	4.9	1.3	3.9	3.5	4.5	7.6	9.2	15.6	2.7	7.6	6.3	2.3	2.0	7.0	6.3	5.5
Sessions	1.3	1.2	1.3	0.6	2.8	1.3	0.6	0.6	2.1	2.8	0.6	0.5	0.8	0.1	0.5	0.2	0.7	2.4
Products x assessors	1.8	2.3	1.6	1.5	1.8	1.0	2.2	1.6	2.9	1.2	1.9	2.2	2.1	2.0	2.8	1.6	2.7	1.3
Products x sessions	0.9	1.5	1.0	0.9	0.9	1.2	1.3	1.1	0.9	1.1	1.0	0.8	1.0	1.3	1.4	1.2	0.9	0.8
Assessors x sessions	1.0	0.7	1.1	2.9	0.8	0.7	1.3	0.9	1.2	0.6	1.9	0.9	0.9	1.6	1.2	1.2	1.0	0.9

the top five American hop cultivars were rated as citrusy compared to three prominent European hop cultivars which brewers felt were not citrusy. The European hops were expected to deliver herbal, floral, spicy and woody aromas. Using input from brewing scientists working for commercial breweries in conjunction with the OSU hop survey, eight hop cultivars were selected for investigation in this study. Cascade, Chinook, Centennial, Citra (all four courtesy of John I. Haas, Yakima, WA) and Simcoe (courtesy of the Craft Brewers Alliance, Portland, OR) were selected based on their flavor profile and current demand by craft brewers seeking American hop aroma. Hallertau Mittelfrueh and Czech Saaz (courtesy of John Barth & Sohn GmbH, Nürnberg, Germany) were selected as representatives of continental-noble hop cultivars while UK East Kent Goldings (courtesy of Boston Beer Company, Boston, MA) was chosen because of its mild aroma and its historic significance to the British hop pedigree. Hops (2009 harvest) were donated to OSU and stored at $-20\text{ }^{\circ}\text{C}$ until brewing in 2010. Bittering acid content and total essential oil content for the hops used in this study are shown in table 2. Hop acids and total essential oil were measured using American Society of Brewing Chemist Standard Methods [10].

2.2 Beer production

Eight single hop beers were brewed in the OSU pilot brewery and hopped using a constant mass approach of three hop additions and fermented using ale yeast. Each single hop beer was brewed in the OSU pilot brewery using a grist comprised of 70 % pale 2-row malt and 30 % liquid adjunct (Clearbrew 60/44 IX, Cargill, Naperville, IL USA). Hop pellets were added to each 2 hL brew using a constant mass approach: 0.6 g/L at 5 minutes into a 60-minute boil, 1.13 g/L at 5 minutes before kettle knock out and 0.45 g/L in the hop back post whirlpool (2.18 g/L total). Dosage using a constant mass was used to demonstrate contributions of each cultivar to aroma on per mass basis, rather than adjusting hopping levels based on alpha acids. Beers were fermented and conditioned at $18\text{ }^{\circ}\text{C}$ over 6 days with an ale yeast (Wyeast 1056, Wyeast Laboratories, Wyeast, OR), and then ramped down to $1\text{ }^{\circ}\text{C}$ over four days. Beers were then filtered, carbonated to 5.5 g/L CO_2 and packaged into brown 350 ml glass bottles. Finished, packaged beers were stored at $1\text{ }^{\circ}\text{C}$ until analysis. The maximum iso-alpha acid concentration as measured by HPLC [10] was 25 mg/L, and finished beers had approximately 5 % ethanol by volume. The eight resultant beers were evaluated using sensory and instrumental analyses.

2.3 Sensory analysis

A quantitative descriptive analysis technique was used for describing and quantifying sensory attributes of single hopped beers. The sensory panel consisted of twelve trained panelists, many of whom had been extensively involved with previous sensory work regarding beer evaluations. Samples of beer ($1\text{ }^{\circ}\text{C}$, 60 ml) were allowed to warm up for 30 minutes prior to evaluation in a $20\text{ }^{\circ}\text{C}$ room and presented to the panelists in 300 ml glasses capped with clear-plastic, odorless lids. Samples were evaluated within two hours of serving and evaluated at ambient temperature. The final descriptive ballot was based on 18 descriptive terms for beer aroma with a focus on hop-derived aromas. The descriptive terms were developed during the training exercises and each term was accompanied by an aroma reference standard in beer to aid panelists in identification and agreement of aroma and descriptors. The descriptive ballot included (in order as they appeared on the ballot): fruit cocktail, Guava, passion fruit, papaya, banana, melon, grapefruit, lemon, estery, green apple, rose, floral, green hop, piney, onion/garlic, soy sauce, buttery, DMS-like aroma (Dimethyl Sulfide). All descriptors were rated on a 16-point intensity scale (0 = none, 15 = extreme intensity). Panelists trained six times over a two-week period prior to data collection. On each day the panel came together, all 8 beers were presented individually to each panelist in a panelist-specific random order.

During testing, panelists evaluated 4 beers, took a brief rest and then evaluated another 4 beers. Each beer was evaluated 5 independent times on 5 separate days/sessions. ANOVA and multiple

Table 2 Hop pellet specifications by cultivar

Cultivar	% alpha acids	Oil content (ml/100g)
Citra	12.3	1.68
Simcoe	12.2	1.64
Hallertau Mittelfrueh	3.8	0.67
Saaz	3.4	0.60
Cascade	5.8	0.82
Centennial	10.9	0.73
Chinook	11.8	0.69
East Kent Goldings	6.9	0.60

comparisons by Tukey's Least Square mean was performed using XLStat 2009. Principle component analysis (PCA) of panelist data, averaged over all replications, was performed using the covariance matrix and a Varimax rotation. Sensory panel analysis was performed using the XLStat sensory package.

2.4 Instrumental analysis of single hop beer

The following fermentation and hop derived aroma compounds were analyzed using a stir bar sorptive extraction-gas chromatography-mass spectrometry (SBSE-GC-MS) method described previously [11, 12, 13]: Ethyl butanoate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, phenylethyl acetate, α -pinene, β -pinene, myrcene, limonene, linalool, E, β -caryophyllene, α -humulene and α -terpineol. All standards were purchased from Sigma Aldrich, (St. Louis, MN USA) and contained less than 2 % impurities. A standard stock solution was made in dichloromethane and added to a 5 % (v/v) ethanol/water solution to obtain the following concentrations for a calibration curve: 1 μ g/L, 5 μ g/L, 10 μ g/L, 25 μ g/L, 50 μ g/L, 100 μ g/L, 250 μ g/L, 500 μ g/L and 1000 μ g/L. All calibration solutions were analyzed according to the SBSE sample preparation and analysis methodology described below and produced a linear response over the concentration range ($R^2 > 0.97$).

A 10 ml beer sample was diluted with 10 ml of water in a 20 ml vial, in which 20 μ L of octyl propionate internal standard solution was added. A stir bar (Twister) coated with poly(dimethylsiloxane) (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc., Baltimore, MD) was used for extraction. The sample was extracted at room temperature with the stir bar for 3 h at a speed of 1000 rpm. After extraction, the stir bar was rinsed with distilled water, dried carefully with paper and placed into a sample holder for GC-MS analysis.

GC-MS analyses were performed using an Agilent 7890 gas chromatograph with a 5975 mass selective detector (Agilent, Santa Clara, CA). Samples were loaded into a thermal desorption unit (TDU) by a multi-purpose autosampler (Gerstel). A cooled injection system (CIS4, Gerstel) was used at the sample inlet. The TDU had an initial temperature of 25 °C. After the sample was loaded, the TDU was heated at a rate of 300 °C/min to a final temperature of 250 °C and held for 2 min. The TDU inlet was in split-less mode during thermal desorption, while the CIS4 was in a solvent vent mode with a venting flow of 50 ml/min for 4.0 min, at a venting pressure of 5 psi. After the solvent vent, the CIS4 was switched to split-less mode for 3.0 min, then changed to split mode with a venting flow of 50 ml/min. The initial temperature of the CIS4 was kept at -80 °C then ramped at a rate of 10 °C/s to a final temperature of 250 °C and held for 10 min.

Compounds were separated with a DB-WAX column (30 m length, 0.25 mm i. d., 0.5 μ m film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40 °C for a 2-minute hold, then to 230 °C at a rate of 4 °C min⁻¹ with a 5-minute hold. A constant helium column flow of 2.5 ml/min was used. A column splitter was used at the end of the column, 1 ml min⁻¹ column flow was introduced to the MS, and the other 1.5 ml min⁻¹ column flow

was vented out. The MS transfer line and ion source temperature were 280 and 230 °C, with an ionization voltage of 70 eV. Analytical standard solutions in 5 % ethanol were used to build a standard curve using response factors from the selected mass ions. Triplicate analysis was performed and the average values were reported. Terpene and terpenoid concentration data were converted to flavor units by dividing the measured concentrations by sensory threshold estimates obtained from literature. Principle components analysis of the peak area data identified those compounds that correlated with differences among the eight hops. Similarly, Generalized Procrustes Analysis was used to visualize the instrumental data with the sensory data and thereby find correlations between specific volatile components and perceived aromas.

3 Results

3.1 Sensory analysis of single hop beers

Panelist analysis was performed using mixed model analysis of variance. Panelists performance scores were based on their ability to repeatedly score treatments, the number of attributes they were able to use to discriminate between treatments, and lack of contribution to interaction effects (panelist by treatment or panelist by rep). Of the 12 panelists three were removed from subsequent analysis due to poor performance scores (data not shown). Results from mixed model analysis of variance post-panelist removal is shown in table 1.

The sensory analysis identified large differences in the aromatic profile and intensities among the eight different cultivars as shown in figure 1. In general, the mean scores were on lower quartile of the scale. This is likely an artifact of comparison of the treatment beers to the spike references, which were intensely aromatic and were used to define the upper end of the sensory scale. ANOVA of sensory results for all sensory descriptors across all treatments showed no significant difference between treatments in the mean sensory scores for fruit cocktail, melon, estery, and soy sauce descriptors (Table 3). In general, the American cultivars, with the exception of Chinook, were more intensely aromatic as compared to the European cultivars, with Citra, Simcoe and Centennial hops rated higher in grapefruit, passion fruit, piney and green hop (Table 3). One should note the presence of buttery and banana descriptors and the unexpectedly low levels of piney and grapefruit descriptors in the Cascade sample. Cascade had a significantly higher mean score for buttery than all the treatments, which may indicate insufficient diacetyl reduction. This sample may not be representative of the characteristic Cascade aroma and as such was viewed as an outlier. Consequently, it was removed from subsequent sensory multivariate statistical analyses.

Mean sensory scores of each attribute for all treatments were used to cluster groups by Agglomerative Hierarchical Clustering analysis (AHC) based on dissimilarity of the treatments (Fig. 2). PCA of the sensory data showed interrelationships among the sensory descriptors and the hop cultivars (Fig. 3 and Fig. 4). The first dimension (accounting for 51 % of the variation) was anchored in the positive direction by piney and green hop descriptors and in the negative direction by DMS-like aroma and melon terms.

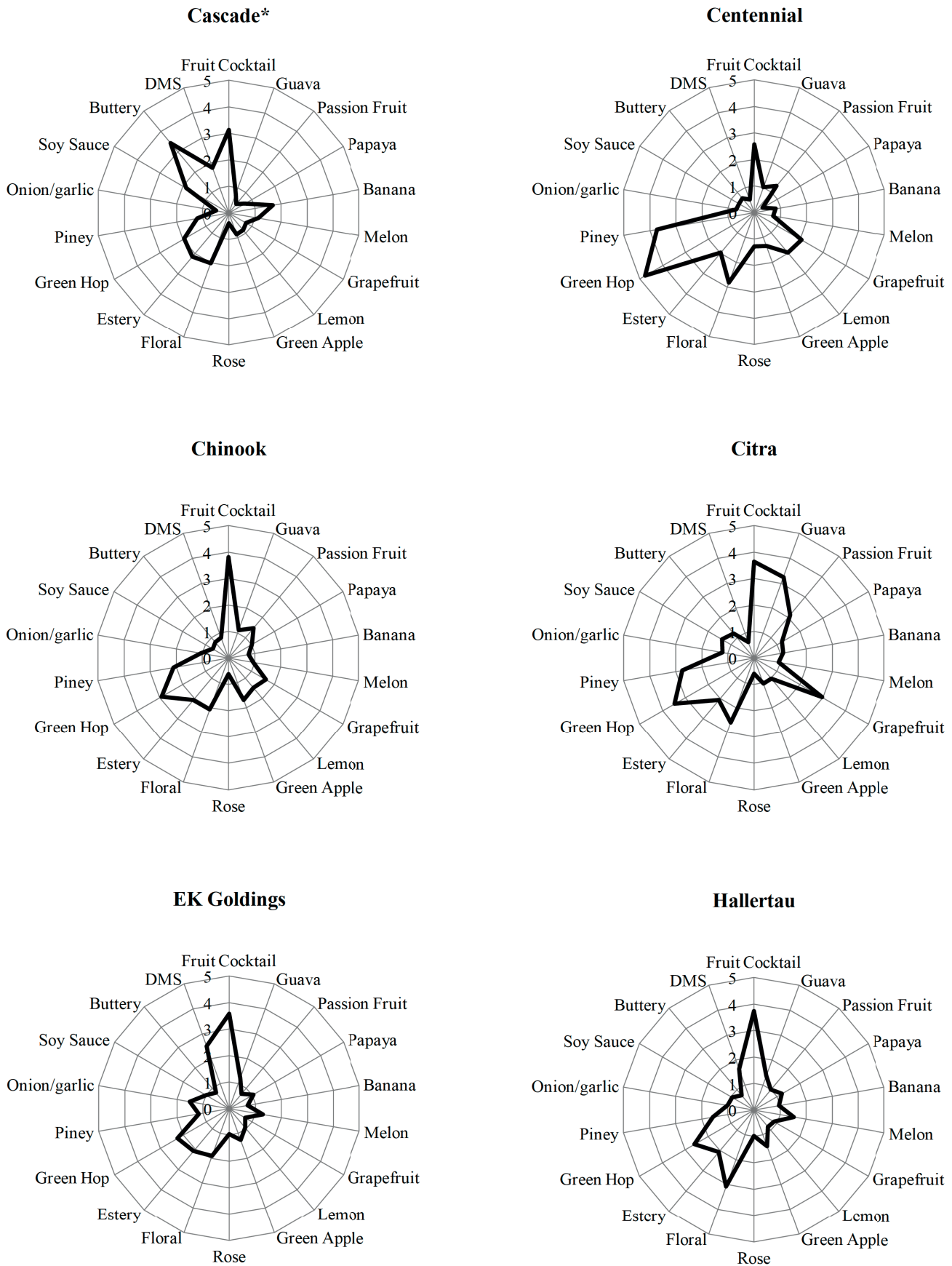


Fig. 1 Spider diagrams of aromatic descriptors for each of the single hop beers. Scale = 0–16 (0–5 shown for detail). Note Cascade showed effects

* Treatment removed from multivariate testing due to sensory defects (buttery)

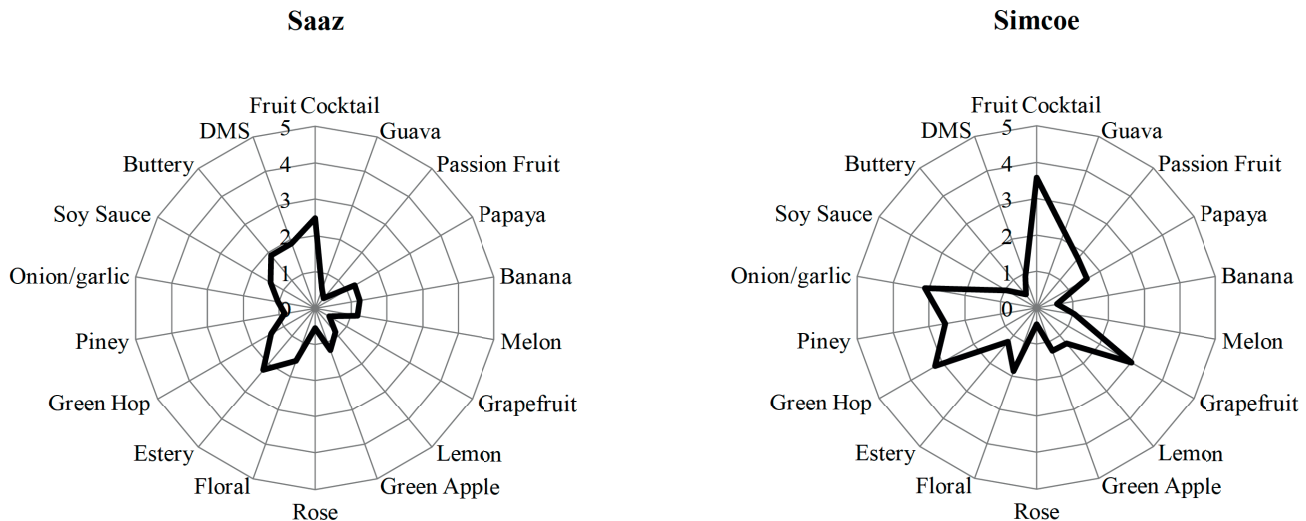


Fig. 1 (continued)

The second dimension (accounting for 23 % of the variation) was anchored in the positive direction by guava. The y-axis serves as a separation between the American (positive dimension) and European (negative dimension) hop cultivars. Centennial (sitting in the lower right quadrant of Fig. 3) had the highest piney and green hop scores but was low in the fruity and tropical fruit aromas. Citra was located in the upper right quadrant of the same figure and was characterized as being very fruity, citrusy, and tropical. Simcoe was positioned along dimension 3 (D3), which was anchored by onion/garlic. Interestingly, Chinook sat near the

middle of the PC space with notes of floral, rose and green apple. The Hallertau Mittelfrueh was similar to the East Kent Goldings, and these two were more floral and rose-like than the Saaz sample and more melon and noticeable levels of DMS-like aroma than beers made with the American cultivars. It should be noted that DMS is not typically associated with hops and may have been produced from insufficient removal of malt derived precursors [14] during wort boiling. Alternatively, the European hops may have contributed an aroma to beer similar to that of DMS.

Table 3 Means and tukey's multiple comparisons of descriptive analysis results

Descriptor	American					European		
	Citra	Cascade*	Centennial	Simcoe	Chinook	EK Golding	Hallertau	Saaz
Fruit cocktail ^{ns}	3.6 ^a	3.2 ^a	2.4 ^a	3.8 ^a	3.4 ^a	3.1 ^a	3.2 ^a	2.6 ^a
Guava	4.0 ^a	0.9 ^c	1.2 ^{bc}	2.5 ^b	1.3 ^{bc}	1.5 ^{bc}	1.3 ^{bc}	0.6 ^c
Passion fruit	2.5 ^a	0.5 ^c	1.3 ^{abc}	2.0 ^{ab}	1.6 ^{abc}	0.7 ^c	0.9 ^{bc}	0.3 ^c
Papaya	1.2 ^a	0.6 ^{ab}	0.3 ^b	1.2 ^a	0.6 ^{ab}	0.7 ^{ab}	0.8 ^{ab}	0.8 ^{ab}
Banana	1.0 ^{ab}	1.5 ^a	0.7 ^{ab}	0.4 ^b	0.4 ^b	0.5 ^b	0.8 ^{ab}	1.2 ^{ab}
Melon ^{ns}	0.6 ^a	1.1 ^a	0.4 ^a	0.7 ^a	0.7 ^a	0.9 ^a	1.0 ^a	1.0 ^a
Grapefruit	2.7 ^a	0.7 ^{bc}	2.0 ^{ab}	3.0 ^a	1.7 ^{abc}	0.6 ^{bc}	1.0 ^{bc}	0.4 ^c
Lemon	1.1 ^{ab}	1.0 ^{ab}	2.1 ^a	1.6 ^{ab}	1.4 ^{ab}	1.1 ^{ab}	1.1 ^{ab}	0.9 ^b
Estery ^{ns}	2.4 ^a	1.9 ^a	2.0 ^a	1.2 ^a	2.1 ^a	2.1 ^a	1.9 ^a	2.1 ^a
Green apple	0.9 ^a	0.7 ^a	1.1 ^a	1.0 ^a	1.4 ^a	0.9 ^a	1.6 ^a	1.2 ^a
Rose	0.8 ^{ab}	0.2 ^b	0.9 ^{ab}	0.5 ^{ab}	0.6 ^{ab}	1.2 ^a	1.2 ^a	0.6 ^{ab}
Floral	2.9 ^{ab}	2.2 ^{ab}	3.3 ^{ab}	1.9 ^b	2.0 ^{ab}	2.2 ^{ab}	3.4 ^a	1.8 ^b
Green hop	3.5 ^{ab}	2.0 ^{bc}	5.2 ^a	3.2 ^b	3.3 ^b	2.8 ^{bc}	3.0 ^{bc}	1.4 ^c
Piney	2.5 ^b	1.1 ^{bc}	4.2 ^a	2.2 ^{bc}	2.4 ^b	1.2 ^{bc}	1.7 ^{bc}	0.7 ^c
Onion/garlic	1.5 ^b	0.6 ^b	0.8 ^b	3.8 ^a	1.3 ^b	1.6 ^b	1.3 ^b	1.0 ^b
Soy sauce ^{ns}	1.2 ^a	1.4 ^a	0.7 ^a	1.0 ^a	0.8 ^a	1.2 ^a	0.9 ^a	1.2 ^a
Buttery	1.2 ^{bc}	3.8 ^a	0.9 ^{bc}	0.6 ^c	0.9 ^{bc}	0.9 ^{bc}	0.8 ^c	2.0 ^b
DMS	0.7 ^b	2.1 ^a	0.6 ^b	0.8 ^b	0.8 ^b	2.6 ^a	1.7 ^{ab}	2.2 ^a

^{ns} = no significant difference between all treatments for that descriptor; means with same letter superscript are not significantly different for that attribute.
 * Treatment removed from multivariate testing due to defects (buttery)

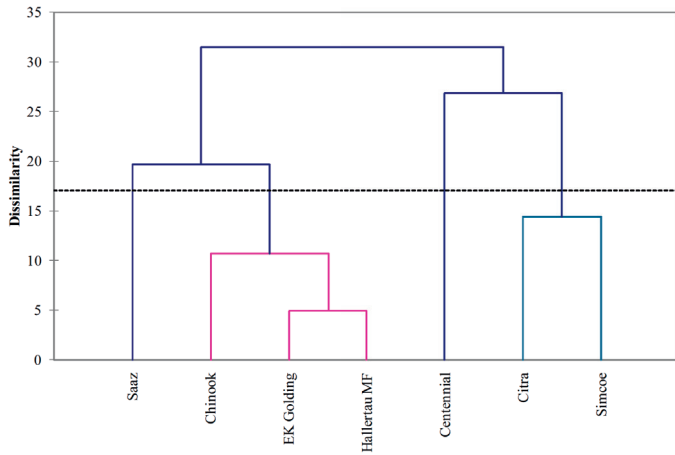


Fig. 2 Agglomerative hierarchical clustering on sensory data

3.2 Instrumental analysis

The GC-MS analysis resulted in the detection of a broad range of over 300 peaks. Some of the initial peak identifications were esters, which were likely fermentation related and not hop related. Concentrations of these esters are shown in figure 5. Linalool, myrcene, α -pinene, β -pinene, limonene, and trans- β -caryophyllene and α -terpineol were identified as hop-derived aroma compounds in the single hopped beers. α -humulene, a major component of hop oil was not detected in any of the beers. Relative contributions to aroma from a compound in a particular matrix can be gauged by their flavor units, which is a function of the measured concentration of a compound in the analytical matrix divided by the accepted sensory threshold in the same matrix. Flavor unit results for the hop-derived compounds investigated here are shown in table 4. It should be noted that flavor units are often disputed due to the variability of sensory detection threshold values reported in the literature and therefore one should avoid placing too much emphasis on them. Furthermore, the character and intensity of a given compound often changes when in the presence of other compounds [15]. Nevertheless, flavor units provide a helpful indicator of the approximate contributions of a compound to aroma.

The principal components analysis of the GC data yielded distinct separations of the hop cultivars (Fig. 6 and Fig. 7, see next page). Centennial and Simcoe anchored the positive and negative ends of Dimension 1 respectively, while Citra and Saaz anchored the positive and negative ends of Dimension 2, respectively. Citra also anchored the positive end of Dimension 3. In much the same manner as in the sensory PCA, the American hop cultivars were positioned on right hand side of the biplot and the European cultivars on the left hand side. The use of Generalized Procrustes Analysis (GPA) as outlined by Noble and Ebeler [16] allowed for the comparison of the GC and sensory data and how they relate to different hop cultivars (Fig. 8, see page 119).

4 Discussion

The sensory analysis data identified prominent differences among the 8 different hop cultivars. The 8 treatments were clustered in

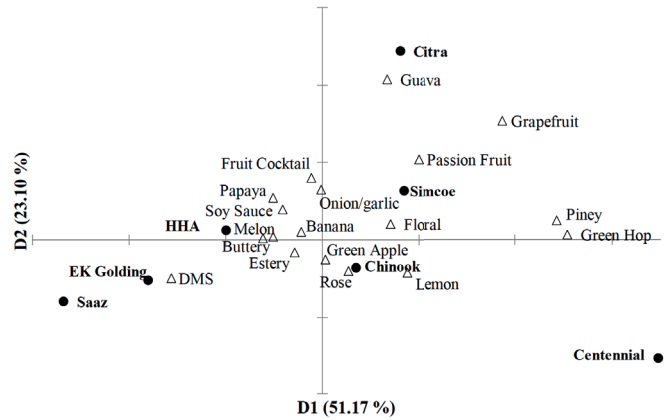


Fig. 3 Principle components analysis (D1 and D2) of sensory descriptive data from single hop beer evaluation. Dimensions 1, 2 and 3 account for 88.5 % of the total variation

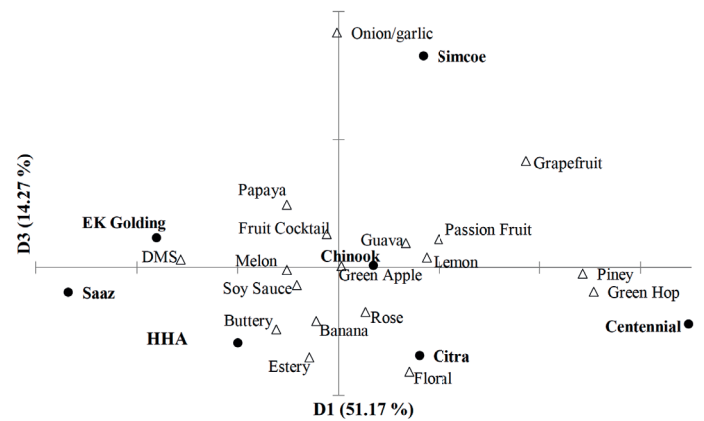


Fig. 4 Principle components analysis (D1 and D3) of sensory descriptive data from single hop beer evaluation. Dimensions 1, 2 and 3 account for 88.5 % of the total variation

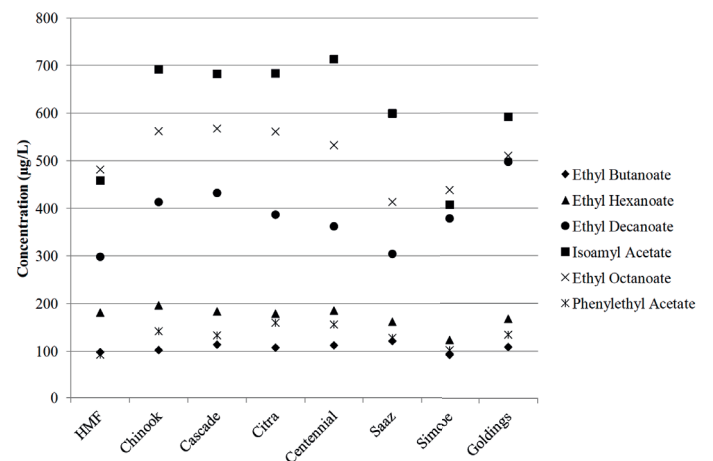


Fig. 5 Esters and high alcohol comparisons among the 8 separate hop treatments

Table 4 Concentration (µg/L) and flavor unit data of hop-derived aroma compounds found in single hopped beers

Compound	Hallertau Mittelfrueh	Chinook	Cascade	Citra	Centennial	Saaz	Simcoe	Goldings	Sensory Threshold (µg/L)
α-pinene	13.1 (2.2)	13.7 (2.3)	13.6 (2.3)	13.7 (2.3)	13.5 (2.3)	13.2 (2.2)	13.0 (2.2)	13.5 (2.3)	6 [28]
β-pinene	21.3 (0.2)	25.1 (0.2)	24.3 (0.20)	25.2 (0.2)	23.8 (0.2)	22.7 (0.2)	24.8 (0.2)	23.6 (0.2)	140 [28]
myrcene	33.2 (2.6)	46.0 (3.5)	61.2 (4.7)	126.8 (9.8)	33.5 (2.6)	27.5 (2.1)	74.2 (5.7)	35.6 (2.7)	13 [28]
limonene	22.1 (2.2)	23.0 (2.3)	22.4 (2.20)	23.2 (2.3)	22.1 (2.2)	22.6 (2.3)	23.6 (2.4)	22.1 (2.2)	10.0 [28]
linalool	36.8 (1.4)	31.0 (1.2)	25.3 (0.9)	91.4 (3.4)	66.3 (2.5)	3.4 (0.1)	69.7 (2.6)	19.2 (0.7)	27 [29]
E, β-caryophyllene	15.1 (0.2)	16.0 (0.3)	15.1 (0.2)	16.0 (0.3)	15.7 (0.2)	15.1 (0.2)	15.1 (0.2)	15.1 (0.2)	64 [28]
α-humulene	< 1 (na)	< 1 (na)	< 1 (na)	< 1 (na)	< 1 (na)	< 1 (na)	< 1 (na)	< 1 (na)	120 [28]
α-terpineol	10.6 (0.0)	10.0 (0.0)	10.9 (0.0)	12.3 (0.0)	10.2 (0.0)	7.1 (0.0)	11.3 (0.0)	9.0 (0.0)	200 [18]

(**Bold**) values represent concentration data. Flavor units are shown below concentrations. na = not applicable (i. e. compound not detected) FU < 1 Little to no flavor contribution, FU = 1–2 Moderate flavor contribution, FU > 2 Significant flavor contribution [28] water; [18] [29] beer

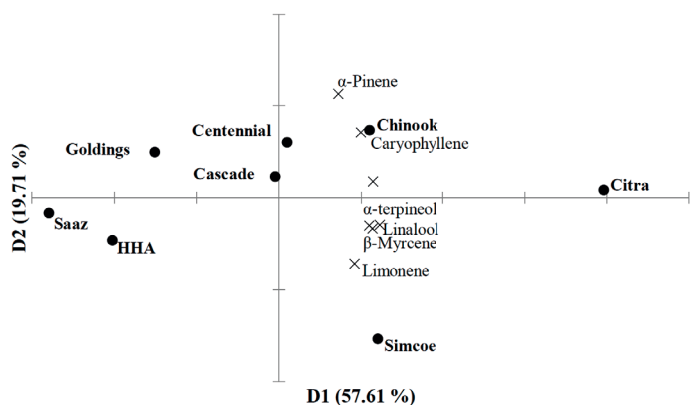


Fig. 6 Principle component analysis of flavor unit data of hop compounds found in beer from GC-MS instrumental analysis. Dimension 1, 2 and 3 account for 89.4 % of total variation

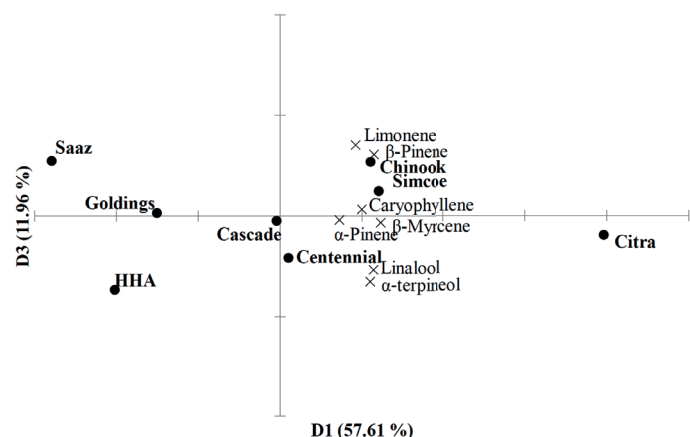


Fig. 7 Principle component analysis of flavor unit data of hop compounds found in beer from GC-MS instrumental analysis. Dimension 1, 2 and 3 account for 89.4 % of total variation

4 groups (Fig. 2) using AHC. When compared to genetic groupings [17] treatments reflected the differences between hops of European descent (Hallertau Mittelfrueh, Saaz, EK Goldings) and American descent (Simcoe, Citra, Chinook, Centennial), with the exception of Chinook being grouped with hops of European descent. In particular, Citra and Simcoe were clustered together, and while their full pedigree is not known they are of a newer breed of post millennium North American hops compared to Cascade, Centennial and Chinook. When averaged across all descriptive terms, Citra and Centennial were rated the highest in aroma while Saaz and East Kent Goldings were rated the lowest (Fig. 1). The American cultivars had the highest scores for grapefruit, passion fruit, piney, and green hop whereas the European cultivars were rated low in all of these categories. Within the American cultivars there exists a range of aromatic differences. Centennial had the highest piney and green hop aromas, but was low in the fruity and tropical fruit aromas. Citra and Simcoe were characterized as being very fruity, citrusy, and tropical (especially Citra), while Chinook had notes of floral, rose and green apple. In contrast, the European cultivars were rated higher than the American counterparts in terms of melon and DMS- like aroma. The Hallertau Mittelfrueh had the highest floral and melon aromas of all the hops. While Saaz and East Kent Goldings had the highest DMS- like aroma scores of the set, they were often rated low in aromatic intensity across nearly all of the attributes. Lack of any appreciable variation in the esters and the higher alcohol phenylethyl alcohol, with the possible exception of Simcoe, indicates that the fermentations were consistent across the 8 hop treatments (Fig. 5). Furthermore, all of these compounds were detected well below sensory detection threshold levels. The estery descriptor was not significant for any of the treatments. It is interesting to note that phenylethyl alcohol has a rose-like aroma [18], and despite the only subtle variation in this compound across the hop treatments, there were differences in the level of the rose

descriptor that was hop cultivar dependent (Table 3). This may be due to other hop-derived compounds that have floral like aromas such as β -citronellol or geraniol [19], which were not measured in this study.

When mean hop scores are plotted against a total oil content (Fig. 9) most do not correlate with increasing hop aroma with the exception of Guava ($R^2 = 0.8$), Passionfruit ($R^2 = 0.6$), and grapefruit ($R^2 = 0.76$) suggesting that these descriptors are correlated with increasing oil content. This is not to say that more oil means more aroma, only that for the cultivars and hopping regimes used in this study, increased oil content was associated with increased higher grapefruit, guava, and passion fruit aromas. Additionally, the high oil contents of Citra and Simcoe hops may be introducing significant leveraging effects on the correlation coefficients.

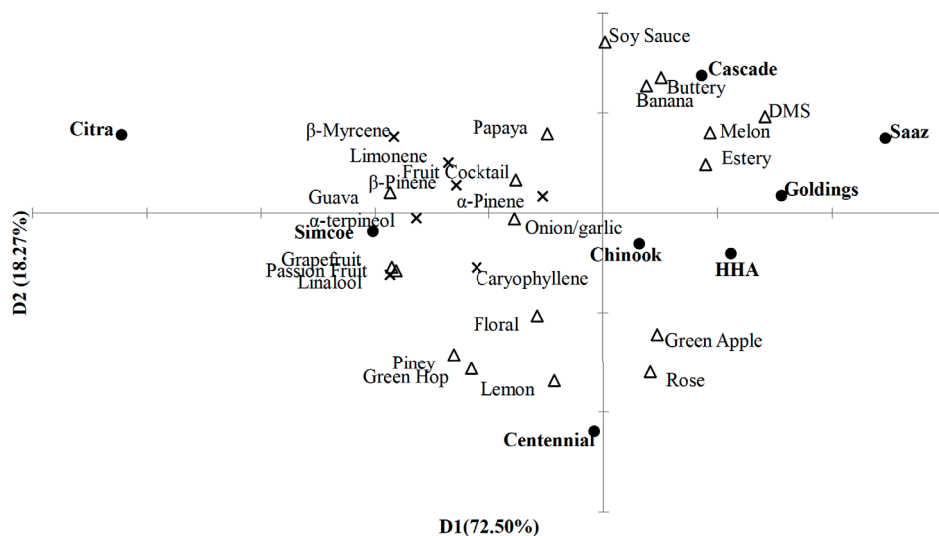


Fig. 8 Generalized procrustes analysis of combined sensory and GC-MS data from single hop beer evaluation. Dimensions 1 and 2 account for 90.78% of the total variation

Principal components analysis of the GC-MS data yielded distinct separations between the American cultivars Centennial, Chinook, Citra, and the European cultivars such as Hallertau Mittelfrueh thereby identifying a clear separation between the citrusy hops and the European (non-citrusy) hop.

The PCA procedure is both a data reduction procedure and a means of studying interrelationships within a complex data set. In terms of data reduction, we used it to find those factors (compounds and their flavor units, in this case) that have the greatest influence on differences in the sample treatments (aromatic components from beer made from individual hop cultivars). For instance, α -pinene and limonene are aligned closely with Chinook and opposite Hallertau Mittelfrueh. We can infer that compounds associated with these hops may be distinguishing features of Chinook hop aroma in beer and relatively less so for Hallertau Mittelfrueh. The close proximity of these peaks in the PCA space indicates they were highly correlated with each other in this study. Finally, since these compounds lie on Dimension 1, which describes the greatest amount of variation in the data set, these compounds may be very important for assessing differences among the set of eight hop cultivars.

Mapping the sensory data to the instrumental data via Generalized Procrustes Analysis revealed interrelationships between the aromatic descriptors and the individual volatile compounds measured by GC-MS. Using the GPA, we can see that Citra, and to a lesser extent Simcoe, are highly correlated with guava, fruit cocktail, and onion/garlic notes and that these aromas are associated with α -pinene, β -pinene, and limonene. The clear separation of Citra and Simcoe from the other hop cultivars reflects the strong pungent aromas characteristic of Citra and Simcoe hops. These descriptors are likely derived from very odor active thiols found in many cultivars of North American descent [20, 21]. Centennial, and to a lesser extent Chinook, were correlated with more rose and green apple like aromas that could not be related to the aroma compounds studied here. A clear separation in the PC space is

shown between the European hops and the modern American hop cultivars Citra and Simcoe. Cascade was characterized by mostly buttery, banana, and melon notes. These aromas, especially buttery and banana, are associated with common beer defects, diacetyl and isoamyl acetate respectively. These defects indicate possible problems during or after fermentation and therefore this particular hop treatment was viewed as an outlier in terms of the sensory analyses. However, this is surprising since Cascade did not have higher ester concentrations than the other treatments, specifically isoamyl acetate, which would indicate inconsistent fermentations. Also, Cascade hops are well known for having a very citrus and floral like quality, yet these attributes were not described in the study. This demonstrates that despite the moderate hopping rates used in this study, strong non-hop-derived aromas can overwhelm and mask hop aroma in beer. Also, previous research has shown that a number of other factors, such as harvest timing and location [22, 23], processing [24, 25], and storage [26] can influence the aroma characteristics for a given sample of hops. Hops used in the study presented here were from a single lot of hops and were not controlled for the factors mentioned above and it is possible that within cultivar variation of hop quality may be a factor in determining the hop aroma in beer. As such, further investigations into the within-cultivar variation effects on hop aroma are warranted.

In this study, hops were added at three different points throughout the brewing process in order to mimic common industry practices. However, this eliminated the possibility to investigate changes in hop aroma due to different hopping regimes. Future studies would benefit from single hop additions and allow for the investigation of differences in hop aroma due to cultivar and addition effects. Furthermore, one should bear in mind that even though the specific hop compounds identified in this study are associated with certain aromas and cultivars, they may not necessarily be responsible for those aromas. Considering the large number of volatile compounds found in hops and beer, it is likely that the compounds examined here do not fully explain the sensory differences. For example,

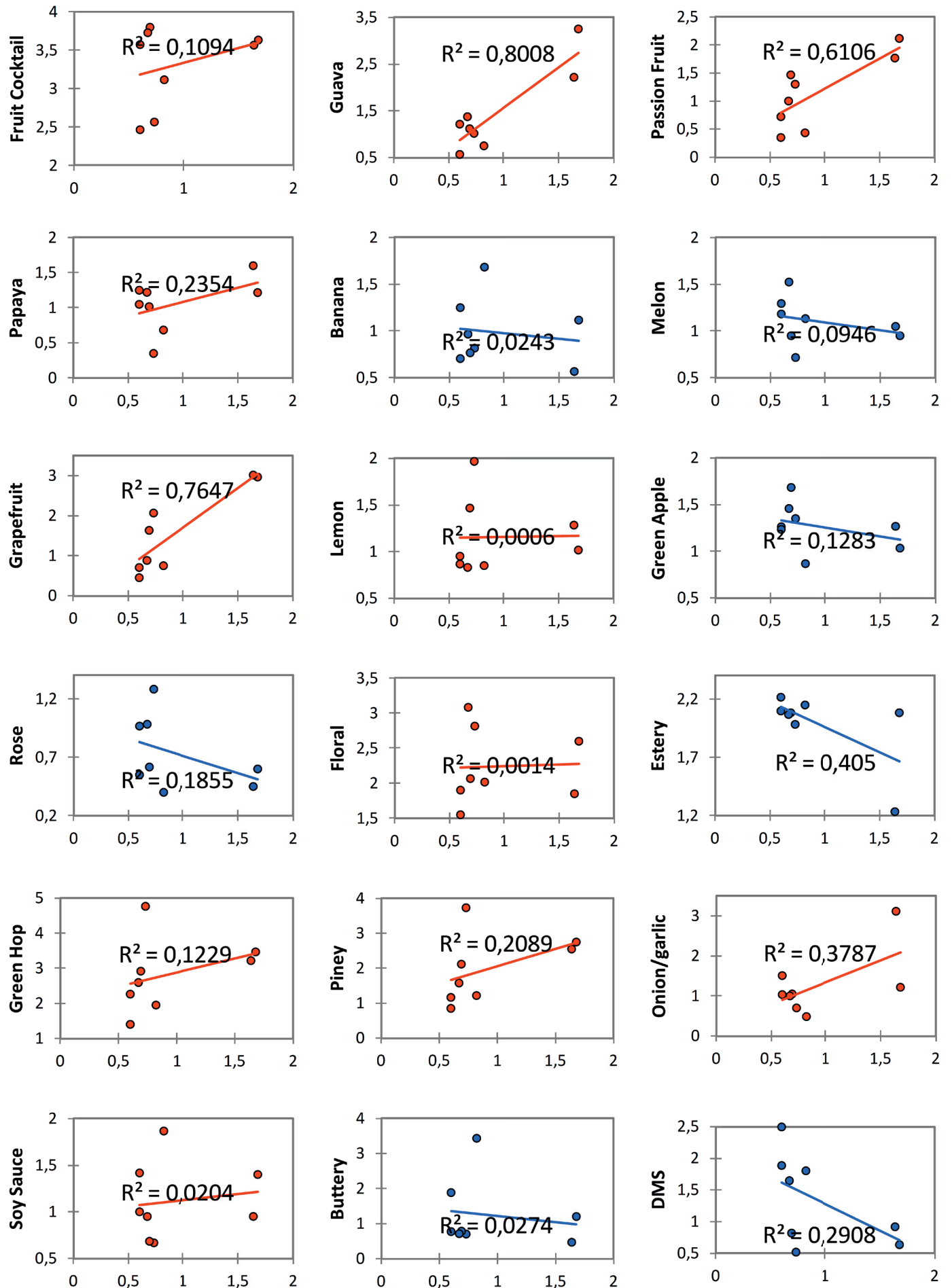


Fig. 9 Scatterplot matrix and correlation coefficients of sensory descriptors vs. essential oil content (ml/100 g hops)

sulfur containing hop compounds have been associated in previous studies as contributors to American hop aroma [27], particularly to the guava and tropical aromas in hops such as Simcoe, Citra and Cascade. Although previous researchers have detected these compounds at trace levels in hops, their contribution to aroma is substantial due to their extremely low flavor thresholds. The compounds investigated here were those primarily reported by hop suppliers on hop specification sheets: myrcene, linalool, humulene, and caryophyllene. However, these specifications are primarily for cultivar identification purposes and not for recipe formulation or to describe the flavor and aroma profile of a given hop. With the popularity of aroma hops increasing, it would be helpful for hop suppliers to provide a more detailed analysis of hop volatiles to aid the brewer in recipe formulation. Nevertheless, while only a handful of compounds were quantified in this study of the hundreds of volatile compounds found in beer, clear differences between the aromas produced by the American and European hop cultivars were found.

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