Control of Hop Aroma Impression of Beer with Blend-Hopping using Geraniol-rich Hop and New Hypothesis of Synergy among Hop-derived Flavour Compounds

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Control of Hop Aroma Impression of Beer with Blend-Hopping using Geraniol-rich Hop and New Hypothesis of Synergy among Hop-derived Flavour Compounds

In previous studies, we found that the concentration of geraniol and β-citronellol in finished beer could be enriched depending on the initial geraniol content in the wort, as a result of single-hopped test-brewing by using geraniol-rich hop. In this study, we tried to confirm the effect of blend-hopping using geraniol-rich Bravo hop on the concentration of monoterpene alcohols during fermentation. As a result, the additional Bravo hop, at only 0.4 g of hop/L dosage, could increase approx. 20 µg/L of geraniol and > 15 µg/L of β-citronellol in blend-hopped beers in comparison with single-hopped control beers. We have also previously demonstrated that the coexistence of linalool, geraniol, and β-citronellol could increase the average scores of 'Citrus' by using the model solution (5 % v/v ethanol, carbonated). In this study, we observed same effect of increase of monoterpene alcohols on the change of flavour characters, first time in hopped beers.

Furthermore, we found that there might be an additive effect between a volatile thiol (4-methyl-4-sulfanylpentan-2-one (4MSP)) and monoterpene alcohols (linalool and geraniol) and that only 1.2 ng/L of 4MSP was enough for this effect. In addition, as a result of model sensory evaluation, it was also found that the 'Tropical' character could be maximized under coexistence of 4MSP and three monoterpene alcohols (linalool, geraniol, and β-citronellol). We assumed that the synergy among volatile thiols and monoterpene alcohols could contribute to such flavours in beers made from certain 'Flavour Hop' varieties, containing both groups of compounds. This result is also useful for determining blend-hopping recipe based on a composition of flavour compounds of each hop variety.

Descriptors: beers, hops, flavour, monoterpene alcohols, biotransformation, linalool, geraniol, β-citronellol, volatile thiols, 4-methyl-4-sulfanylpentan-2-one, additive effect, synergy

1 Introduction

In recent years, a group of hops giving characteristic 'Varietal Aroma', for example citrus-like and/or exotic fruit-like (tropical) flavours, to finished beer has been bred and widely used for craft beers all around the world [3, 12, 25, 30–32, 41]. Such hops are newly categorized as 'Flavour Hops' [25, 36], 'Special Flavour hops' or 'Impact Hops' [11]. We have focused on certain hop varieties, for example Bravo, Cascade, Citra, Mosaic and Nelson Sauvin, and revealed the contribution of monoterpene alcohols and volatile thiols to the hop-derived varietal aromas, the behaviour of these compounds during beer production and the mechanism of varietal aroma formation based on the synergy among various flavour compounds [44–50]. However, mechanisms contributing to the formation of varietal aroma derived from each flavour hop variety have not been fully revealed yet.

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Monoterpene alcohols (linalool, geraniol, β-citronellol, nerol, and α-terpineol; Fig. 1), derived from hops, have been investigated by many researchers [6–10, 16–21, 24, 28, 35, 37–42, 47–50]. In previous studies, we focused on biotransformation of hop-derived monoterpene alcohols (Fig. 1) and their contribution to the flavour of hopped beer. As a result, in general, the geraniol drastically decreases during first 3–4 days of fermentation. The β-citronellol was almost absent in hop and wort and gently increased during fermentation, because of the biotransformation from geraniol to β-citronellol by brewing yeast. The concentration of geraniol and β-citronellol in finished beer could be enriched depending on the initial geraniol content in the wort by using geraniol-rich hop [47–49]. In addition, we found that there was an additive effect among linalool, geraniol, and β-citronellol [47], and that the flavour impression became lime-like (citrus) by coexistence of these three monoterpene alcohols [48]. We also demonstrated (Fig. 2) that European hop varieties (Hallertauer Tradition (HHT), Hallertauer Magnum (HHM), and Saaz (CSA)) contained very small amounts of geraniol. On the other hand, most American hop varieties, including Cascade, Citra, Mosaic, Apollo, Amarillo, and others, contained relatively large amounts of geraniol. In particular, the geraniol level in Bravo was highest among the U.S. varieties [49].

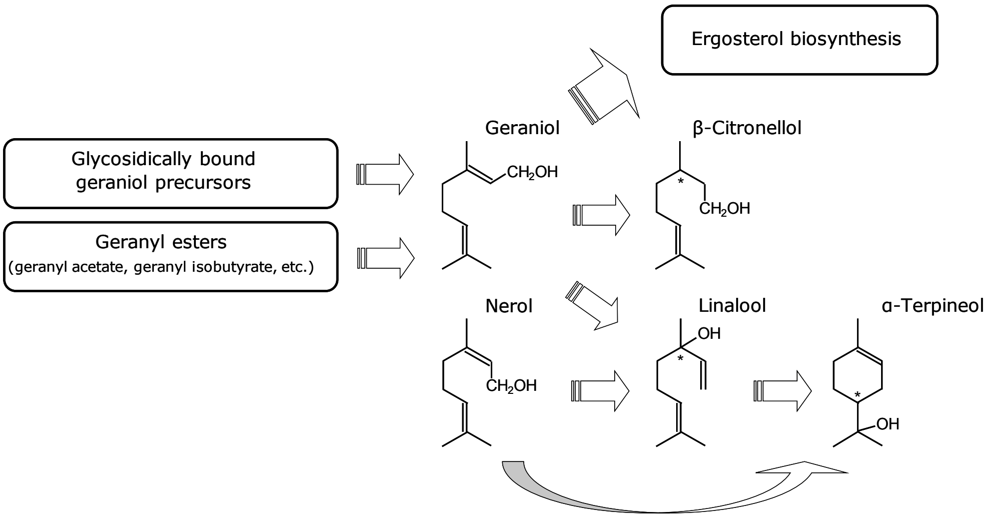

Fig. 1 Biotransformation pathway of monoterpene alcohols by brewing yeast (On the basis of ref. 8, 9, 18–19, 24, 47–49, 53). The asterisk indicate a chiral center. (This figure was previously shown in reference 50)

It is well-known that certain volatile thiols (4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH), 3-sulfanylpentan-1-ol (3SP), and 3-sulfanylhexyl acetate (3SHA)) could contribute to the varietal aroma of wines, especially Sauvignon Blanc wine [5, 33–34, 51–52]. These compounds were present in very small amounts in the wines. However, they contributed to the wine varietal aroma, for example, box-tree like, grapefruit-like and passion fruit-like aroma.

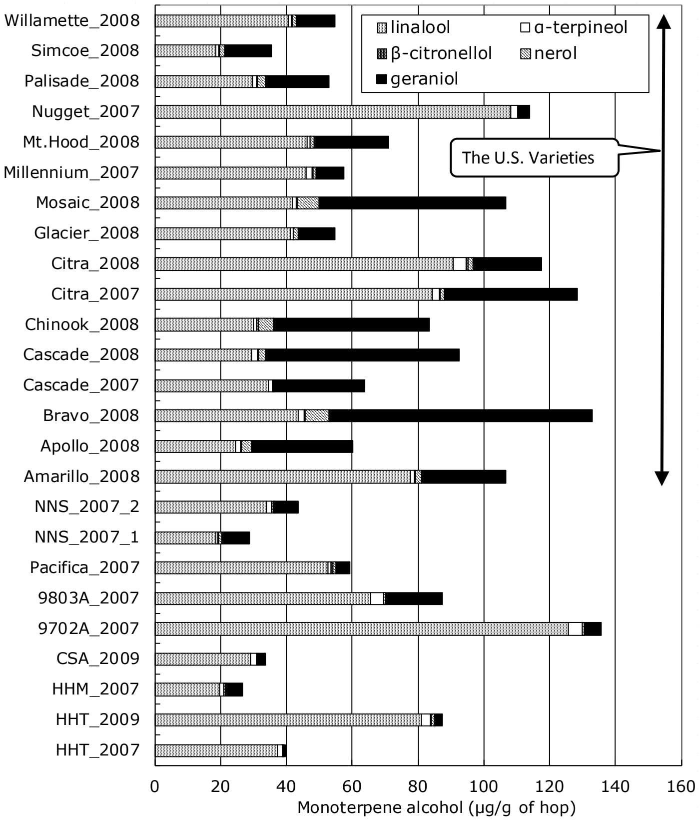

Fig. 2 Composition of monoterpene alcohols (μg/g of hop) in various hops : HHT, Hallertauer Tradition; HHM, Hallertauer Magnum; CSA, Czech Saaz; NNS, New Zealand Nelson Sauvin. (This figure was previously shown in ref. 49)

Because of their very low threshold values and characteristic flavours, beer researchers have also focused on these compounds [4, 13–14, 22–23, 26–27, 39–40, 43–46]. Figure 3 shows major volatile thiols derived from hops. Of these thiols, 3-sulfanyl-4-methylpentan-1-ol (3S4MP) and 3-sulfanyl-4-methylpentyl acetate (3S4MPA), having grapefruit-like flavour similar to that of 3SH, were identified for the first time in Nelson Sauvin hops and beer [44–46]. In addition, we found that 3S4MP could enhance the flavour intensity of other hop-derived flavour compounds, for example 3S4MPA, isobutyric ester (2-methylbutyl isobutyrate), linalool and geraniol, and that 3S4MP might contribute to the varietal aroma of Nelson Sauvin as a key compound having two roles, its own characteristic flavour and its function as a flavour enhancer [45–46].

In this study, we tried to brew test-beers with a certain variety hop and the Bravo hop, which contained highest amount of geraniol among the U.S. hop varieties. The effect of blend-hopping on the concentration of monoterpene alcohols during fermentation and the change in flavour profile of finished beers are discussed. In addition, we proposed new hypothesis of synergy among several hop-derived flavour compounds.

2 Materials and methods

2.1 Hop raw materials

Hallertauer Tradition (HHT) was grown and pelletized in Germany in 2007 (type 90 pellet). Apollo, Bravo, Cascade, Mosaic (HBC369) and Simcoe were grown and harvested in the United States in 2008 (hop powder). Citra was harvested and pelletized in the United States in 2008 (type 90 pellet).

2.2 Pilot-scale brewing

Beers were made with the same recipe according to the standard method of the Production & Technology Development Centre, Sapporo Breweries, Ltd. Briefly, the wort was prepared using commercially available 67 % malts, 33 % adjuncts (starch, corn, and rice), and hops in a 400-L pilot-scale apparatus. Boiling period was 90 min. For prevention of over boiling, the HHT hop was added at the beginning of boiling (hop at 0.2 g/L). Cooled wort was collected to fermentation tanks (30 L/tank) and media bottles (900 mL/bottle). For each hop flavouring with single hop variety (Apollo, Bravo, Cascade, Citra, Mosaic and Simcoe), 24.8 g of hop was added to each bottle (sealed with aluminum foil) and autoclaved at 105 °C for 5 min. After cooling, the hop-flavoured wort sample was mixed with 30 L of wort in each fermentation tank. This condition corresponded to that of the late-hopping, with hop at 0.8 g/L. For hop flavouring with blend-hopping, the set of 24.8 g of the Apollo hop and 12.4 g of the Bravo hop or the set of 24.8 g of the Simcoe hop and 12.4 g of the Bravo hop was used for the same procedure. The dosage of the Bravo hop corresponded to 0.4 g/L. Subsequently, the fermentation was started by adding lager yeast (brewery-collected *Saccharomyces pastorianus*) at 15.0 x 10^6 cells/mL to the wort. The temperature of the fermentation was maintained at 10–12 °C (primary fermentation) for 7 days. After fermentation, the fermented wort was transferred to another storage tank under a CO_2 atmosphere and the maturation was carried out at 13 °C for 8 days, then at 0 °C for 2–3 weeks. Filtration and bottling were done using the pilot scale equipment under air-free conditions.

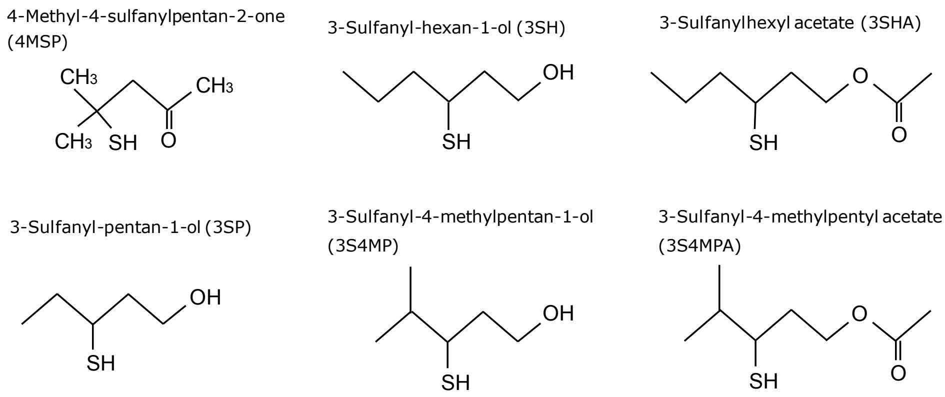

Fig. 3 Major volatile thiols derived from hops (On the basis of ref. 4, 13–14, 22–23, 26–27, 39–40, 43–46)

2.3 Standard products

Linalool (> 98 %, racemic mixture) and β-citronellol (> 92 %, racemic mixture) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Geraniol (98 %) was purchased from Aldrich Chemical Company Inc. (Milwaukee, US). 4-methyl-4-sulfanylpentane-2-one (1 % solution in propylene glycol) was purchased from Interchim (Montluçon, France).

2.4 Quantification of hop-derived monoterpene alcohols by solid phase microextraction-gas chromatography-mass spectroscopy (SPME-GC-MS)

GC-MS analyses were carried out using a 6890N gas chromatograph (Agilent Technologies). The carrier gas was helium, with a column-head pressure of 15 psi and flow rate of 1.8 mL/min. The detector was a mass spectrometer (MS 5973; Agilent Technologies) functioning in the EI mode (70 eV) and was connected to the GC by a transfer line heated to 280 °C. For analysis of wort, fermenting beer, and finished beer, 8 mL of each sample was put into a 20-mL glass vial including 3 g of sodium chloride at 0 °C on ice water. The vial, including a sample, was sealed with a magnet cap. The vial was preincubated with stirring at 40 °C for 15 min using a Combi-PAL autosampler (CTC Analytics). After preincubation, an SPME fibre (polydimethylsiloxane (PDMS), 100-µm film thickness; Supelco) was inserted into the head space of the vial and adsorption was carried out for 15 min. After the adsorption, the SPME fibre was injected into a splitless injector (260 °C; purge time = 3 min, purge flow = 20 mL/min) at oven temperature (50 °C) onto a type HP-1MS capillary column (30 m, 0.25-mm i.d., 1.0-µm film thickness) (Agilent Technologies). For all the analyses, the temperature program was as follows: 50 °C for 1 min, raised at 5 °C/min to 250 °C, followed by a 1-min isotherm. The monoterpene alcohols (linalool, β-citronellol and geraniol) were quantified in the SIM mode, selecting the following ions: *m/z* 93 (for geraniol) and 109 (for linalool and β-citronellol). Calibration curves were determined using water (including 5 % ethanol) containing these monoterpene alcohols at final concentrations ranging from 0 to 10 µg/L. All calibration produced a linear response with an R^2 value > 0.98 over the concentration range analysed. The analysis was performed in duplicate.

2.5 Sensory evaluation

2.5.1 Sensory evaluation of flavour profiles of test-brewed beers

Each sensory evaluation was performed by approximately 40–50 well-trained panelists. The profile of flavour characters of each test-brewed beer was assessed, as follows. A 50–100 mL aliquot of each sample beer was presented in a glass and the six flavour characters (flowery, fruity, citrus, tropical, green, and sulfur) were scored from 0 (no flavour) to 3 (strong flavour) in intervals of 1.0.

2.5.2 Determining flavour thresholds

Each sensory evaluation was performed by 10–13 well-trained panelists. Perception threshold of 4-methyl-4-sulfanylpentane-2-one (4MSP) was assessed by a forced-choice ascending concentration series method of limits [1]. Briefly, the directional triangular tests of six increasing concentrations in model carbonated dilute alcohol solution (5 % v/v ethanol). The 50 mL of each sample solution was presented in plastic cups. The best estimate threshold was calculated for each panelist as the geometric mean of the highest concentration missed and the next highest concentration. The group threshold was calculated as the geometric mean of the best estimate thresholds of the panelists.

2.5.3 Study of additive effect between volatile thiol and monoterpene alcohols

Evaluation of an additive effect was performed according to the methods previously described [45–47]. Namely, in order to assess an additive effect among three monoterpene alcohols (linalool, geraniol, and β-citronellol), triangular tests were carried out in model carbonated dilute alcohol solution (5 %v/v ethanol), as follows. A control solution containing the estimated threshold concentration (1.2 ng/L) of 4MSP was compared with test solutions containing the same concentration of 4MSP together with 3 µg/L of linalool, 5 µg/L of geraniol or 5 µg/L of β-citronellol. The 50 mL of each sample solution was presented in plastic cups. The significance of the results was determined according to the binominal law.

2.5.4 Sensory evaluation of synergy among volatile thiol and monoterpene alcohols

Each sensory evaluation was performed by 10–13 well-trained panelists. The change of flavour characters by synergy among volatile thiols (4MSP) and monoterpene alcohols was assessed in a model solution (5 % v/v ethanol, carbonated), as follows. Three test solutions were prepared. A '4MSP' solution contained 40 ng/L of 4MSP. A 'LGC mix' solution contained 70 µg/L of linalool, 50 µg/L of geraniol and 30 µg/L of β-citronellol. A '4MSP + LGC mix' solution contained 40 ng/L of 4MSP, 70 µg/L of linalool, 50 µg/L of geraniol and 30 µg/L of β-citronellol. A 50 mL aliquot of each sample solution was presented in a plastic cup and the six flavour characters (flowery, fruity, citrus, tropical, green, and sulfur) were scored from 0 (no flavour) to 3 (strong flavour) in intervals of 1.0.

3 Results and discussions

3.1 Effect of blend-hopping on the behaviour of monoterpene alcohols during fermentation

In previous studies, we have investigated the behaviour of monoterpene alcohols (linalool, geraniol, and β-citronellol) during fermentation by using test-brewed beer flavoured with only single hop variety [47–49]. In this study, we tried to confirm the effect of blend-hopping using geraniol-rich hop on the behaviour of monoterpene alcohols during fermentation. We selected Bravo as a geraniol-rich hop, containing geraniol at largest level in figure 2. In addition, recent results from our laboratory showed that Bravo contained volatile thiols only at trace levels (data not shown). Therefore, we thought that this hop was suitable for selective increase of geraniol. In previous study, the finished beer late-hopped with Bravo (hop dosage; 0.8 g of hop/L) has contained approx. 70 µg/L of geraniol and approx. 50 µg/L of β-citronellol [49]. Therefore, we thought that an addition of this hop at 0.4 g of hop/L dosage could increase 20–30 µg/L of geraniol and β-citronellol in finished beer. We selected Apollo and Simcoe as control varieties in the brewing trial with blend-hopping. Among the U.S. hop varieties, Apollo contained geraniol at relatively high level and Simcoe contained this at relatively low level, respectively (Fig. 2). Figure 4 showed behaviours of linalool, geraniol, and β-citronellol during fermentation of single-hopped (Apollo or Simcoe) and blend-hopped (Apollo + Bravo or Simcoe + Bravo) beers. As a result, the additional Bravo hop, at only 0.4 g of hop/L dosage, could increase approx. 20 µg/L of geraniol and > 15 µg/L of β-citronellol in blend-hoped beers in comparison with single-hopped beers made from Apollo or Simcoe (Fig. 4).

3.2 Effect of blend-hopping on the flavour impression of beer

Figure 5 shows the six flavour characters (flowery, fruity, citrus, tropical, green, and sulfur) of test-brewed beers made with single-

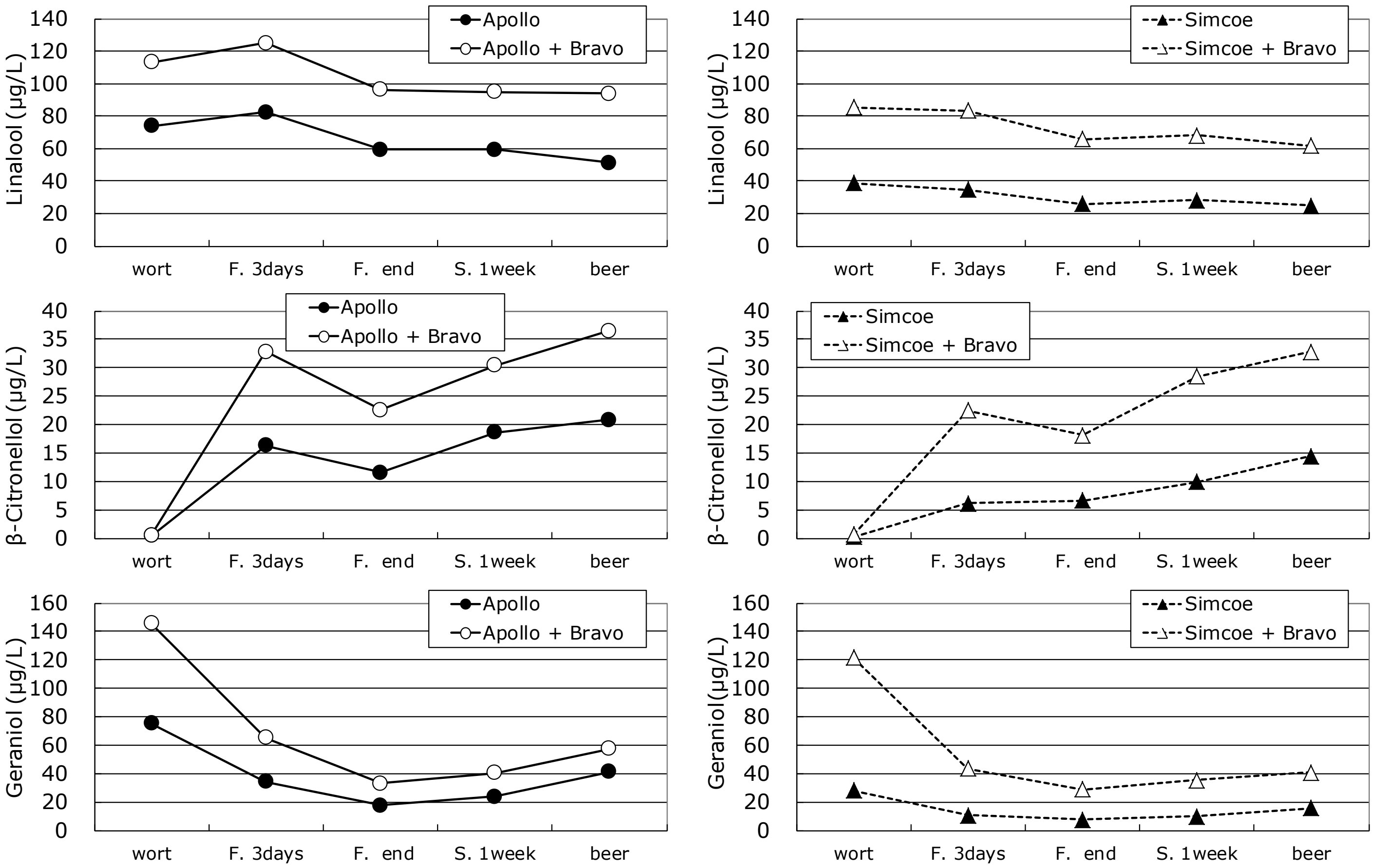

Fig. 4 Comparison of monoterpene alcohols (µg/L) during fermentation with blend-hopping: F., fermentation; S., Storage; Flavouring hop dosage; ● Apollo (Apollo 0.8 g/L); ○ Apollo + Bravo (Apollo 0.8 g/L + Bravo 0.4 g/L); ▲ Simcoe (Simcoe 0.8 g/L); △ Simcoe + Bravo (Simcoe 0.8 g/L + Bravo 0.4 g/L)

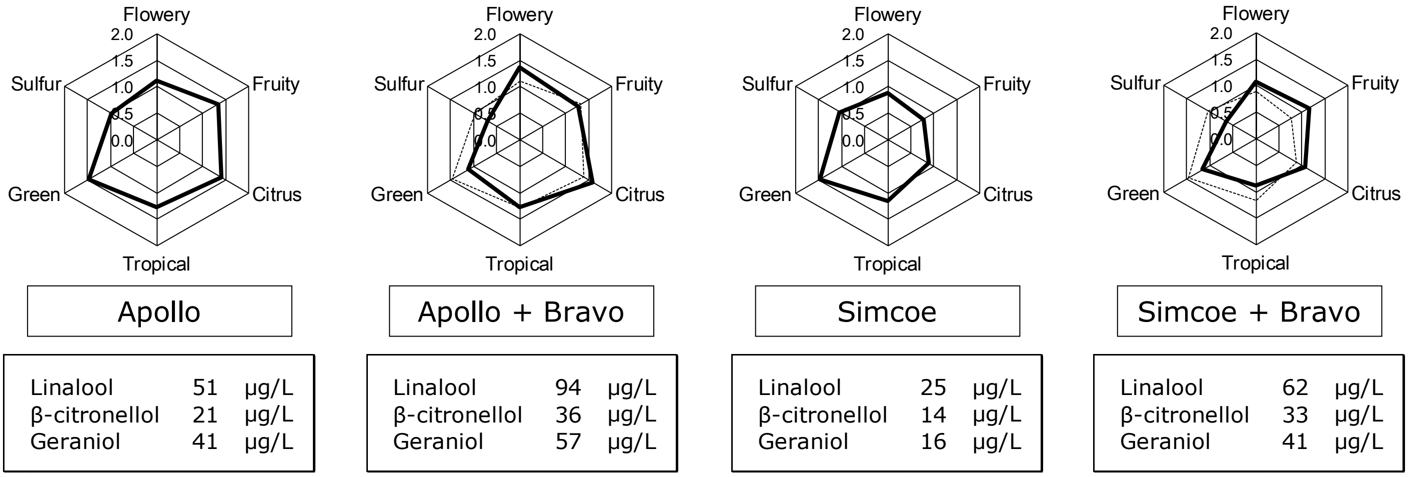

Fig. 5 Flavor profiles of test-brewed beers made with single-hopping (Apollo and Simcoe) and blend-hopping (Apollo + Bravo and Simcoe + Bravo): dotted line, profile of corresponding single-hopped beer

hopping (Apollo or Simcoe) and blend-hopping (Apollo + Bravo or Simcoe + Bravo). In the case of Apollo beers, the average scores of 'Flowery' and 'Citrus' increased by blend-hopping with Bravo. In the case of Simcoe beers, the average scores of 'Flowery', 'Fruity' and 'Citrus' increased by blend-hopping. In addition, total shape of flavour profile was also drastically changed by blend-hopping. We have previously demonstrated that the coexistence of linalool, geraniol, and β-citronellol could increase the average scores of 'Citrus' by using the model solution (5 % v/v ethanol, carbonated) [48]. In this study, we observed same effect of increase of monoterpene alcohols on the change of flavour characters, first time in hopped beers.

3.3 Flavour impressions of beers brewed by single-hopping with several flavour hop varieties

Figure 6 shows the six flavour characters (flowery, fruity, citrus, tropical, green, and sulfur) of test-brewed beers made with single-hopping (Bravo, Cascade, Citra, and Mosaic). In the case of Bravo beer, the average scores of 'Flowery', 'Fruity', 'Citrus', and 'Green' were relatively high and those of 'Tropical' and 'Sulfur' were relatively low. We thought that the characters of 'Flowery', 'Fruity', 'Citrus', and 'Green' might mainly depend on three monoterpene alcohols in this beer, because this beer had lime-like flavour similar to the flavour of the mixture of three monoterpene alcohols (linalool, geraniol, and β-citronellol). The Cascade beer had relatively high average scores of 'Flowery', 'Fruity', and 'Citrus'. The Citra beer indicated very high scores of 'Citrus', 'Tropical', and 'Green'. The Mosaic beer showed characteristic 'Fruity' and 'Tropical' characters.

Remarkably, the 'Citrus' scores in the Cascade beer, the Citra beer, and the Mosaic beer were higher than that in the Bravo beer, though the Bravo beer contained monoterpene alcohols at very high levels (78 µg/L of linalool, 69 µg/L of geraniol, and 36 µg/L of β-citronellol). In general, the 'Citrus' character in beer includes various citrus fruit nuances, for example lemon-like, lime-like, orange-like, grapefruit-like, and so on. Several researchers have pointed out an occurrence of 3SH, having grapefruit-like flavour, in the Cascade beer and the Citra beer [4, 13–14, 26–27]. Therefore, it is thought that a part of the 'Citrus' character in these beers might derived from not only monoterpene alcohols but also volatile thiols.

However, the 'Citrus' character in test-brewed Citra beer (Fig. 6) was felt lime-like rather than grapefruit-like. Therefore, we assumed that there might be unknown flavour enhancer for lime-like character derived from monoterpene alcohols.

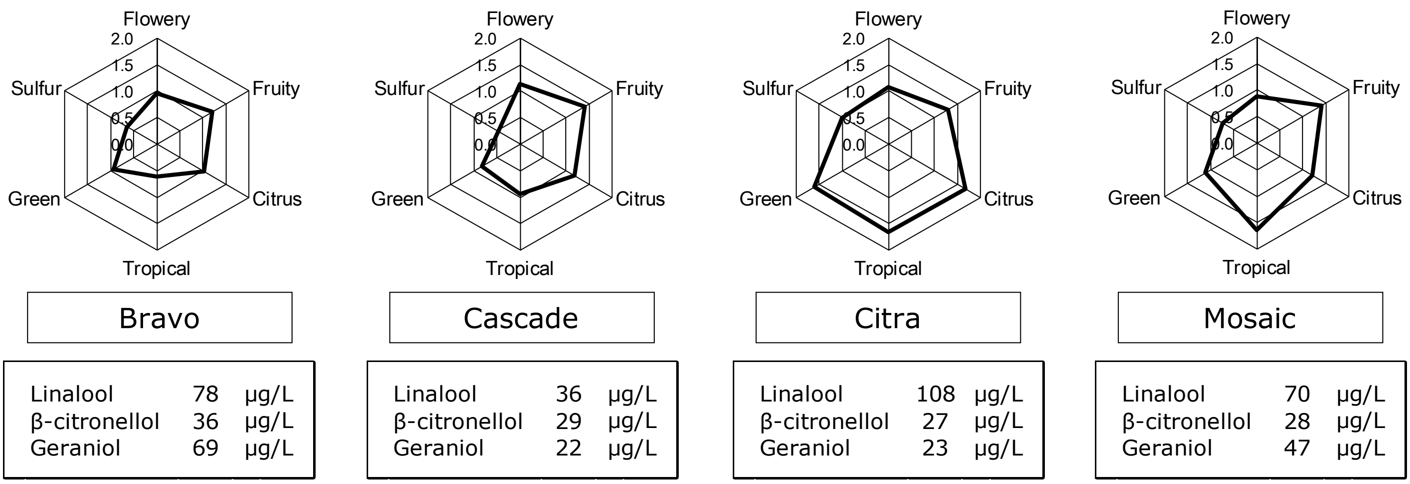

Fig. 6 Flavor profiles of test-brewed beers made with single-hopping using Bravo, Cascade, Citra and Mosaic

Table 1 **Olfactory descriptions and perception thresholds of the monoterpene alcohols (µg/L) and 4-methyl-4-sulfanylpentan-2-one (ng/L) in model solution (5 % v/v ethanol, carbonated)**

ref compd		olfactory descriptions	olfactory perception threshold[a] model solution[b]
linalool	(µg/L)	lavender	3[c,d]
β-citronellol	(µg/L)	lemon, lime	9[c,d]
geraniol	(µg/L)	rose	7[d]
4-methyl-4-sulfanylpentan-2-one (4MSP)	(ng/L)	blackcurrant, box-tree	1.2

[a] Flavor threshold determined by 10–13 panelists
[b] Model carbonated dilute ethanol solution (5 % v/v Ethanol, Carbonated)
[c] Determined using racemic mixture
[d] Previously reported in ref 47

3.4 Additive effect between 4MSP and monoterpene alcohols

In previous study, we have already observed that a certain volatile thiol, 3S4MP, could enhance intensities of other flavour compounds, for example 3S4MPA, 2-methylbutyl isobutyrate, linalool, and geraniol [45, 46]. However, 3S4MP has very characteristic grapefruit-like (‘Citrus’) flavour similar to the flavour of 3SH.

Another volatile thiol, 4MSP, has been focused on as an important contributor to the characteristic aroma derived from flavour hops. This compound commonly contained in many flavour hop varieties, for example Amarillo [4], Apollo [22], Cascade [13, 14, 22, 26, 27, 39, 40], Citra [27], Mosaic [4], Nelson Sauvin [13, 14, 27, 45, 46], Simcoe [22], Summit [22], and Topaz [22]. The flavour of 4MSP was commented as blackcurrant-like and/or muscat-like (not ‘Citrus’) flavour [4, 13, 14, 22, 26, 40]. Therefore, we try to confirm whether 4MSP could have similar enhancing effect or not, for further understanding of the mechanism of hop-derived beer flavour.

Firstly, we determined group threshold of 4MSP in model solution (5 % v/v ethanol, carbonated) by using our own panelists. The group thresholds of three monoterpene alcohols (linalool, geraniol, and β-citronellol) have been determined by same panelists [47]. Table 1 lists the olfactory descriptions and group thresholds of monoterpene alcohols and 4MSP. The aroma of 4MSP is somewhat reminiscent of blackcurrant and/or box-tree, and its threshold was estimated at 1.2 ng/L, similar level to previously reported its threshold (0.8 ng/L) in model solution simulating wine (12 % v/v ethanol) [52]. Secondly, in order to assess a possible additive effect between 4MSP and three monoterpene alcohols, triangular tests were designed according to the method used in previous study [46] and carried out, as shown in table 2.

A control model solution (5 % v/v ethanol, carbonated) containing 1.2 ng/L of 4MSP (perception threshold of 4MSP in model solution) was compared with test solutions containing 1.2 ng/L of 4MSP together with 3 µg/L of linalool, 5 µg/L of geraniol, or 5 µg/L of β-citronellol. The concentrations of linalool corresponded to its threshold level and those of geraniol and β-citronellol were lower than their thresholds. The concentrations of these monoterpene alcohols in model solutions were adjusted to same concentrations used in previous study [46]. In general, in a triangular test corresponding to the threshold of a certain compound, about half of panelists judge correctly. It is not at significant level. In model solution shown in table 2, there were significant differences with risks of 1 % between the control solution and the test solution containing linalool or geraniol, and there was no significant difference between the control solution and the test solution containing β-citronellol. These results suggested that odours of linalool and geraniol might be enhanced by the occurrence of 4MSP at a threshold level. Therefore, we concluded that there might be an additive effect between 4MSP and monoterpene alcohols (linalool and geraniol) and that only 1.2 ng/L of 4MSP was enough for this effect.

In previous studies [2, 15, 34], it has been reported that there was an additive effect among same class of compounds, having similar chemical structures and similar odours, for example 3-sulfanypentan-1-ol, 3-sulfanylhexan-1-ol, and 3-sulfanylheptan-1-ol [34]. We have also reported similar additive effect among monoterpene alcohols (linalool, geraniol, and β-citronellol), having similar chemical structures [47]. On the other hand, it was thought that certain volatile thiols (3S4MP and 4MSP) could enhance flavour intensities of various flavour compounds, having different chemical structures and different odours.

3.5 Synergy of 4MSP and monoterpene alcohols

In addition, we try to confirm an effect of 4MSP on flavour profile, for further understanding of characteristics of 4MSP. A test was

Table 2 **Triangular test involving 12 panelists (in model solution (5 % v/v ethanol, carbonated))**

test solution	control solution	correct answers/total answers	p
1.2 ng/L 4MSP + 3 µg/L linalool	1.2 ng/L 4MSP	9/12	0.01
1.2 ng/L 4MSP + 5 µg/L β-citronellol	1.2 ng/L 4MSP	3/12	–
1.2 ng/L 4MSP + 5 µg/L geraniol	1.2 ng/L 4MSP	9/12	0.01

conducted to assess changes of flavour character by synergy of 4MSP and monoterpene alcohols. Figure 7 shows the six flavour characters (flowery, fruity, citrus, tropical, green, and sulfur) of model solutions, evaluated by the panelists. The model solution '4MSP' (containing 40 ng/L of 4MSP) was designed based on previously reported concentrations of 4MSP in test-brewed beers made with thiol-containing flavour hops [22, 26–27] and recent results from our laboratory (data not shown). The model solution 'LGC mix' (containing 70 µg/L of linalool, 50 µg/L of geraniol, and 30 µg/L of β-citronellol) was designed to simulate the composition of the three monoterpene alcohols in test-brewed beers made with geraniol-rich flavour hops. The model solution '4MSP + LGC mix' contained all four compounds.

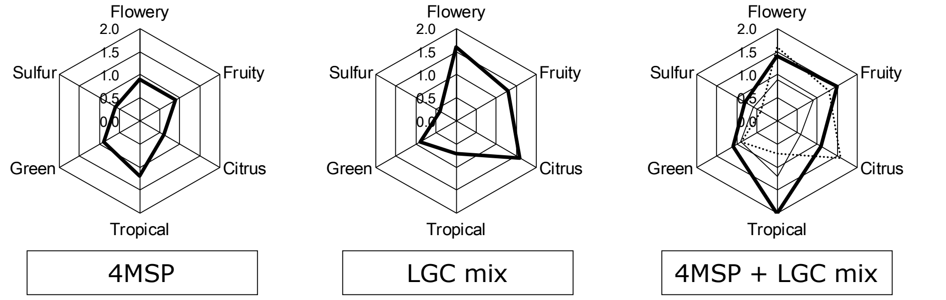

Fig. 7 Flavor profiles of model solutions (5 % v/v ethanol, carbonated) containing 4MSP and/or three monoterpene alcohols: 4MSP, containing 40 ng/L of 4MSP; LGC mix, containing 70 µg/L of linalool, 50 µg/L of geraniol and 30 µg/L of β-citronellol; hair line, profile of 4MSP model solution; dotted line, profile of LGC mix model solution

As a result, in the flavour profile of the model solution '4MSP', the average scores of 'Citrus' and 'Sulfur' were relatively low and the one of 'Tropical' was relatively high. In the case of 'LGC mix', the average scores of 'Flowery', 'Fruity', 'Citrus', and 'Green' were relatively high and those of 'Tropical' and 'Sulfur' were relatively low. This result is similar to that shown in our previous study [48]. The total shape of the flavour profile of '4MSP + LGC mix' was similar to that of '4MSP', however, the intensities of 'Flowery', 'Fruity', 'Citrus', and 'Tropical' clearly increased by mixing of 4MSP and three monoterpene alcohols. Surprisingly, the intensity of 'Tropical' character maximized by coexistence of these all compounds.

In the field of beer investigation, 4MSP has been mainly focused on because of its characteristic blackcurrant-like and/or muscat-like flavour [4, 13, 14, 22, 26, 40]. However, it has been reported that 4MSP was contained in mango and contributed to tropical aroma of this fruit [29]. Therefore, we assumed that 4MSP could also contribute to tropical, mango-like flavour derived from 'Flavour hops'.

It has been reported that newly bred 'Flavour Hop' varieties contained not only monoterpene alcohols but also volatile thiols (4, 13, 14, 46), and that these varieties gave characteristic exotic-fruit like, tropical flavours to finished beers, for example guava-like flavour derived from Citra [30, 31], mango-like flavour from Mosaic [32], and so on. We assumed that the synergy among volatile thiols and monoterpene alcohols could contribute to such flavours in beers made from certain 'Flavour Hop' varieties.

4 Conclusions

4.1 Control of hop aroma impression of beer with blend-hopping using geraniol-rich hop

In previous studies, we found that the concentration of geraniol and β-citronellol in finished beer could be enriched depending on the initial geraniol content in the wort, as a result of single-hopped test-brewing by using geraniol-rich hop [47–49]. In this study, we tried to confirm the effect of blend-hopping using geraniol-rich Bravo hop on the behaviour of monoterpene alcohols during fermentation. As a result, the additional Bravo hop, at only 0.4 g of hop/L dosage, could increase approx. 20 µg/L of geraniol and > 15 µg/L of β-citronellol in blend-hopped beers in comparison with single-hopped control beers.

We have also previously demonstrated that the coexistence of linalool, geraniol, and β-citronellol could increase the average scores of 'Citrus' by using the model solution (5 % v/v ethanol, carbonated) [48]. In this study, we observed same effect of increase of monoterpene alcohols on the change of flavour characters, first time in hopped beers.

In addition, total shape of flavour profile was also drastically changed by blend-hopping. It is thought that this result could evidence a change of flavour impression by blend-hopping, which had been experimentally carried out by craft brewers.

4.2 New hypothesis of synergy among hop-derived flavour compounds

We found that there might be an additive effect between 4MSP and monoterpene alcohols (linalool and geraniol) and that only 1.2 ng/L of 4MSP was enough for this effect. It was thought that certain volatile thiols (3S4MP [46] and 4MSP) could enhance flavour intensities of various flavour compounds, having different chemical structures and different odours.

In addition, as a result of model sensory evaluation, it was also found that the 'Tropical' character could be maximized under coexistence of 4MSP and three monoterpene alcohols (linalool, geraniol, and β-citronellol). We assumed that the synergy among volatile thiols and monoterpene alcohols could contribute to such flavours in beers made from certain 'Flavour Hop' varieties, containing both groups of compounds. This result is also useful for determining blend-hopping recipe based on a composition of flavour compounds of each hop variety.

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5 Literature

1. American Society for Testing and Materials. Subcomittee E-18. Standard Practice E-679 for Determination of Odor and Taste Thresholds by a Forced-Choice Ascending Concentration Series Method of Limits. ASTM: Philadelphia, PA, 1979.
2. Bailly, S.; Jerkovic, V.; Marchand-Brynaert, J. and Collin, S.: Aroma extraction dilution analysis of Sauternes wines. Key role of polyfunctional thiols. J. Agric. Food Chem. **54** (2006), pp. 7227-7234.
3. Beatson, R. A.; Ansell, K. A. and Graham, L. T.: Breeding, development, and characteristics of the hop (*Humulus lupulus*) cultivar 'Nelson Sauvin'. New Zealand Journal of Crop and Horticultural Science **31** (2003), pp. 303-309.
4. Cibaka, M.-L. K.; Gros, J.; Nizet, S. and Collin, S.: Quantitation of selected terpenoids and mercaptans in the dual-purpose hop varieties Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace. J. Agric. Food Chem. **63** (2015), pp. 3022–3030.
5. Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N. and Dubourdieu, D.: Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. Flavour Fragr. J. **10** (1995), pp. 385-392.
6. Dresel, M.; Van Opstaele, F.; Praet, T.; Jaskula-Goiris, B.; Van Holle, A.; Naudts, D.; De Keukeleire, D.; De Cooman, L. and Aerts, G.: Investigation of the impact of the hop variety and the hopping technology on the analytical volatile profile of single-hopped worts and beers. BrewingScience – Monatsschrift für Brauwissenschaft **66** (2013), pp. 162-175.
7. Dresel, M.; Praet, T.; Van Opstaele, F.; Van Holle, A.; Naudts, D.; De Keukeleire, D.; De Cooman, L. and Aerts, G.: Comparison of the analytical profiles of volatiles in single-hopped worts and beers as a function of the hop variety. BrewingScience – Monatsschrift für Brauwissenschaft **68** (2015), pp. 8-28.
8. Forster, A. and Gahr, A.: On the fate of certain hop substances during dry hopping. BrewingScience – Monatsschrift für Brauwissenschaft **66** (2013), pp. 93-103.
9. Forster, A.; Gahr, A. and Van Opstaele, F.: On the transfer rate of geraniol with dry hopping. BrewingScience – Monatsschrift für Brauwissenschaft **67** (2014), pp. 60-62.
10. Forster, A. and Gahr, A.: A comparison of the analytical and brewing characteristics of Cascade and Comet hop varieties as grown in Yakima (USA) and Hallertau (Germany). BrewingScience – Monatsschrift für Brauwissenschaft **67** (2014), pp. 137-148.
11. Hieronymus, S.: A taste for hops – Exploring the history and mystery of Flavor Hops. All About Beer Magazine **34** (2013). (http://allaboutbeer.com/article/flavor-hops/)
12. Graves, I. R.; Brier, M. B.; Chandra, G. S. and Alspach, P. A.: Kettle hop flavour from New Zealand hop (*Humulus lupulus* L.) cultivars. Proceedings of the 27th Convention of the Institute and Guild of Brewing, Asia Pacific Section, Adelaide, Australia, 17–22 March 2002 (CD-ROM).
13. Gros, J.; Nizet, S. and Collin, S.: Occurrence of odorant polyfunctional thiols in the super alpha Tomahawk hop cultivar. Comparison with the thiol-rich Nelson Sauvin bitter variety. J. Agric. Food Chem. **59** (2011), pp. 8853-8865.
14. Gros, J.; Peeters, F. and Collin, S.: Occurrence of odorant polyfunctional thiols in beers hopped with different cultivars. first evidence of an *S*-cysteine conjugate in hop (*Humulus lupulus* L.). J. Agric. Food Chem. **60** (2012), pp. 7805–7816.
15. Guadagni, D. G.; Buttery, R. G.; Okano, S. and Burr, H. K.: Additive effect of sub-threshold concentrations of some organic compounds associated with food aromas. Nature **200** (1963), pp. 1288-1289.
16. Hao, J.; Dong, J.; Yin, H.; Yan, P.; Ting, P. L.; Li, Q.; Tao, X.; Yu, J.; Chen, H. and Li., M.: Optimum method of analyzing hop derived aroma compounds in beer by Headspace Solid-Phase Microextraction (SPME) with GC/MS and their evolutions during Chinese lager brewing process. J. Am. Soc. Brew. Chem. **72** (2014), pp. 261-270.
17. Inui, T.; Tsuchiya, F.; Ishimaru, M.; Oka, K. and Komura, H.: Different beers with different hops. Relevant compounds for their aroma characteristics. J. Agric. Food Chem. **61** (2013), pp. 4758–4764.
18. King, A. and Dickinson, J. R.: Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis*. Yeast **16** (2000), pp. 499-506.
19. King, A. and Dickinson, J. R.: Biotransformation of hop aroma terpenoids by ale and lager yeasts. FEMS Yeast Research **3** (2003), pp. 53-62.
20. Kishimoto, T.; Wanikawa, A.; Kono, K. and Shibata, K.: Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. J. Agric. Food Chem. **54** (2006), pp. 8855-8861.
21. Kishimoto, T.; Wanikawa, A.; Kono, K. and Aoki, K.: Odorants comprising hop aroma of beer: hop-derived odorants increased in the beer hopped with aged hops. Proc. 31st EBC Congr., (2007), pp. 226-235 (CD-ROM).
22. Kishimoto, T.; Kobayashi, M.; Yako, N.; Iida, A. and Wanikawa, A.: Comparison of 4-mercapto-4-methylpentan-2-one contents in hop cultivars from different growing regions. J. Agric. Food Chem. **56** (2008), pp. 1051-1057.
23. Kishimoto, T.; Morimoto, M.; Kobayashi, M.; Yako, N. and Wanikawa, A.: Behaviors of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate during brewing processes. J. Am. Soc. Brew. Chem. **66** (2008), 192-196.
24. Lam, K. C.; Foster II, R. T. and Deinzer, M. L.: Aging of hops and their contribution to beer flavor. J. Agric. Food Chem. **34** (1986), pp. 763-770.
25. Lutz, A.; Kammhuber, K. and Seigner, E.: New Trend in Hop Breeding at the Hop Research Center Huell. BrewingScience – Monatsschrift für Brauwissenschaft **65** (2012), pp. 24-32.
26. Morimoto, M.; Kishimoto, T.; Kobayashi, M.; Yako, N. and Wanikawa, A.: Effects of Bordeaux mixture (copper sulfate) treatment on blackcurrant/muscat-like odors in hops and beer. J. Am. Soc. Brew. Chem. **68** (2010), pp. 192-196.
27. Ochiai, N.; Sasamoto, K. and Kishimoto, T.: Development of a method for the quantitation of three thiols in beer, hop, and wort samples by Stir Bar Sorptive Extraction with in situ derivatization and Thermal Desorption–Gas Chromatography–Tandem Mass Spectrometry. J. Agric. Food Chem. **63** (2015), pp. 6698–6706.
28. Peacock, V. E.; Deinzer, M. L.; Likens, S. T.; Nickerson, G. B. and McGill, L. A.: Floral hop aroma in beer. J. Agric. Food Chem. **29** (1981), pp. 1265-1269.
29. Pino, J. A. and Mesa, J.: Contribution of volatile compounds to mango (*Mangifera indica* L.) aroma. Flavour Fragrance J. **21** (2006), pp. 207–213.

30. Probasco, G.; Perrault J. and Varnum, S.: Citra – a new special aroma hop variety. World Brewing Congress 2008 Proceedings, Honolulu, Hawaii, 2008, Poster 115 (CD-ROM).
31. Probasco, G.; Varnum, S.; Perrault J. and Hysert, D.: Citra – a new special aroma hop variety. MBAA Tech. Quart. **47** (2010), pp. 17-22.
32. Probasco, G.; Perrault J. and Varnum, S.: Mosaic HBC 369 – a new flavor hop variety. World Brewing Congress 2012 Proceedings, Portland, Oregon, 2012, Poster 147.
33. Roland, A.; Schneider, R.; Razungles, A. and Cavelier, F.: Varietal thiols in wine: Discovery, analysis and applications. Chemical Reviews **111** (2011), pp. 7355-7376.
34. Sarrazin, E.; Shinkaruk, S.; Tominaga, T.; Bennetau, B.; Frérot, E. and Dubourdieu, D.: Odorous impact of volatile thiols on the aroma of young botrytized sweet wines: Identification and quantification of new sulfanyl alcohols. J. Agric. Food Chem. **55** (2007), pp. 1437-1444.
35. Schmidt, C. and Biendel, M.: Headspace Trap GC-MS analysis of hop aroma compounds in beer. BrewingScience – Monatsschrift für Brauwissenschaft **69** (2016), pp. 9-15.
36. Schönberger, C.: Unnachahmliche Noten. Flavour Hops – Herausstellungsmerkmal Hopfen. Brauindustrie **97** (2012), pp. 28-30.
37. Seaton, J. C.; Moir, M. and Sugget, A.: The refinement of hop flavour by yeast action. Proceedings of the 17th Convention of the Institute of Brewing, Australia and New Zealand Section, Perth, Australia, (1982), pp. 117-124.
38. Sharp, D. C.; Shaun Townsend, M.; Qian, Y. and Shellhammer, T. H.: Effect of harvest maturity on the chemical composition of Cascade and Willamette hops. J. Am. Soc. Brew. Chem. **72** (2014), pp. 231-238.
39. Steinhaus, M.; Wilhelm, W. and Schieberle, P.: Comparison of the most odor-active volatiles in different hop varieties by application of a comparative aroma extract dilution analysis. Eur. Food. Res. Technol. **226** (2007), pp. 45-55.
40. Steinhaus, M. and Schieberle, P.: Transfer of the potent hop odorants linalool, geraniol and 4-methyl-4-sulfanyl-2-pentanone from hops into beer. Proc. 31st EBC Congr., (2007), pp. 1004-1011 (CD-ROM).
41. Steyer, D.; Clayeux, C. and Laugel, B.: Characterization of the terpenoids composition of beers made with the French hop varieties: Strisselspalt, Aramis, Triskel and Bouclier. BrewingScience – Monatsschrift für Brauwissenschaft **66** (2013), pp. 192-197.
42. Steyer, D. and Clayeux C.: Genetic metabolism of hop terpenoid compounds by yeast in beer. 35th EBC Congr., (2015), Lecture 20.
43. Takasumi, K.; Takoi, K.; Koie, K.; Kaneko, T. and Nakamura, T.: Development of new quantitation method for polyfunctional thiols and its application for investigation of the characteristic aroma of "Flavor Hops". World Brew. Congr., (2016), Poster 135.
44. Takoi, K.; Tominaga, T.; Degueil, M.; Sakata, D.; Kurihara, T.; Shinkaruk, S.; Nakamura, T.; Maeda, K.; Akiyama, H.; Watari, J.; Bennetau, B. and Dubourdieu, D.: Identification of novel unique flavor compounds derived from Nelson Sauvin hop and development of new product using this hop. Proc. 31st EBC Congr., (2007), pp. 241-251 (CD-ROM).
45. Takoi, K.; Degueil, M.; Shinkaruk, S.; Thibon, C.; Maeda, K.; Ito, K.; Bennetau, B.; Dubourdieu, D. and Tominaga, T.: Identification and characteristics of new volatile thiols derived from the hop (*Humulus lupulus* L.) cultivar Nelson Sauvin. J. Agric. Food Chem. **57** (2009), pp. 2493-2502.
46. Takoi, K.; Degueil, M.; Shinkaruk, S.; Thibon, C.; Kurihara, T.; Toyoshima, K.; Ito, K.; Bennetau, B.; Dubourdieu, D. and Tominaga, T.: Specific flavor compounds derived from Nelson Sauvin hop and synergy of these compounds. BrewingScience – Monatsschrift für Brauwissenschaft **62** (2009), pp. 108-118.
47. Takoi, K.; Koie, K.; Itoga, Y.; Katayama, K.; Shimase, M.; Nakayama, Y. and Watari, J.: Biotransformation of hop-derived monoterpene alcohols by lager yeast and their contribution to the flavor of hopped beer. J. Agric. Food Chem. **58** (2010), pp. 5050-5058.
48. Takoi, K.; Itoga, Y.; Koie, K.; Kosugi, T.; Shimase, M.; Katayama, K.; Nakayama, Y. and Watari, J.: Contribution of geraniol metabolism to citrus flavour of beer: Synergy of geraniol and β-citronellol under co-existence with excess linalool. J. Inst. Brew. **116** (2010), pp. 251-260.
49. Takoi, K.; Itoga, Y.; Takayanagi, J.; Kosugi, T.; Shioi, T.; Nakamura, T. and Watari, J.: Screening of Geraniol-rich Flavor Hop and Interesting Behavior of β-Citronellol During Fermentation under Various Hop-Addition Timings. J. Am. Soc. Brew. Chem. **72** (2014), pp. 22-29.
50. Takoi, K.; Tokita, K.; Sanekata, A.; Usami, Y.; Itoga, Y.; Koie, K.; Matsumoto, I. and Nakayama, Y.: Varietal difference of hop-derived flavour compounds in late-hopped/dry-hopped beers. BrewingScience – Monatsschrift für Brauwissenschaft **69** (2016), pp. 1-7.
51. Tominaga, T.; Furrer, A.; Henry, R. and Dubourdieu, D.: Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon Blanc wines. Flavour Fragr. J. **13** (1998), pp. 159-162.
52. Tominaga, T.; Murat, M. -L. and Dubourdieu, D.: Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon Blanc. J. Agric. Food Chem. **46** (1998), pp. 1044-1048.
53. Vaudano, E.; Moruno, E. G. and Stefano, R. D.: Modulation of geraniol metabolism during alcohol fermentation. J. Inst. Brew. **110** (2004), pp. 213-219.

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