Dry Hopping with the Dual-Purpose Varieties Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace: Minor Contribution of Hop Terpenol Glucosides to Beer Flavors

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ABSTRACT

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The dual-purpose hop varieties Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace were recently shown to contain unusually high amounts of some discriminating terpenoids, polyfunctional thiols, and precursors of the latter (cysteine and glutathione adducts). The present work aimed to investigate the terpenol glucoside fraction in hops and its potential contribution to beer after a dry hopping process. Terpenols were quantified by stir-bar sorptive extraction GC-MS in five pilot monovarietal dry-hopped beers. In all of them, linalool and geraniol were found above their sensory thresholds (72–178 and 7–57 µg/L, respectively, for a threshold of 8 µg/L for linalool and 4 µg/L for geraniol). β-Citronellol also exceeded its threshold when the Amarillo, Citra, or Sorachi Ace cultivars were used. The hop glucoside potential was analyzed by GC-MS after enzymatic degradation. A relative hydrolysis efficiency factor was applied to our data to take into account that the commercial β-glucosidase releases octan-1-ol, used here as an internal standard, 2.8 times more efficiently than geraniol. β-Glucosidase treatment caused the release of linalool, α-terpineol, β-citronellol, and geraniol from all five dual-purpose cultivars, but in much lower amounts than the corresponding free terpenols (0.6–28.6 mg/kg of aglycons versus 7.8–109.2 mg/kg of free forms). Further quantitative analyses focusing on more traditional aromatic and bitter hops are now needed to compare their glucoside fractions with those here investigated.

Keywords: Hop (*Humulus lupulus* L.), Terpenic alcohols, Flavor precursors, Dry hopping, Beer

Hop (*Humulus lupulus* L.) breeding has yielded dual-purpose cultivars producing hop cones characterized by both high bitter acid (>7% humulones) and high flavoring compound contents.

Terpenes ($(C_5H_8)_n$) and their oxygenated analogs (usually referred to as terpenoids) have long been used to discriminate between aromatic hops (containing less than 5–7% α-acids) and bitter hops (containing more) (3,4,24,27). Farnesene and bergamotene have emerged as markers of the former, whereas high amounts of esters such as 3-methylbutyl isobutyrate have been found only in the latter. The arrival of dual-purpose hop varieties has completely modified such classifications. Despite its 12.3% α-acid level, Sorachi Ace contains more farnesene than the prestigious aromatic cultivar Saaz. Recently, Gros et al. (11) and Kankolongo Cibaka et al. (16) showed that the monoterpenic alcohols linalool (floral/lavender/coriander/citrus-like flavor), geraniol (floral/rose-like flavor), and β-citronellol (lemon/lime-like flavor) can be used to distinguish dual-purpose hop varieties from others. Linalool has been found at 100–312 mg/kg in Nelson Sauvin, Tomahawk, and Amarillo hops, whereas geraniol characterizes Tomahawk, Amarillo, Citra, and Mosaic hops (69–247 mg/kg). The highest β-citronellol content (33 mg/kg) has been found in the Sorachi Ace variety.

Together with polyfunctional thiols, monoterpenic alcohols most probably impart citrus notes to beers hopped with dual-purpose varieties. Indeed, taking into account a maximum dilution factor of 500 between hop and beer (hopping rate close to 2 g/L in Europe, often much more in the United States), terpenoids should logically be found above their sensory thresholds: 8 µg/L for a mixture of linalool enantiomers (14), 4 µg/L for geraniol (20), and 8 µg/L for β-citronellol (32). Moreover, synergistic effects have been shown to exist among terpenoids: Takoi et al. (32) found linalool perception to be enhanced by the presence of 5 µg/L of geraniol and citronellol. Recently, Sanekata (28) evidenced an unexpected role of geranic acid in beers late-hopped with the Sorachi Ace variety, owing to synergy with other terpenoids.

Linalool is a chiral compound whose (*R*) and (*S*) enantiomers are found in hops at an approximate distribution ratio of 95:5 (7,12,31). Racemization occurs, however, during wort boiling (31). This racemization of linalool can still occur during beer aging (13). More than 80% (*R*)-enantiomer characterizes dry-hopped beers (31). (*R*)-Linalool is much more odorant than its enantiomer (threshold in beer = 1 µg/L [20] or 2.2 µg/L [31], versus 80 times as much for the (*S*)-isomer [31]).

Flavor-active compounds are very different from hops to beer. First, small, lipophilic hop odorants are strongly degraded or lost during wort boiling (21). This is the case of the major hop monoterpene, myrcene, which according to Forster and Gahr (6) remains present at near-threshold concentrations (9.5 µg/L) (19) only in dry-hopped beers. Second, many bioconversions occur during fermentation. Lam et al. (23) described the biosynthesis of geraniol through geranial reduction or geranyl acetate and geranyl isobutyrate hydrolysis. Geraniol and nerol can be reduced to (*R*)-β-citronellol or isomerized to linalool and α-terpineol (10,18, 23,32). They can also be esterified by *Saccharomyces cerevisiae* to geranyl, neryl, or citronellyl acetates (17).

Hop terpenoid glycosides constitute an additional source of terpenoids in beer. These odorless, nonvolatile precursors have been evidenced in hops and water-soluble hop products (pellets, ethanolic extracts, and spents) (8,9,22,25). Goldstein et al. (9) claimed that hop glycosides are responsible for the kettle hop flavor of beer, after both chemical and biochemical hydrolyses. β-Glucosidases or yeast can release free geraniol, linalool, and α-terpineol from hop extracts (8,9,22). Murakami et al. (26) have shown that these glycosides can also occur in plant parts other than hop cones. More recently, Kollmannsberger et al. (22) compared different hop products and found more glycosides in spent hops (residual hop fraction after supercritical CO_2 extraction of humulones) and ethanolic extracts than in CO_2 extracts. They also found the contribution of glucosides to beer flavor to depend on the hop variety used. The lowest amount of linalool glucoside was found in the bitter Hallertau Magnum hop variety, whereas Saaz

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exhibited much higher amounts of glucosides, especially bound norisoprenoids (22,30). Unfortunately, quantitative data are lacking in most of these publications, and little is yet known about the relative abilities of brewing yeasts to release aglycons during primary fermentation or maturation. It was recently suggested that in the presence of *Brettanomyces* yeasts, the nutrient depletion encountered in maturation might favor terpenol and thiol release in a dry-hopped beer (2).

The aim of the present study was to assess to what extent the free and bound terpenoid profiles of five dual-purpose hop varieties might predict the flavor of the derived dry-hopped beers. Both hops and derived dry-hopped beers were analyzed. Bound terpenoids of the Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace dual-purpose cultivars were quantitated after β-glucosidase treatment. The same hops were used to produce pilot monovarietal dry-hopped beers in which terpenols were quantified.

EXPERIMENTAL

Chemicals

1-*O*-Octyl-β-D-glucopyranoside (the internal standard [IST] for β-glucosidase assays), β-glucosidase (from almonds, ≥6 U/mg), Amberlite XAD 2 resin, diethyl ether inhibitor-free (>99.9%), myrcene, linalool, α-terpineol, β-citronellol, geraniol, geranyl acetate, phenol glucoside, dodecane (the external standard for β-glucosidase assays), and 2-acetylthiophene (IST for terpenoid extractions from beer) were purchased from Sigma-Aldrich (Bornem, Belgium). Geraniol glucoside was purchased from Carbosynth (Compton, U.K.). Methanol, ethanol, diethyl ether (99.9%), and 37% HCl were obtained from VWR (Leuven, Belgium). Milli-Q water was used (Millipore, Bedford, MA, U.S.A.). NaOH, NaCl, sodium acetate (99%), and sodium sulfate (99%) were supplied by Acros Organics (Geel, Belgium). Polyvinylpolypyrrolidone was supplied by Spindal (Gretz-Armainvilliers, France).

Hop Samples

Amarillo, Citra, Mosaic, and Sorachi Ace bred in the United States were provided by Yakima Chief (Ottignies-Louvain-la-Neuve, Belgium). Hallertau Blanc hops were supplied by Hopsteiner (Mainburg, Germany). Saaz hops were purchased from Brouwland (Beverlo, Belgium).

Pilot Beer Production

All studied beers were produced in a 60 L microbrewery (Coenco, Oostkamp, Belgium) with the same brewing protocol. For the production of all pilot beers, 12.3 kg of pilsen malt (two-row spring malt, from Boortmalt, Antwerpen, Belgium) was brewed in 33.9 L of water, according to the following mashing program: 60 min at 60°C and 25 min at 72°C. The wort was then heated to 78°C and filtered through the lauter tun. After sparging, 62 L of wort with a density of 12°P was obtained. The wort was boiled with 126 mg/L of Tomahawk CO_2 extract (just used here as a bitterness contributor) for 90 min (8–11% evaporation), and the final density was adjusted to 12°P by addition of water. The fermentation was conducted in cylindroconical fermenters with an ale-type yeast (INBR Bras212, propagated in a glucose/maltose/yeast extract/peptone medium). This strain was pitched at 7.5×10^6 cells/mL. Fermentation was carried out at 22°C for 7 days. Dry hopping was applied to each beer with a single hop variety, except for the control beer, which was not dry hopped. The selected hop varieties were Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace, respectively, for the dry-hopped beers A-DHB, C-DHB, HB-DHB, M-DHB, and SA-DHB. To allow comparisons, the same hopping rate was applied (2 g/L, unmilled hop pellets in a cotton net). Hoppings were conducted in the presence of yeast at a constant temperature of 16°C for 14 days. The beer was then kept at 4°C for 7 days. All the beers (including the control beer) were filtered on plates (8 μm pores followed by 0.5 μm pores, Buon Vino, Cambridge, ON, Canada) under a CO_2 atmosphere; the beer was carbonated at 5 g/L and stored at 4°C. To compare the flavors issued solely from dry hopping with those coming from combined late and dry hoppings, a sixth pilot beer (SA-LH+DHB) was obtained, with addition of Sorachi Ace hops both to the wort at the end of boiling (2 g/L) and during maturation (2 g/L). The main characteristics of the pilot beers obtained are presented in Table I. The differences between bitterness values may be explained by the fact that for dry-hopped beers, nonisomerized α-acids and humulinones significantly contribute to the measured absorbance at 275 nm (ASBC method) (1).

Extraction of Terpenoids from Beer by the Stir-Bar Sorptive Extraction Method

IST (500 μL) (50 mg/L of 2-acetylthiophene in ethanol) was added to 50 mL of degassed beer. The sample was diluted with 50 mL of saturated aqueous sodium chloride solution in the extraction vial, and a polydimethylsiloxane (PDMS)-coated stir bar was added (Twister, 0.5 mm film thickness, 10 mm length, Gerstel, Mülheim an der Ruhr, Germany). After the vial was capped, the PDMS-coated bar was used to stir the mixture for 2 h at 20°C in order to extract the aroma compounds by adsorption. The stir bar was then removed from the extraction vial, soaked briefly in Milli-Q water, and dried with a lint-free tissue. The odorant compounds were thermally desorbed from the stir bar into a thermal desorption unit (TDU; Gerstel) online with the gas chromatography–mass spectrometry (GC-MS) system. Reconditioning of the PDMS-coated bars after GC-MS was performed first by stirring ultrapure water (100 mL) for 30 min and then by stirring acetonitrile (100 mL) for 90 min. The stir bars were then removed from the washing solvent and dried on a clean surface at room temperature before thermal conditioning for 30 min at 260°C under a flow of helium.

TDU and Cooled Injection System Conditions

Thermal desorption of the odorant compounds from the stir bar was carried out in the TDU in splitless mode, for which the temperature was programmed to rise at the rate of 150°C/min from 25 to 250°C (held for 5 min). Liquid nitrogen was used to trap the desorbed compounds in the cooled injection system (CIS4 inlet) at –100°C. For injection of the cryofocused odorant compounds

TABLE I
Main Characteristics of Pilot Beers[a]

Characteristic	A-DHB	C-DHB	HB-DHB	M-DHB	SA-DHB	SA-LH+DHB	Control
Ethanol (%, v/v)	4.3	4.0	4.0	3.6	5.1	4.3	4.0
pH	4.2	4.5	4.1	4.2	4.4	4.2	4.2
Bitterness (°EBU)	13.5	16.6	11.8	12.5	16.1	20.2	8.5

[a] The measured values are means of duplicates. The variation coefficients of these measurements were below 15%. Dry-hopped beers A-DHB, C-DHB, HB-DHB, M-DHB, and SA-DHB were made with Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace, respectively. For SA-LH+DHB, Sorachi Ace was added both at the end of boiling and during maturation.

onto the analytical column, the CIS4 inlet temperature was programmed to rise at the rate of 720°C/min from –100 to 250°C (held for 5 min).

GC Combined with Electronic Impact MS

Flavors were analyzed with a wall-coated open tubular apolar capillary column (CP-Sil 5 CB, 50 m × 0.32 mm i.d., 1.2 µm film thickness) on an Agilent 6890N GC. Injections were carried out at 250°C in splitless mode. The carrier gas was helium, and the pressure was set at 134 kPa. The oven temperature was programmed to rise from 36 to 85°C at 20°C/min, then to 145°C at 1°C/min, and finally to 250°C (held for 30 min) at 3°C/min. The column was connected to a single quadrupole mass spectrometer (Agilent 5973N MSD) operating in single ion monitoring (SIM) mode with electron ionization at 70 eV. The following *m/z* values were monitored: 56 (for octan-1-ol), 69 (for β-citronellol, geraniol, and geranyl acetate), 93 (for myrcene, linalool, and α-terpineol), and 111 (for 2-acetylthiophene). Chromatograms were recorded throughout elution with Gerstel Maestro software (version 1.3).

Quantitation of Terpenoids in Beer

The standard addition method was performed for each compound (A), and the following equation was used for quantitation in beer: concentration of A (in µg/L) = IST concentration (in µg/L) × (A area/IST area) × relative standard addition slope.

Sensory Evaluation of Dry-Hopped Beers

To roughly assess the aroma profile of each pilot beer, descriptive flavor analyses were performed in a single session by 13 previously trained panelists (members of our brewing department, all familiar with beer sensorial descriptors). The overall dry-hopped beer flavors were broken down into 12 aroma descriptors (litchi, lemon, tangerine, grapefruit, passionfruit, grapes, blackcurrant, grenadine, fruity, green, coriander, and rose). The sensory panel was asked to assess the intensities of each odor characteristic in the beers (one trial per beer). The odor intensities were rated from 0 to 3 on the following scale: 0 = not perceivable, 0.5 = weak, 1 = normal, 2 = strong, and 3 = very strong. The odor characteristics between the different beers were compared by calculating the mean of assigned intensity values (generally in the same range for most of the panelists).

Extraction of Terpenoid Glycosides from Hop Samples

Glycosides were extracted from beer according to the procedure described by Scholtes et al. (30). Milled hops (20 g) were spiked with 1 mL of IST (1 g/L of 1-*O*-octyl-β-D-glucopyranoside in H_2O/MeOH, 80:20 v/v), and 250 mL extraction solvent (H_2O/MeOH, 80:20 v/v) was added. Extraction was performed by mixing with an Omni mixer (Omni International, Kennesaw, GA, U.S.A.) for 10 periods of 30 s each, interrupted by 10 resting periods of 30 s each. The mixture was then centrifuged for 10 min at 10,000 rpm. The supernatant was recovered, and the extraction was repeated on the residual solid with 250 mL of the hydroalcoholic solvent. The two supernatants were combined, filtered with a Büchner funnel, and concentrated by rotary evaporation down to 300 mL volume. The polyphenols present in the extract were partially removed by adding 6 g of polyvinylpolypyrrolidone and stirring for 60 min. After a second Büchner filtration, the hop extract was divided equally between two Schott flasks. Amberlite XAD 2 resin (6 g, rinsed beforehand with approximately 400 mL of Milli-Q water) was poured into each flask. After shaking for 2 h, the mixtures were loaded into glass columns, and the upper liquid phases were removed. Each column was then rinsed consecutively with 50 mL of water and 25 mL of diethyl ether. Glycosides were finally eluted with 25 mL of methanol. The solvent was evaporated under reduced pressure, and the obtained solid extracts were stored at –20°C before β-glucosidase assays.

β-Glucosidase Treatment of Hop Glycoside Extracts and Standards

Each glycoside extract was solubilized in 25 mL of acetate buffer (pH 5). Commercial β-glucosidase (14 mg) was added, and incubation was performed at 35°C for 2 h. A control without enzyme was conducted in parallel. Terpenoids were extracted three times with 15 mL of diethyl ether inhibitor-free. The combined organic phases were dried with anhydrous sodium sulfate, 0.5 mL of external standard (20 mg/L of dodecane in diethyl ether inhibitor-free) was added, and the extract was concentrated to 0.5 mL in a Danish-Kuderna apparatus at 39°C. The obtained extracts were stored at –80°C, and 1 µL was injected into the GC-MS system (splitless mode, the split being turned on after 0.5 min; same GC-MS conditions as described earlier; *m/z* values of 67, 71, and 137 for SIM monitoring of 8-hydroxylinalool). The released IST aglycon was octan-1-ol, resulting from the hydrolysis of 1-*O*-octyl-β-D-glucopyranoside. To assess relative hydrolysis efficiencies of the commercial enzyme on different glucosides, the above-described assay was also applied to a model medium containing the IST (1-*O*-octyl-β-D-glucopyranoside), phenol glucoside, and geraniol glucoside (1, 0.3, or 0.1 mg of each compound). This experiment led us to apply a corrective factor of 2.8 (=0.14/0.05) in the subsequent quantitation equation, taking into account that 14% of the IST was degraded when hydrolyzing 1 mg, for only 5% in the case of geraniol glucoside (Table II). Because pure standards of other terpenol glucosides were commercially unavailable, the same factor was applied for all of them.

Quantitation of Terpenoids Released from Hops by Enzymatic Treatment

The following equation was used to quantify released compound X, using 1-*O*-octyl-β-D-glucopyranoside as the IST: Concentration of X (in mg/kg) = 2.8 × concentration of IST (in mg/kg) × (molecular weight of IST aglycon/molecular weight of IST) × (peak area of X/peak area of released IST aglycon) × (mass response coefficient of released IST aglycon/mass response coefficient of X).

Statistical Analyses

All analytical measurements were carried out in duplicate. Multiple comparisons of means were performed with Tukey's test. Values not sharing any common letter in the same row of the ta-

TABLE II
Rate of Almond β-Glucosidase Catalyzed Conversion of 1-*O*-Octyl-β-D-glucopyranoside (Internal Standard [IST]), Phenol Glucoside, and Geraniol Glucoside to the Corresponding Aglycon; Hydrolysis Efficiencies Relative to the IST Are Given in Parentheses

		Conversion rate, % (relative hydrolysis efficiency)		
Assay number	Amount of each spiked glucoside	Octanol glucoside, IST	Phenol glucoside	Geraniol glucoside
1	1 mg	14 (1.0)	10 (0.71)	5 (0.36)
2	0.3 mg	12 (1.0)	8 (0.67)	6 (0.50)
3	0.1 mg	9 (1.0)	6 (0.67)	5 (0.56)

bles are significantly different ($P < 0.05$). Principal component analysis (PCA) was applied to the data of the analyzed beers as samples and the organoleptic descriptors as variables. The PCA was done using the correlation matrix instead of the covariance matrix to limit the possible influence of different scales in the data. The analysis was performed on averages, and varimax rotation was not used. All statistical analyses, including multivariate analysis, were carried out with SAS software version 9.4 (SAS Institute, Cary, NC, U.S.A.).

RESULTS AND DISCUSSION

The terpenoid glucosides present in six hop varieties (including Saaz used here as a reference) were quantified by GC-MS after purification and hydrolysis by a commercially available β-glucosidase. To calculate their concentrations, a coefficient was used to take into account the relative efficiencies of 1-*O*-octyl-β-D-glucopyranoside (IST) and terpenol glucoside hydrolysis: as depicted in Table II, and probably because of more steric hindrance, geraniol proved to be 2.8 times less efficiently released from its glucoside than octanol (relative hydrolysis efficiency to the IST = 0.36 for 1 mg of glucoside). In all cases, the conversion rates remained low (5–14%).

The amounts of bound linalool, α-terpineol, β-citronellol, and geraniol present in the five dual-purpose investigated hops, as determined here by glucoside extraction and hydrolysis, were further compared with the previously published free fractions (16) (Table III, Fig. 1). Surprisingly, all four aglycons were found in lesser quantity than the free forms (0.8–86%), although in higher amounts than in the Saaz hop reference.

The Sorachi Ace variety, which in the previous study appeared the richest in free terpenes and terpenoids (8,522 mg/kg), also showed the highest amount of total aglycons (52.6 mg/kg), followed by Citra (34.2 mg/kg), Amarillo (20.2 mg/kg), Mosaic (8.1 mg/kg), and Hallertau Blanc hops (6.4 mg/kg). Yet the published levels of free linalool and β-citronellol in this variety are 57.9 and 33.3 mg/kg, compared with only 7.0 and 28.6 mg/kg for the bound forms as quantified here.

The same Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace dual-purpose hop varieties were selected to dry hop (at 2 g/L) green beers that had been bittered only with CO_2 hop extracts in the boiling kettle. The obtained beers were named A-DHB, C-DHB, HB-DHB, M-DHB, and SA-DHB, respectively, to indicate dry hopping with Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace. For comparison with the dry-hopped beer SA-DHB, another beer, named SA-LH+DHB, was produced by combining late (2 g/L) and dry hopping (2 g/L) with Sorachi Ace. Finally, a control with no added pellets was also investigated.

As depicted in Figure 2, 12 typical "dry hopping" descriptors were assessed by our 13 panelists: litchi, lemon, tangerine, grapefruit, passionfruit, grapes, blackcurrant, grenadine, fruity, green, coriander, and rose. These odors most probably result from the coexistence of hop-derived terpenoids with polyfunctional thiols (15), C13 norisoprenoid derivatives, and esters. A-DHB was characterized by an overall fruity flavor, with a predominance of litchi and grape aromas, together with notes of passionfruit, grapefruit, and lemon. HB-DHB was perceived similarly to A-DHB, except for the litchi-like flavor. C-DHB was characterized by an overall citrus and tropical fruit flavor, with a predominance of blackcur-

TABLE III
Concentrations (mg/kg of Hops) of Free Monoterpene Alcohols and Corresponding Aglycons Released from β-Glucosidase Treatment Applied to Purified Hop Glycoside Extracts[a]

		Amarillo		Citra		Hallertau Blanc		Mosaic		Sorachi Ace		Saaz	
Substance	**Odor**	**Free**	**Aglycon**	**Free**	**Aglycon**	**Free**	**Aglycon**	**Free**	**Aglycon**	**Free**	**Aglycon**	**Free**	**Aglycon**
Linalool	Floral/citrus	109.2	1.4bc	18.6	2.8b	18.6	0.6c	58.0	0.8c	57.9	7.0a	26.2	0.0c
α-Terpineol	Pine/lilac	na	5.6a	na	2.5b	na	1.1b	na	1.4b	na	5.6a	na	0.0c
β-Citronellol	Lemon/lime	10.6	1.7b	7.8	3.1b	13.0	0.8b	10.0	0.3b	33.3	28.6a	1.0	2.3b
Geraniol	Floral/rose	105.1	11.5b	68.9	25.8a	16.6	3.9b	83.2	5.6b	18.9	11.5b	12.9	2.1b
8-Hydroxylinalool[b]	Citrus	na	1.9b	na	2.8b	na	na	na	na	na	na	na	7.8a
Total[c]		224.9	20.2bc	95.3	34.2b	48.2	6.4c	151.2	8.1c	110.1	52.6a	40.1	4.4c

[a] Measured concentrations were determined by GC-MS. Free forms were previously measured by Kankolongo Cibaka et al. (16) and Gros et al. (11). na = Data not available. Values of aglycons in the same row that do not share a common letter are significantly different ($P < 0.05$).
[b] Measured in internal standard equivalents.
[c] Excluding 8-hydroxylinalool.

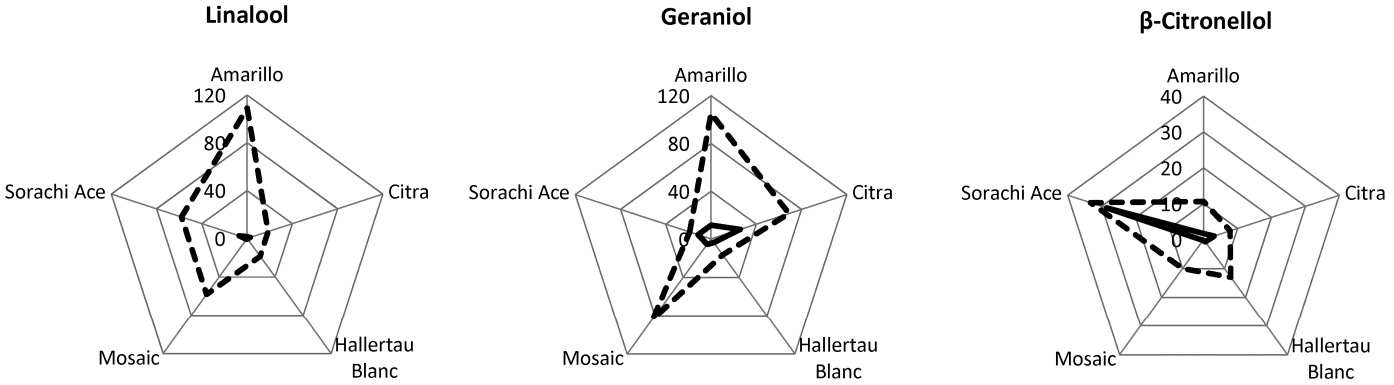

Fig. 1. Concentrations (mg/kg of hops) of free monoterpenols (dashed line) and of the corresponding aglycons (solid line) released through β-glucosidase treatment applied to purified hop glycoside extracts.

rant notes. The green flavors were more perceived in M-DHB and SA-DHB, together with coriander in the latter. The grape and citrus notes were perceived with the highest intensity in SA-LH+DHB. The combination of late and dry hopping led to a more complex overall green, floral, and fruity flavor in SA-LH+DHB than in SA-DHB. PCA was performed on these data with the analyzed beers as samples and the organoleptic descriptors as variables. Figure 3 shows the distribution along the first two components, which together explained 87% of the data dispersion. A-DHB and HB-DHB emerged, logically, in the same map area. The panelists were also asked to rank the dry-hopped beers according to their preference, and A-DHB emerged as the beer preferred above all the others.

After sensory analysis, the beer terpenoid profiles were determined by Twister-GC-MS and compared with the flavor potential of the corresponding hop varieties (Table IV).

Linalool was found in all investigated beers, at concentrations above its odor perception threshold of 8 µg/L (Fig. 4). The highest concentrations of linalool were found in C-DHB and A-DHB (178 and 150 µg/L, respectively). In five beers, the linalool concentration was in the range of what we expected on the basis of both free and bound linalool content in hops (Table IV). One exception was the beer dry-hopped with Citra, whose linalool content was higher than expected from both the free and bound forms. Biochemical terpenoid interconversions probably explain our results (for example, linalool could derive from geraniol, linalyl, or geranyl acetates) (5,23). The contribution of 8-hydroxylinalool glucoside, previously evidenced by Kollmannsberger et al. (22), should also be deeply investigated. As depicted in Table III, some preliminary analyses confirm, at least, its occurrence in hops (1.9, 2.8, and 7.8 mg/kg IST equivalents in Amarillo, Citra, and Saaz, respectively).

Geraniol concentration ranged from 7 to 57 µg/L (Fig. 4) and thus exceeded its odor perception threshold (4 µg/L) in all beers. The highest concentration was found in A-DHB (57 µg/L). Here, the free and bound forms found in hops could explain the whole content of beer (Table IV). Forster et al. (5) have suggested that the fluctuation of geraniol transfer rates in different dry-hopped beers might be partly owing to the release of geraniol through geranyl acetate hydrolysis.

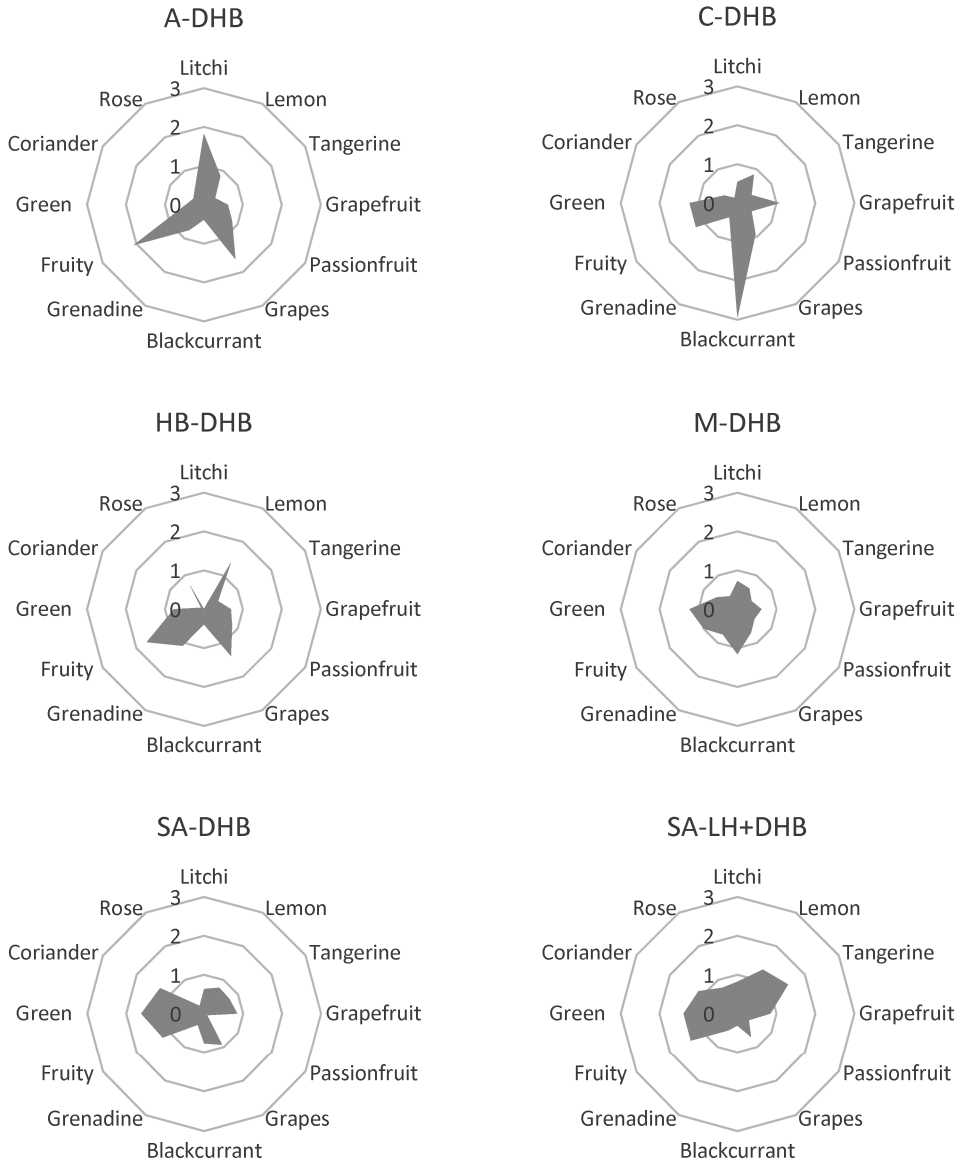

Fig. 2. Sensory profiles of dry-hopped beers. Dry-hopped beers A-DHB, C-DHB, HB-DHB, M-DHB, and SA-DHB were made with Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace, respectively. For SA-LH+DHB, Sorachi Ace was added both at the end of boiling and during maturation.

Concentrations of β-citronellol (Fig. 4) were also found at levels above the odor perception threshold (8 µg/L) in A-DHB, C-DHB, and SA-DHB (79, 12, and 12 µg/L, respectively). Although low contents (4 µg/L) were found in HB-DHB and M-DHB, β-citronellol might even contribute to their citrus/floral flavor, thanks to synergistic effects occurring with linalool and geraniol, as proposed by Takoi et al. (32). The β-citronellol transfer rate ranged from 5 to 58% in all beers except A-DHB, for which we found, as for linalool in C-DHB, values well above what we expected (Table IV).

The recorded α-terpineol concentrations (3–43 µg/L) did not exceed the odor perception threshold of this compound: 330 µg/L, which is high compared with other monoterpenols.

To assess the terpenoid contents achieved by combining late with dry hopping, this combination of hopping procedures was applied to obtain SA-LH+DHB (Sorachi Ace added at 4 g/L in total, instead of 2 g/L). Not surprisingly, this beer was characterized by a higher linalool content (121 µg/L) than SA-DHB (88 µg/L). Yet it was found to contain less α-terpineol, β-citronellol, and geraniol. The biggest difference between the two beers concerned myrcene, which surprisingly required boiling for efficient extraction (88 µg/L in SA-LH+DHB and 5 µg/L in SA-DHB). Myrcene reached a concentration above its odor threshold (9.5 µg/L) in SA-LH+DHB only.

CONCLUSIONS

In conclusion, assessing the terpenoid profile of a dry-hopped beer on the basis of hop analyses alone remains delicate. Terpenoid glucosides were found in lower amounts than the corresponding free forms in all the dual-purpose hop varieties here investigated. Yet their concentrations were revealed to be higher than those found in the Saaz cultivar. Further research should now be conducted to confirm these observations on other traditional aromatic and bitter hops.

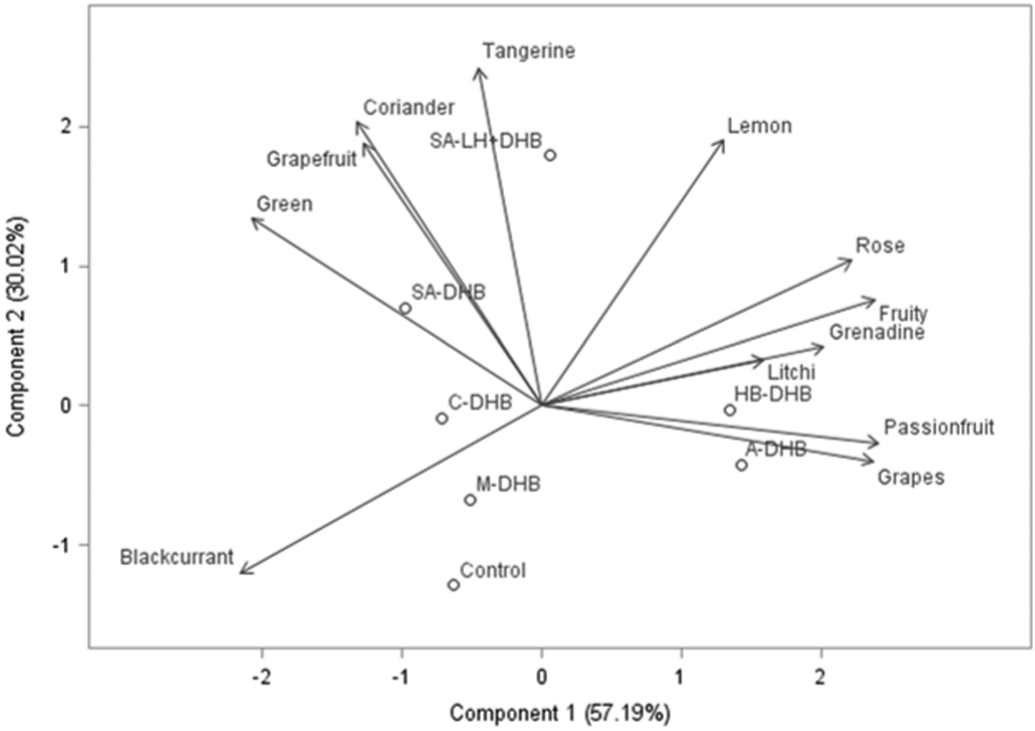

Fig. 3. Biplot of the first two principal components. Beers are presented as scores and odor descriptors as variables. Dry-hopped beers A-DHB, C-DHB, HB-DHB, M-DHB, and SA-DHB were made with Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace, respectively. For SA-LH+DHB, Sorachi Ace was added both at the end of boiling and during maturation.

TABLE IV
Concentrations (µg/L) of Terpenes and Free Terpenoids in Pilot Beers (Expected Values in Parentheses)[a]

Substance	Odor	A-DHB		C-DHB		HB-DHB		M-DHB		SA-DHB		SA-LH+DHB		Control	Threshold (µg/L)[b]
Myrcene	Lime/resinous	7c		5c		7c		9c		5c		88a		20b	9.5
Linalool	Floral/citrus	150b	(221)	178a	(43)	72d	(38)	76d	(118)	88d	(130)	121c	(260)	49e	8
α-Terpineol	Pine/lilac	43a		11c		3e		3e		23b		8d		3e	330
β-Citronellol	Lemon/lime	79a	(25)	12b	(22)	4c	(28)	4c	(21)	12b	(124)	2c	(248)	3c	8
Geraniol	Floral/rose	57a	(233)	33b	(190)	14b	(42)	7de	(177)	31b	(61)	11cd	(122)	4e	4
Geranyl acetate	Floral/lavender	10a		2e		10a		8b		2e		4d		5c	…

[a] Measured concentrations were determined by GC-MS. Values in the same row that do not share a common letter are significantly different ($P < 0.05$). Expected values are estimated on the basis of the free terpenoid contents previously measured by Kankolongo Cibaka et al. (16) and the aglycons measured here. A recovery factor of 100% from hops after dry hopping or combined late and dry hopping is assumed for the estimations. Dry-hopped beers A-DHB, C-DHB, HB-DHB, M-DHB, and SA-DHB were made with Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace, respectively. For SA-LH+DHB, Sorachi Ace was added both at the end of boiling and during maturation.

[b] Threshold references: Kishimoto et al. (19) for myrcene, Kaltner et al. (14) for linalool, Schmidt and Biendl (29) for α-terpineol, Takoi et al. (32) for β-citronellol, and Kishimoto et al. (20) for geraniol.

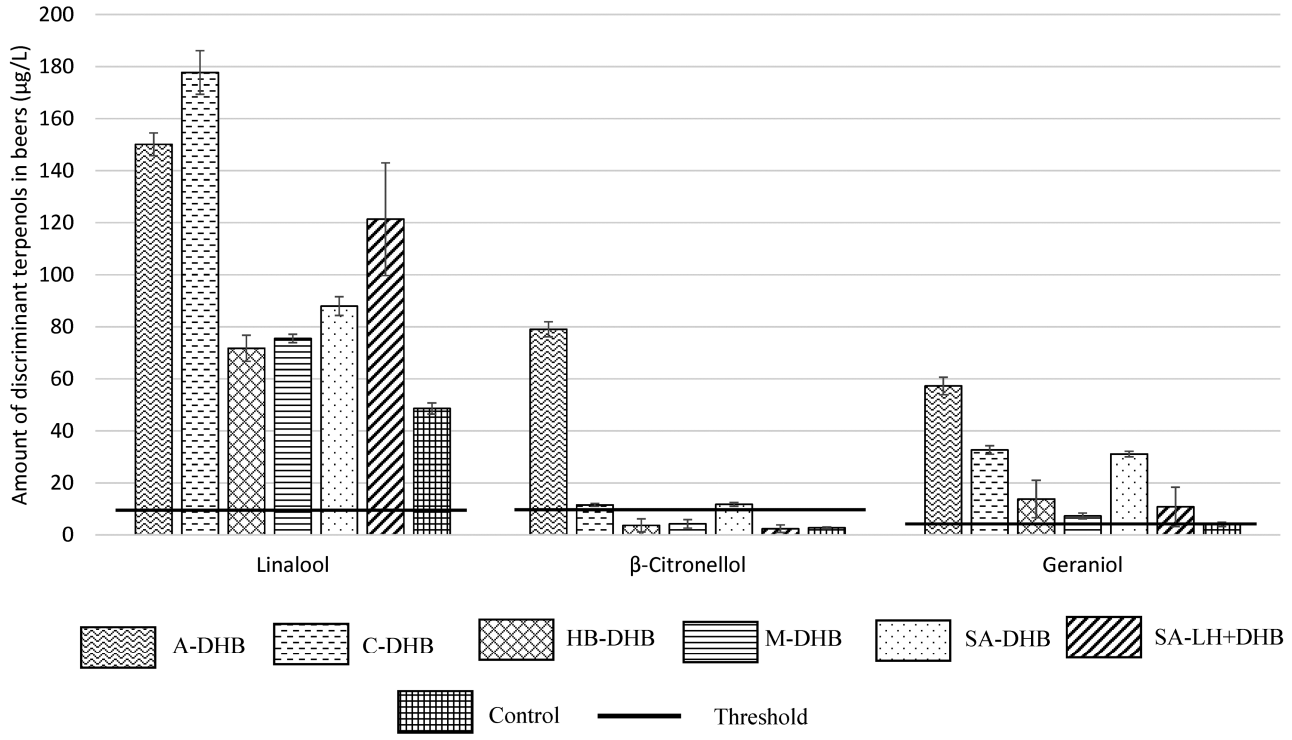

Fig. 4. Measured concentrations of discriminant monoterpenols in the pilot beers. Error bars shown are the standard deviation on the average of duplicate measurements. Dry-hopped beers A-DHB, C-DHB, HB-DHB, M-DHB, and SA-DHB were made with Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace, respectively. For SA-LH+DHB, Sorachi Ace was added both at the end of boiling and during maturation.

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