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Hypothesis of synergy between Sorachi Ace-derived geranic acid and various flavour compounds contributing to characteristic beer aroma

The hop variety Sorachi Ace was bred and first licenced in Japan by Sapporo Breweries Ltd. in 1984. Recently, this hop has been used as a ‘flavour hop’ to give beer a unique characteristic aroma, for example, woody, pine-like, citrus, dill-like and lemon grass-like. In previous study, we identified geranic acid as one of the unique compounds comprising Sorachi Ace. As a result of model sensory evaluations, we reported that geranic acid itself had little flavour, but that this compound could enhance the flavour intensities of other hop-derived flavour compounds in spite of its very low flavour intensity. In this study, the mechanism of Sorachi Ace varietal aroma was further investigated using sensory omission test. The selected 16 compounds were separated to five groups, ‘carboxylic acids’, ‘alcohols’, ‘aldehydes’, ‘esters’, and ‘hydrocarbons’. As a result of the omission test, it was concluded that all groups could affect to the varietal aroma of Sorachi Ace, despite the concentrations of several compounds were at very low levels. In addition, an effect of blend-hopping with Sorachi Ace and other flavour hop varieties was investigated. It was surprisingly found that blend-hopping between Sorachi Ace and certain hop varieties (Kazbek, Hallertau Blanc, and Cascade) could negatively affect to the flavour impression of test-beers. On the other hand, blend-hopping between Sorachi Ace and Citra or Mosaic could positively affect to the flavour impression of test-beers. Because of high concentrations of 4-Methyl-4-sulfanylpentan-2-one (4MSP) derived from these hops, it is assumed that 4MSP containing hops could be effective choice for blending with Sorachi Ace.

Descriptors: beers; hops; flavour; geranic acid; terpenoids; synergy

1 Introduction

Of all raw materials used for beers, hops (*Humulus lupulus* L.) especially contain many types of flavour compounds. Various terpenoids, esters, aldehydes, ketones, and sulphur compounds were well known as the major flavour compounds in hop cones. Until now, our group have focused on several groups of hop-derived flavour compounds, for example monoterpene alcohols, volatile thiols, branched-chain esters, and branched-chain fatty acids, and revealed their contribution 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 [1–13]. However, hop breeders are still developing characteristic new varieties, so that the mechanisms for forming the varietal aromas of various hops aren't fully investigated yet.

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Sorachi Ace hop can impart pine-like, woody, dill-like, citrus, and lemongrass-like flavours owing to its unique varietal aroma and used for brewing craft beers. Although Sorachi Ace is now mainly grown in the U.S., it was originally bred in Sorachi-gun, Hokkaido, Japan, around 30 years ago by Sapporo Breweries Ltd., and named after its place of origin. The initial breeding process was carried out using a Brewer's Gold female hop plant and a Saaz male hop plant in 1975. From these crosses, the 70K-SH6 female hop plant was selected and crossed with a Beikei male hop plant. Finally, Sorachi Ace was produced (Fig. 1), and the variety was registered in 1984 in Japan [14]. Subsequently, this hop was brought to the U.S. in 1994 by a Japanese hop breeder and is now grown on commercial-scale farms in the states of Washington and Oregon. In the 2010s, Sorachi Ace gained much attention because of its very characteristic varietal aroma and has been widely used for craft beers around the world.

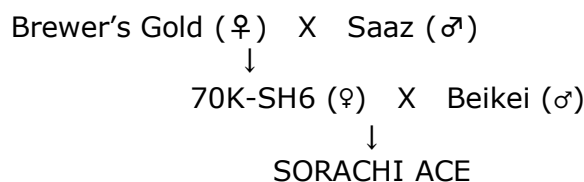


Fig. 1 Crossing history of the Sorachi Ace hop

Several researchers reported the composition of hop-derived flavour compounds in various hops and beers, including the Sorachi Ace hop and beer [11–12,15–18]. However, the key compounds contributing to the varietal aroma of Sorachi Ace have not been fully revealed. In recent times, we investigated characteristic flavour compounds derived from Sorachi Ace and found that a unique variety-specific compound, geranic acid, was present at a significant level only in the test-beer brewed with this hop (Fig. 2) [19]. Geranic acid was also found in lemongrass, ginger, lemon oil, tomato, and wine grape, which are raw materials used for wine making [20–27]. Methyl geranate, which is a methyl ester of geranic acid, has been reported as a compound derived from hop oil [15–16,28–29]. In the 1960s, Roberts et al. assumed geranic acid to be a possible precursor of methyl geranate, which they first identified in hop oil [28–29]. Peacock et al. analyzed three commercial beers and detected geranic acid in only one of the beer samples at a trace level (1 µg/L) [30]. Our previous study was the first to report the presence of hop-derived geranic acid in Sorachi Ace beers at a significant level [19].

It is assumed that the biosynthesis of geraniol derivatives in a hop cone occurs as follows. Geraniol is first oxidized to geranial, which is subsequently oxidized to geranic acid, and finally, geranic acid is esterified to methyl geranate (Fig. 3). In the case of other plant, the sedge *Cyperus iria* followed the biosynthesis pathway from farnesol to methyl farnesoate [23]. The structure of farnesol is similar to that of geraniol; therefore, not only farnesol (C15) but also geraniol (C10) could follow this reaction pathway. For example, Gewürztraminer grapes contain methyl farnesoate, methyl geranate, and geranic acid [21]. It is thought that the hop plant also adopts this biosynthesis pathway due to the presence of methyl geranate in hops. As described above, it could be confirmed that geranic acid is very specific to Sorachi Ace beers (Fig. 2). In addition, the beers brewed with other hops often contained methyl geranate at quantifiable levels, while the Sorachi Ace beers contained this compound at trace levels [19].

Furthermore, sensory evaluation techniques revealed that geranic acid has very unique characteristics. In previous study, selected 16 hop-derived terpenoids were spiked to kettle-hopped beer for simulating the dry-hopped Sorachi Ace beer and our panellists evaluated six flavour characters (flowery, fruity, lemon, tropical, green, and woody) in the model beers. In the case of the first model beer, which was spiked with only geranic acid and nerolic acid, all the scores were at low levels. In the second model beer, which contained 14 hop-derived terpenoids, except for geranic acid and nerolic acid, the scores of flowery, fruity, lemon, green, and woody were clearly higher. In the third model beer, which was spiked with all the compounds, the flowery, fruity, lemon, tropical, and green

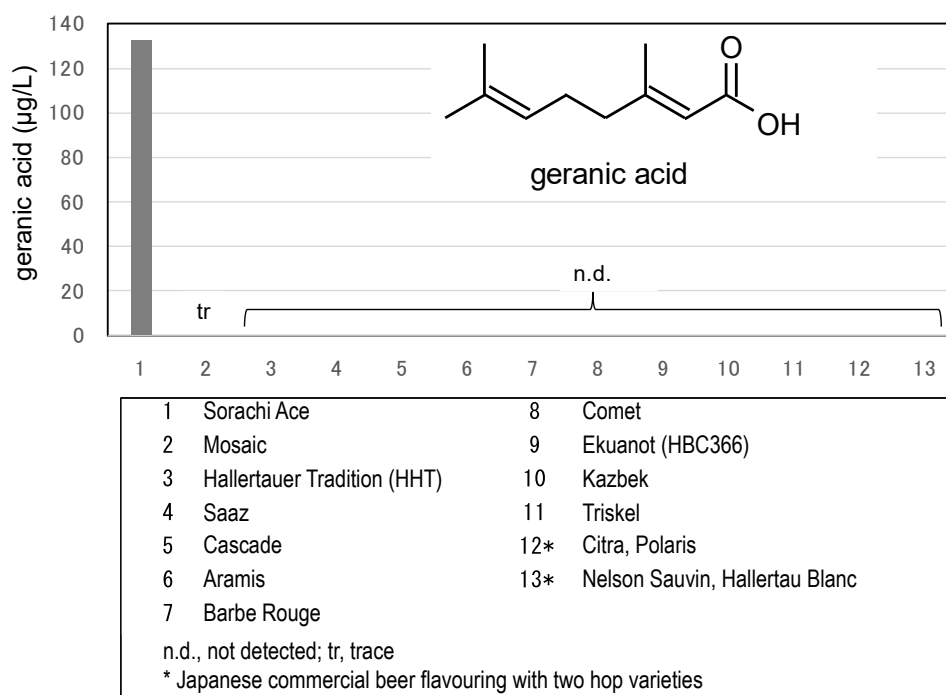


Fig. 2 Concentrations of geranic acid in test-brewed beers made using various hops (these data were previously published in ref 19)

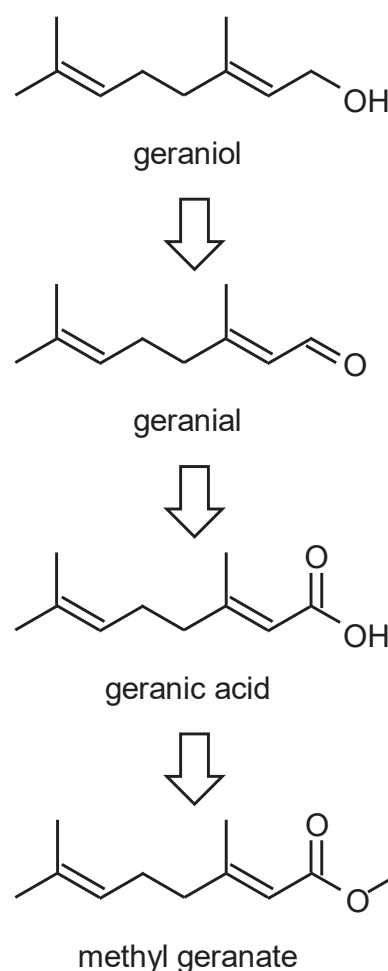


Fig. 3 Possible biosynthesis pathway of geraniol, geranial, geranic acid, and methyl geranate in plants

characters were at higher scores than those in the second model beer. The characters of the dry-hopped Sorachi Ace beer could be successfully reconstructed by spiking with all 16 hop-derived terpenoids, including geranic acid. From these results, it is thought that geranic acid is not odour-active but functions as an enhancer for hop-derived terpenoids at subthreshold levels [19].

In this study, the mechanism of Sorachi Ace varietal aroma was further investigated using sensory omission test. In addition, an effect of blend-hopping with Sorachi Ace and other flavour hop varieties was evaluated by test-brewing and sensory profile test.

2 Materials and methods

2.1 Hop raw materials

Hop variety, harvest year, form, and grown area of all hop samples were as follows: The Sorachi Ace used in this study were harvested in 2014 (Type 90 pellet); the Cascade, Citra, and Mosaic hops (Type 90 pellet) were harvested in the U.S. in 2012, 2013, and 2014, respectively. The Kazbak (Type 90 pellet) was harvested in 2014 in the Czech Republic. The Hallertauer Tradition (HHT), and Hallertau Blanc hops (Type 90 pellet) were harvested in Germany in 2015 and 2012, respectively.

2.2 Commercial Beer

Commercial-scale kettle-hopped beer was brewed by Sapporo Breweries Ltd. This beer was used for sensory evaluation as described in the following section (sensory omission test in 2.6.1).

2.3 Pilot-scale brewing

2.3.1 Test-beer dry-hopped with Sorachi Ace for investigating sensory omission test.

A test-beer dry-hopped with Sorachi Ace (Beer SA) was the same one brewed for researching the characteristic flavour compound of 'Sorachi Ace' hop in our previous paper [19]. This was produced according to the standard method prescribed by the Production & Technology Innovation Department, Sapporo Breweries Ltd. The wort was briefly prepared using commercially available malts and hops in a 100-L pilot scale apparatus. The boiling period was 90 min. The Sorachi Ace hop (0.09 g of hop/L) was firstly added at the beginning of the boiling period. Each cooled wort was transferred to a fermentation tank (100 L/tank), and fermentation was initiated by adding 15.0×10^6 cells/mL of lager yeast (*Saccharomyces pastorianus*) to the wort. For hop-flavouring or the so-called dry-hopping, the Sorachi Ace hop (1.5 g of hop/L) was added to the cooled wort together with yeast. The fermentation temperature was maintained at 10–12 °C (primary fermentation). After transferring the fermented wort to another storage tank under CO₂ atmosphere, maturation was carried out at 12 °C for 6 days (conditioning period) and then at 0 °C for 2–3 weeks. Filtration and bottling were carried out using the pilot-scale equipment under anti-oxidative conditions. All the hop-flavouring conditions are listed in table 1.

Table 1 Conditions of using hops in test-brewed beers

	hop dosage (g of hop/L)		
	bittering	late-hopping	dry-hopping
beer	variety dosage	variety dosage	variety dosage
SA	Sorachi Ace 0.09	– –	Sorachi Ace 1.5
B1-1	Sorachi Ace 0.09	Sorachi Ace 1.0	Sorachi Ace 0.5
B1-2	Sorachi Ace 0.09	Sorachi Ace 1.0	Kazbek 0.5
B1-3	Sorachi Ace 0.09	Sorachi Ace 1.0	Hallertau Blanc 0.5
B1-4	Sorachi Ace 0.09	Sorachi Ace 1.0	Cascade 0.5
B1-5	Sorachi Ace 0.09	Sorachi Ace 1.0	Citra 0.5
B1-6	Sorachi Ace 0.09	Sorachi Ace 1.0	Mosaic 0.5
B2-1	HHT 1.30	– –	Mosaic 0.5
B2-2	Sorachi Ace 0.09	Sorachi Ace 1.0	Mosaic 0.5

2.3.2 Test beers blend-hopping with Sorachi Ace and various flavour hop varieties.

A series of test-brewed beers (B1-1 to B1-6) were produced according to the same method, except that their hop-flavouring conditions were different. The Sorachi Ace hop (0.09 g of hop/L) was firstly added at the beginning of the boiling period. For late-hopping, same hop was added 5 min prior to the end of the boiling period (1.0 g of hop/L cooled wort). For dry-hopping, each flavour hop (0.5 g of hop/L) was added to the cooled wort together with yeast. In addition, another series of test-brewed beers (B2-1 to B2-2) were produced. For B2-1, the HHT hop (1.3 g of hop/L) was firstly added at the beginning of the boiling period. For dry-hopping, Mosaic hop (0.5 g of hop/L) was added to the cooled wort together with yeast. For B2-2, the Sorachi Ace hop (0.09 g of hop/L) was firstly added at the beginning of the boiling period. For late-hopping, same hop was added 5 min prior to the end of the boiling period (1.0 g of hop/L cooled wort). For dry-hopping, Mosaic hop (0.5 g of hop/L) was added to the cooled wort together with yeast. These beers were produced to evaluate an effect of blend-hopping between the Sorachi Ace and other flavour hop varieties. All the hop-flavouring conditions are listed in table 1.

2.4 Chemicals

2.4.1 Reference Compounds.

The chemicals used to quantitate terpenoids were as follows. Geranic acid (> 85 %, racemic mixture), and myrcene (> 90 %) were purchased from Sigma-Aldrich Japan Co., Ltd. (Tokyo, Japan). Linalool (> 96 %), α -terpinene (> 90 %), γ -terpinene (> 95 %), α -terpineol (> 95 %, racemic mixture), β -citronellol (> 92 %, racemic

mixture), geraniol (> 97 %), nerol (> 98 %), citral (> 98 %, racemic mixture), (+)-limonene (> 95 %), terpinolene (> 85 %), and benzyl acetate (> 99 %) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Methyl geranate (> 94 %, mixture of isomers) was purchased from Thermo Fisher Scientific Inc. (NYSE: TMO).

The chemicals used to quantitate volatile thiols were as follows. 4-Methyl-4-sulfanylpentan-2-one (4MSP) was purchased from Combi-Blocks (San Diego, CA, USA). 3-Sulfanylhexan-1-ol (3SH) was purchased from Alfa Aesar (Lancashire, United Kingdom). 3-Sulfanylhexyl acetate (3SHA) was purchased from Matrix Scientific (Columbia, SC, USA). 3-Sulfanylpropyl hexanoate (3SPH) was purchased from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan). 3-Sulfanyl-4-methylpentan-1-ol (3S4MP) and 3-sulfanyl-4-methylpentyl acetate (3S4MPA) were synthesized as described in a previous study [2].

2.4.2 Miscellaneous Chemicals.

Acetonitrile, anhydrous sodium sulfate, dichloromethane, ethyl acetate, phosphoric acid, sodium chloride, sodium hydrogen carbonate, and thioglycerol were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). 5,5'-Dithiobis

(2-nitrobenzoic acid) was purchased from Tokyo Chemical Industry (Tokyo, Japan).

2.5 Analysis of hop-derived flavour compounds

2.5.1 Determination of the Ratio of Isomers in the Reference Compounds by Gas Chromatography–Flame Ionization Detection (GC-FID)

The reference compounds of citral, geranic acid, and methyl geranate contained isomers. Citral is a mixture of geraniol and nerol. Commercial geranic acid and methyl geranate contained small amounts of neric acid and methyl nerolate, respectively. All the reference compounds contained small amounts of contaminants and/or degradation products. For isomer analysis, GC-FID measurements were conducted on a 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) under the same analysis conditions described in our previous paper [19]. The calculated isomer ratios are listed in table S1 (see page 131).

2.5.2 Quantitation of Hop-Derived Compounds by solid phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS)

Table 2 Concentrations of spiked compounds in model beers for sensory omission test

			isomer ratio ^a (%)	beer SA (dry-hopped with Sorachi Ace)	concentrations of spiked compounds						perception threshold
					model beer P all compounds	model beer Q - geranic acid	model beer R - alcohols	model beer S - aldehydes	model beer T - esters	model beer U - hydrocarbons	
alcohols	linalool	(µg/L)	–	97	97	97	–	97	97	97	3 ^d
	α-terpineol	(µg/L)	–	20	20	20	–	20	20	20	450 ^d
	β-citronellol	(µg/L)	–	13	13	13	–	13	13	13	9 ^d
	geraniol	(µg/L)	–	98	98	98	–	98	98	98	7 ^d
	nerol	(µg/L)	–	9.6	9.6	9.6	–	9.6	9.6	9.6	80 ^d
aldehydes	geranial	(µg/L)	53	2.7	2.7 ^c	2.7 ^c	2.7 ^c	–	2.7 ^c	2.7 ^c	30 – 32 ^e
	neral	(µg/L)	27	tr (0.46) ^b	1.4 ^c	1.4 ^c	1.4 ^c	–	1.4 ^c	1.4 ^c	30 – 32 ^e
carboxylic acids	geranic acid	(µg/L)	70	178	178 ^c	–	178 ^c	178 ^c	178 ^c	178 ^c	2200 ^f
	neric acid	(µg/L)	20	n.d.	51 ^c	–	51 ^c	51 ^c	51 ^c	51 ^c	–
esters	methyl geranate	(µg/L)	71.5	1.2	1.2 ^c	1.2 ^c	1.2 ^c	1.2 ^c	–	1.2 ^c	–
	methyl nerolate	(µg/L)	28.5	tr (0.01) ^b	0.5 ^c	0.5 ^c	0.5 ^c	0.5 ^c	–	0.5 ^c	–
hydrocarbons	α-terpinene	(µg/L)	–	tr (0.20) ^b	0.2	0.2	0.2	0.2	0.2	–	–
	γ-terpinene	(µg/L)	–	tr (0.03) ^b	0.03	0.03	0.03	0.03	0.03	–	260 ^g
	(+)-limonene	(µg/L)	–	tr (0.26) ^b	0.26	0.26	0.26	0.26	0.26	–	200 ^g
	terpinolene	(µg/L)	–	tr (0.06) ^b	0.06	0.06	0.06	0.06	0.06	–	41 ^g
	myrcene	(µg/L)	–	10	10	10	10	10	10	10	–

tr, trace; n.d., not detected

^a isomer ratio in standard substances (geranial, geranic acid, and methyl geranate), omitting degradation products and/or contaminants.

^b concentrations of 'tr' compounds were calculated according to the calibration curves in SPME-GC-MS for profile sensory test.

^c concentrations of spiked standard substances (geranial, geranic acid, and methyl geranate) were adjusted according to the concentrations of major isomers.

^d perception threshold reported in ref 6.

^e perception threshold for citral reported in ref 31.

^f perception threshold reported in ref 19.

^g perception threshold reported in ref 32.

2.5.2.1 Quantitation of Terpene Alcohols, Aldehydes, and Esters.

The quantitation of terpene alcohols, aldehydes, and esters was performed on a 6890N GC with a 5973N MS (Agilent Technologies) under the same analysis conditions described in our previous paper [19]. The monitored ions for selected ion monitoring (SIM) mode, range of the calibration curve, and the ratios of the isomers in the reference compounds are listed in table S1.

2.5.2.2 Quantitation of Geranic Acid and Nerolic Acid.

Geranic acid and nerolic acid were quantitated on a 6890N GC with a 5973N MS (Agilent Technologies) under the same analysis conditions described in our previous paper [19]. The monitored ions for selected ion monitoring (SIM) mode, range of the calibration curve, and the ratios of the isomers in the reference compounds are listed in table S1.

2.5.2.3 Analysis of Volatile Thiols by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS)

The specific extraction of volatile thiols from hops and beers using an ASPEC GX-274 (Gilson, Middleton, WI, USA) and a solid phase extraction cartridge (Meta-Sep IC-Ag; GL Sciences, Tokyo, Japan) and the quantitation of volatile thiols via GC-MS/MS analysis with

an Agilent 7890A gas chromatograph coupled to a 7000B triple quadrupole mass spectrometer (Agilent Technologies) were performed according to the methods reported by Takazumi et al. [5]. The conditions for the selected reaction monitoring (SRM) mode are listed in table S2 (see page 132).

2.6 Sensory evaluation

2.6.1 Omission test

Each sensory evaluation was performed by 8 well-trained panelists. In order to analyse the synergy between geranic acid and hop-derived terpenoids, omission tests were carried out on model beers; the procedure is as follows (Table 2). Japanese commercial pilsner-type beer made with kettle-hopping was used as the control beer. The concentrations of the targeted compounds in the simulated model beers were similar to their concentrations in the dry-hopped Sorachi Ace beer (2.2.1). The concentrations of not only the major compounds but also the minor compounds were calculated at trace levels from the calibration curves in SPME-GC-MS, in order to simulate Sorachi Ace beer as closely as possible. As shown in table 2, 5 mixtures were used for this omission tests; a mixture of carboxylic acids contained geranic acid and nerolic acid; a mixture of alcohols, linalool, geraniol, nerol, β -citronellol, and α -terpineol; a mixture of aldehydes, geraniol and neral; a mixture of esters, methyl geranate and methyl nerolate; a mixture of hydrocarbons,

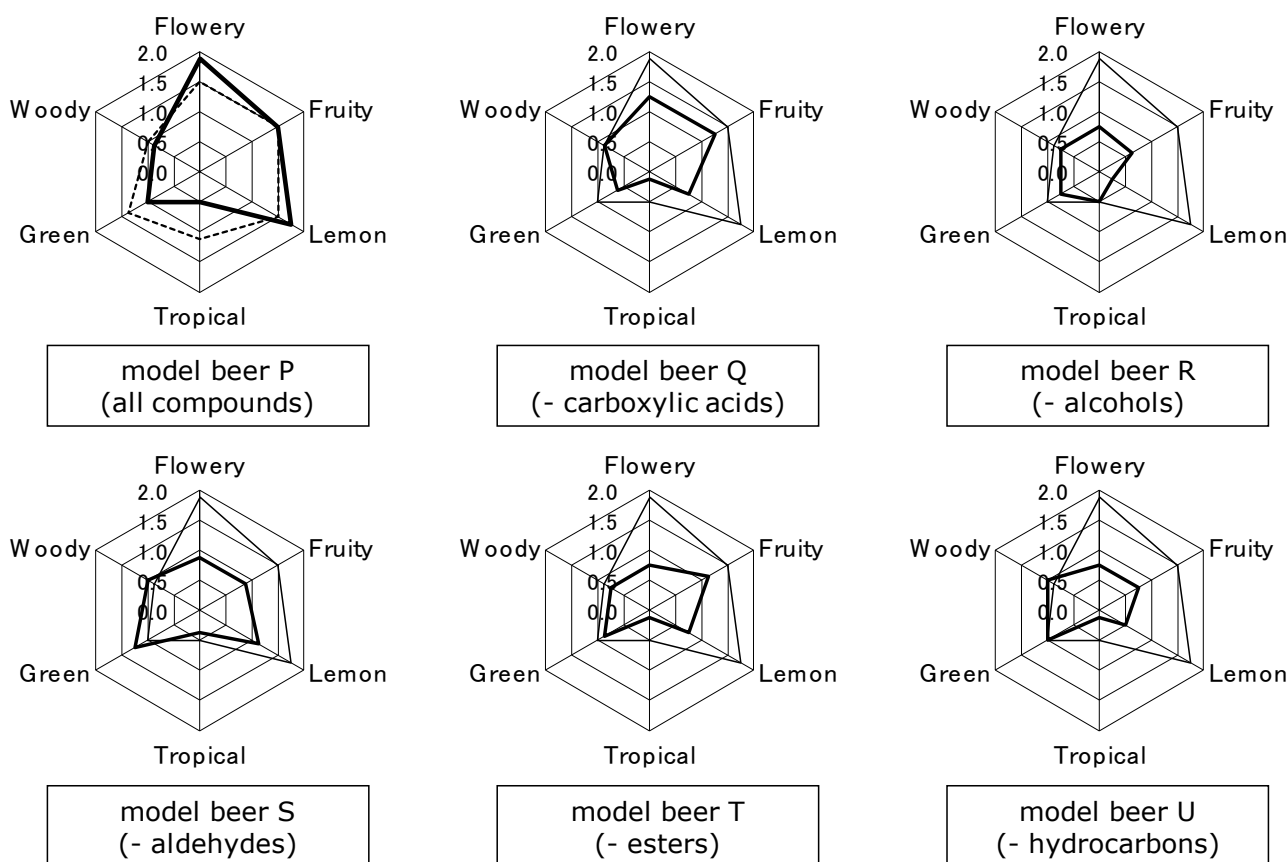


Fig. 4 Flavour profile of model beers containing a mixture of hop-derived flavour compounds: Beer SA was dry-hopped with Sorachi Ace (indicated as dotted line in model beer P); all model beers prepared by spiking reference compounds to Japanese commercial kettle-hopped beer; model beer P, all compounds (indicated as hair line in model beer Q to U); model beer Q, all compounds except for carboxylic acids; model beer R, all compounds except for alcohols; model beer S, all compounds except for aldehydes; model beer T, all compounds except for esters; model beer U, all compounds except for hydrocarbons

Table 3 Average scores of model beers in sensory omission test

	model beer P all compounds	model beer Q - carboxylic acids		model beer R - alcohols		model beer S - aldehydes		model beer T - esters		model beer U - hydrocarbons	
	intensity ^a	intensity ^a	<i>p</i> ^b	intensity ^a	<i>p</i> ^b	intensity ^a	<i>p</i> ^b	intensity ^a	<i>p</i> ^b	intensity ^a	<i>p</i> ^b
flowery	1.88	1.25	0.049*	0.75	0.00004***	0.88	0.001**	0.75	0.007**	0.75	0.007**
fruity	1.50	1.25	0.351	0.63	0.0002***	0.88	0.049*	1.13	0.197	0.75	0.020*
lemon	1.75	0.75	0.018*	0.25	0.014*	1.13	0.140	0.75	0.086	0.50	0.019*
tropical	0.50	0.13	0.080	0.50	1.000	0.38	0.685	0.13	0.080	0.13	0.197
green	1.00	0.63	0.080	0.75	0.170	1.25	0.516	0.88	0.598	1.00	1.000
woody	0.88	0.88	1.000	0.75	0.685	1.00	0.685	0.75	0.598	1.00	0.732

^a mean intensity value of the scores from eight well-trained panellists

^b paired t-test comparing model beer Q-U against beer P

*, significant difference with a risk of 5 %; **, significant difference with a risk of 1 %; ***, significant difference with a risk of 0.1 %

limonene, terpinolene, α -terpinene, γ -terpinene, and myrcene. The reconstructed model beer P was prepared by spiking the control beer with all mixtures. Of all mixtures, the mixture of carboxylic acids was omitted in the model beer Q; the mixture of alcohols, in the model beer R; the mixture of aldehydes, in the model beer S; the mixture of esters, in the model beer T; the mixture of hydrocarbons, in the model beer U. These samples were stored at 4 °C, and 70 mL of each sample was poured in uncovered glass vessels (total volume = 150 mL) immediately before presenting them to the panellists. Later, the six flavour characters (flowery, fruity, lemon, tropical, green, and woody) of each sample were scored from 0 (no flavour) to 3 (strong flavour) at intervals of 0.5. The mean intensity value of each characteristic was calculated, and paired t-tests were conducted on Microsoft Excel 2013.

2.6.2 Profile test

The test-brewed beers in 2.2.2 (Table 1) were evaluated using the same profile test described in 2.5.1. The results were shown as spider charts. The mean intensity value of each characteristic was calculated, and paired t-tests were conducted on Microsoft Excel 2013.

3 Results and discussions

3.1 Study of the synergy among various hop-derived terpenoids

It is proposed that geranic acid is a key compound responsible for the varietal aroma of the Sorachi Ace hop, and that geranic acid exhibits very unique characteristics; by itself, it was less odour-active but functioned as an enhancer for hop-derived terpenoids at subthreshold levels. In the previous study [19], the flavour characters of the dry-hopped Sorachi Ace beer could be successfully reconstructed by spiking with 16 hop-derived terpenoids including geranic acid to the kettle-hopped beer having no hoppy flavour (The spider chart of 'model beer P' in Fig. 4 indicated the same data).

In this study, the mechanism of the varietal aroma of Sorachi Ace were further investigated in detail using sensory omission test. The selected 16 compounds were separated to five groups as follows:

'carboxylic acids' contained geranic acid and nerolic acid; 'alcohols', linalool, geraniol, nerol, β -citronellol, and α -terpineol; 'aldehydes', geranial and neral; 'esters', methyl geranate and methyl nerolate; 'hydrocarbons', limonene, terpinolene, α -terpinene, γ -terpinene, and myrcene. In the omission test, each group of compounds were omitted to investigate a contribution of each group under the presence of geranic acid. The spiking condition of all model beers were listed in table 2.

Figure 4 shows the six flavour characters (flowery, fruity, lemon, tropical, green, and woody) of all model beers. As described above, model beer P containing all compounds could simulate the dry-hopped Sorachi Ace beer (beer SA). In the chart of model beer Q, which was omitted the group 'carboxylic acids', the average scores of 'flowery', 'fruity', 'lemon', 'tropical', and 'green' decreased. Especially, the scores of 'flowery' and 'lemon' were significantly lower with a risk of 5 %, when compared to model beer P (Table 3). This result reconfirmed the enhancing effect of geranic acid. In the chart of model beer R, which was omitted the group 'alcohols', the average scores of 'flowery', 'fruity', and 'lemon' drastically decreased. The scores of 'flowery' and 'fruity' were significantly lower with a risk of 0.1 %, and the one of 'lemon' with a risk of 5 %, when compared to model beer P (Table 3). This result clearly indicated the contribution of monoterpene alcohols to hoppy beer aroma. In model beer S, which was omitted the group 'aldehydes', the average scores of 'flowery', 'fruity', and 'lemon' decreased. The scores of 'flowery' and 'fruity' were significantly lower with a risk of 1 % and 5 %, respectively (Table 3). In model beer T, which was omitted the group 'esters', the average score of 'flowery' was significantly lower with a risk of 1 % (Table 3). In the chart of model beer U, which was omitted the group 'hydrocarbons', the average scores of 'flowery', 'fruity', and 'lemon' drastically decreased. The score of 'flowery' was significantly lower with a risk of 1 %, and the ones of 'fruity' and 'lemon' with a risk of 5 % (Table 3). From these results, it was concluded that all groups could contribute to the varietal aroma of Sorachi Ace.

In this study, we selected 16 hop-derived compounds, not only the geraniol-derivatives shown in figure 3 but also terpene hydrocarbons, myrcene, α -terpinene, γ -terpinene, (+)-limonene, and terpinolene, as these compounds, which exhibit resinous and/or woody odours, can be enriched in beers by dry-hopping; further,

Table 4 Concentrations of hop-derived flavour compounds in test-beers blend-hopped with Sorachi Ace and various flavour hop varieties

test-beer			B1-1	B1-2	B1-3	B1-4	B1-5	B1-6	B2-1	B2-2	
hopping condition	bittering late-hopping dry-hopping		Sorachi Ace Sorachi Ace Sorachi Ace	Sorachi Ace Sorachi Ace Kazbek	Sorachi Ace Sorachi Ace Hallertau Blanc	Sorachi Ace Sorachi Ace Cascade	Sorachi Ace Sorachi Ace Citra	Sorachi Ace Sorachi Ace Mosaic	HHT - Mosaic	Sorachi Ace Sorachi Ace Mosaic	perception threshold
alcohols	linalool	(µg/L)	62.5	48.8	44.7	48.5	91.5	63.0	36.0	74.5	3 ^a
	α-terpineol	(µg/L)	11.1	11.2	9.0	8.2	8.4	9.8	2.4	8.3	450 ^a
	β-citronellol	(µg/L)	15.9	18.8	20.1	27.3	16.2	27.8	19.5	26.3	9 ^a
	geraniol	(µg/L)	15.4	19.5	21.4	26.2	18.1	29.7	25.2	28.6	7 ^a
	nerol	(µg/L)	4.0	3.5	3.0	3.8	5.3	5.9	5.0	7.0	80 ^a
aldehydes	geranial	(µg/L)	tr	tr	tr	tr	tr	tr	tr	tr	30 – 32 ^b
	neral	(µg/L)	n.d.	n.d.	n.d.	tr	n.d.	n.d.	n.d.	n.d.	30 – 32 ^b
carboxylic acids	geranic acid	(µg/L)	115	89	85	116	72	73	tr	65	2200 ^c
	neric acid	(µg/L)	tr	tr	tr	tr	tr	tr	tr	tr	–
esters	methyl geranate	(µg/L)	1.3	1.7	1.9	4.6	25.4	24.6	29.0	27.4	–
	methyl nerolate	(µg/L)	tr	tr	0.1	tr	0.3	0.6	0.9	0.9	–
hydrocarbons	α-terpinene	(µg/L)	tr	tr	tr	tr	tr	tr	tr	0.3	–
	γ-terpinene	(µg/L)	tr	tr	tr	tr	tr	tr	tr	tr	260 ^d
	(+)-limonene	(µg/L)	tr	tr	tr	tr	tr	tr	tr	tr	200 ^d
	terpinolene	(µg/L)	tr	tr	tr	tr	tr	tr	tr	tr	41 ^d
	myrcene	(µg/L)	tr	tr	tr	tr	tr	tr	1.4	4.0	100 ^d
volatile thiols	4-methyl-4-sulfanyl-pentan-2-one	(ng/L)	tr	tr	2.0	1.5	23	16	15	17	1.2 ^e
	3-sulfanyl-4-methyl-pentyl acetate	(ng/L)	tr	tr	17.5	tr	tr	13.9	11.7	10.7	120 ^f
	3-sulfanyl-4-methyl-pentan-1-ol	(ng/L)	tr	20	547	7	24	332	298	272	40 ^f
	3-sulfanylhexyl acetate	(ng/L)	tr	tr	tr	tr	tr	tr	tr	tr	4 ^g
	3-sulfanylhexan-1-ol	(ng/L)	72	75	79	75	74	76	85	76	60 ^g

tr, trace; n.d., not detected

^aperception threshold reported in ref 6.

^bperception threshold for citral reported in ref 31.

^cperception threshold reported in ref 19.

^dperception threshold reported in ref 32.

^eperception threshold reported in ref 10.

^fperception threshold reported in ref 2.

^gperception threshold reported in ref 37.

their structures are similar to those of geraniol derivatives. In order to evaluate any possible synergy between subthreshold compounds, the concentrations of several compounds contained at trace levels in the dry-hopped beer (Beer SA) were calculated using the HS-SPME-GC-MS calibration curves, and these compounds were used to spike the model beers (Table 2). (Of these selected compounds, the spiked concentrations of neral, neric acid, and methyl nerolate did not correspond with those calculated for beer SA, as we used commercially available reference compounds of citral, geranic acid, and methyl geranate, which also contained their isomers.)

Of all groups, ‘aldehydes’ could affect both ‘flowery’ and ‘fruity’ characters, ‘esters’ could affect ‘flowery’ character, and ‘hydrocarbons’ could affect ‘flowery’, ‘fruity’, and ‘lemon’ characters, despite their very low concentrations. The flavour threshold of citral, mixture of geranial and neral, was reported as 30-32 µg/L [31] (There was no report described the threshold of methyl geranate and methyl

nerolate). The flavour thresholds of (+)-limonene, terpinolene, γ-terpinene, and myrcene were reported as 200, 41, 260, and 100 µg/L, respectively [32]. Although the concentrations of these compounds might be regarded as below their threshold levels, it was suggested that all of these groups could affect to the varietal aroma of Sorachi Ace.

3.2 Blend-hopping with Sorachi Ace and other flavour hop varieties

As described above, it was proposed new mechanism for the Sorachi Ace varietal aroma relating geranic acid and various hop-derived terpenoids. In recent studies, several mechanisms for hop aromas have been discussed. Their key flavour compounds are as follows: monoterpene alcohols [6–7], volatile thiols [2–4], and branched-chain fatty acids [13] and so on. Therefore, we next tried to investigate an effect of blend-hopping with Sorachi Ace and other

hop varieties containing such key compounds.

3.2.1 Test-beers blend-hopped with Sorachi Ace and other hop varieties

A series of test-brewed beers (B1-1 to B1-6) were produced for evaluating the effect of blend-hopping. In this series, Sorachi Ace was commonly used for late-hopping (1.0 g of hop/L cooled wort). We selected Sorachi Ace (B1-1, control), Kazbek (B1-2), Hallertau Blanc (B1-3), Cascade (B1-4), Citra (B1-5), and Mosaic (B1-6) for dry-hopping (0.5 g of hop/L). All the hop-flavouring conditions are listed in table 1. The compositions of hop-derived flavour compounds in all test-beers were shown in table 4. Our well-trained panellists evaluated these test-beers by sensory profile test.

Figure 5 shows the six flavour characters (flowery, fruity, lemon, tropical, green, and woody) of all blend-hopped test-beers. In the chart of model beer B1-2, which was dry-hopped with Kazbek, the average scores of 'fruity', 'green', and 'woody' decreased. Especially, the score of 'fruity' was significantly lower with a risk of 5 %, when compared to test-beer B1-1, which brewed only with Sorachi Ace (Table 5). In test-beer B1-3, which was dry-hopped with Hallertau Blanc, the average scores of 'fruity', 'lemon', and 'woody' decreased. The scores of 'fruity' and 'lemon' were significantly lower with a risk of 1 % and 5 %, respectively (Table 5). In test-beer B1-3, the scores of all characters were at very low levels. In test-beer B1-4, which was dry-hopped with Cascade, the aver-

age scores of 'fruity', 'green', and 'woody' decreased. The score of 'woody' was significantly lower with a risk of 5 % (Table 5). It was surprisingly found that blend-hopping with certain hop varieties could negatively affect to the flavour impression of finished beers.

On the other hand, in the chart of test-beer B1-5, which was dry-hopped with Citra, the average scores of 'flowery', 'fruity', 'lemon', and 'tropical' increased. Finally, in test-beer B1-6, which was dry-hopped with Mosaic, the average scores of 'fruity', 'lemon', and 'tropical' increased. Especially, the score of 'tropical' was significantly higher with a risk of 0.1 %, when compared to test-beer B1-1.

In the series B1-1 to B1-6, all test-beers were commonly late-hopped with Sorachi Ace and dry-hopped various varieties. In other words, each effect of variety-specific compounds would be compared in a presence of Sorachi Ace-derived compounds. The observed negative effect in B1-2, B1-3, and B1-4 couldn't be sufficiently explained by the compositions of flavour compounds in these samples (Table 4). Further investigation would be needed. In B1-5 and B1-6, 4-methyl-4-sulfanylpentan-2-one (4MSP), which could impart tropical flavour to beer by synergy [4], was present in both beers (Table 4). 4MSP could also functioned as an enhancer for monoterpene alcohols [4]. It is thought that the sensory effect of 4MSP could function under the condition of blend-hopping with Sorachi Ace.

3.2.2 Test-beers blend-hopped with Sorachi Ace and Mosaic

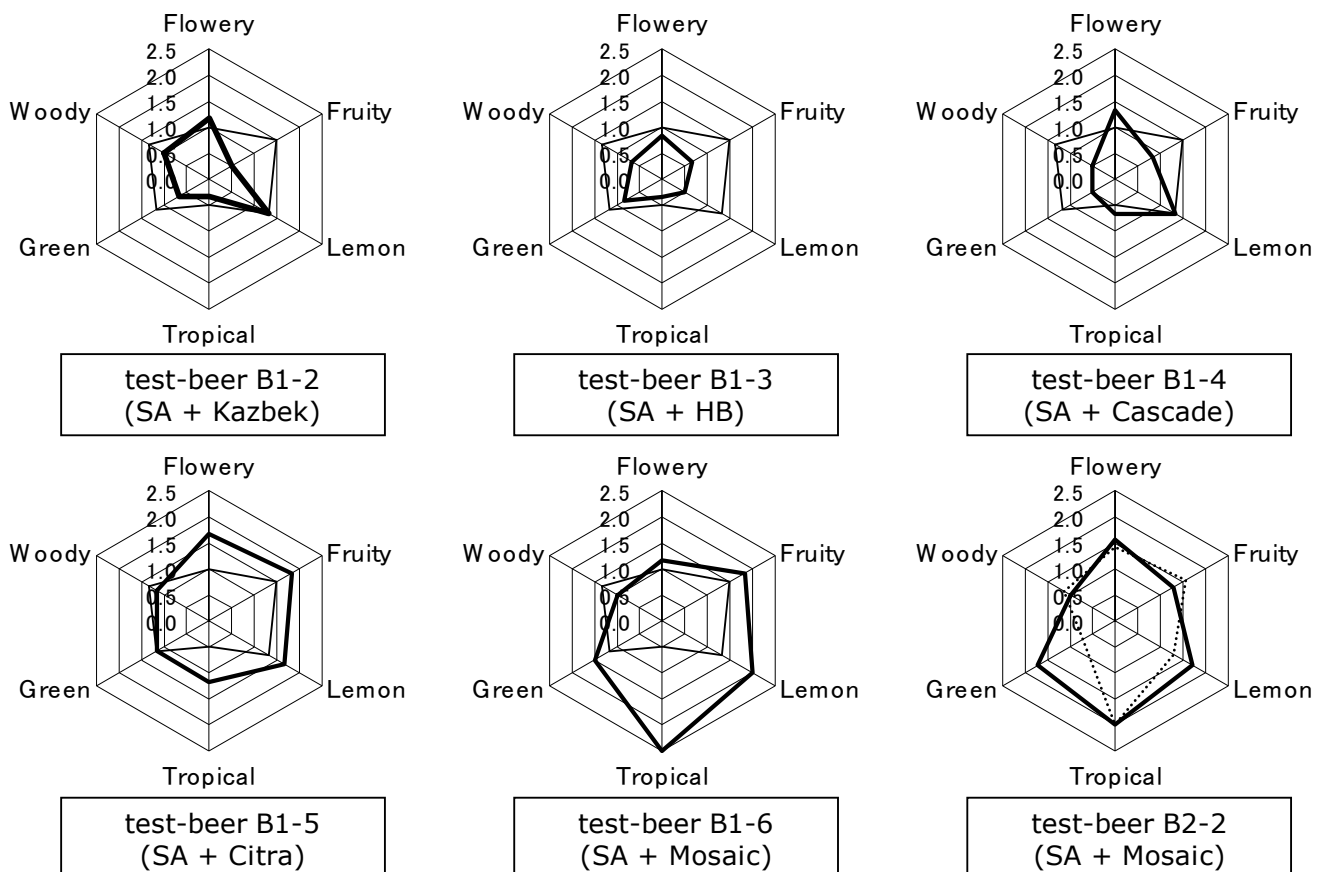


Fig. 5 Flavour profile of test-beers blend-hopped with Sorachi Ace and other flavour hop varieties: the conditions of blend-hopping was shown in Table 1; hair line in the charts of test-beer B1-2 to B1-6 indicated the data of B1-1; dotted line in the charts of test-beer B2-2 indicated the data of B2-1; SA, Sorachi Ace; HB, Hallertau Blanc

Table 5 Average scores of test-beers with blend-hopping in sensory profile test

test-beer		B1-1			B1-2			B1-3			B1-4			B1-5			B1-6			B2-1			B2-2		
hopping condition	bittering late-hopping dry-hopping	Sorachi Ace Sorachi Ace Sorachi Ace Sorachi Ace	Sorachi Ace Sorachi Ace Kazbek			Sorachi Ace Sorachi Ace Hallertau Blanc			Sorachi Ace Sorachi Ace Cascade			Sorachi Ace Sorachi Ace Citra			Sorachi Ace Sorachi Ace Mosaic			HHT - Mosaic			Sorachi Ace Sorachi Ace Mosaic				
		intensity	intensity	<i>p</i> ^a	intensity	<i>p</i> ^a	intensity	<i>p</i> ^a	intensity	<i>p</i> ^a	intensity	<i>p</i> ^a	intensity	<i>p</i> ^a	intensity	<i>p</i> ^a	intensity	intensity	<i>p</i> ^b						
flowery		1.00	1.17	0.695	0.83	0.611	1.33	0.363	1.67	0.175	1.17	0.695	1.43	1.57	0.604										
fruity		1.50	0.50	0.012*	0.67	0.004**	0.83	0.175	1.83	0.363	1.83	0.363	1.57	1.57	1.000										
lemon		1.33	1.33	1.000	0.50	0.042*	1.33	1.000	1.67	0.465	2.00	0.175	1.29	1.57	0.631										
tropical		0.50	0.33	0.611	0.33	0.611	0.67	0.695	1.17	0.102	2.50	0.0006***	2.00	2.00	1.000										
green		1.17	0.67	0.076	0.83	0.576	0.50	0.102	1.17	1.000	1.50	0.465	0.86	1.71	0.078										
woody		1.33	1.00	0.530	0.67	0.102	0.50	0.042*	1.17	0.611	1.00	0.175	1.00	1.00	1.000										

^apaired t-test comparing model beer B1-2 to B1-5 against beer B1-1 by six well-trained panellists

^bpaired t-test comparing model beer B2-2 against beer B2-1 by seven well-trained panellists

*, significant difference with a risk of 5 %; **, significant difference with a risk of 1 %; ***, significant difference with a risk of 0.1 %

For further investigation of blend-hopping with Sorachi Ace and Mosaic, another series of test-brewed beers (B2-1 to B2-2) was produced. For B2-1, HHT hop (1.3 g of hop/L) was used for kettle-hopping, and Mosaic hop (0.5 g of hop/L) was for dry-hopping. The hopping condition of B2-2 was the same one of B1-6, the Sorachi Ace hop was used for kettle-hopping (0.09 g of hop/L), late-hopping (1.0 g of hop/L cooled wort), and Mosaic hop was used for dry-hopping, (0.5 g of hop/L). These beers were produced to evaluate an effect of blend-hopping between the Sorachi Ace and Mosaic (Table 1). In the chart of model beer B2-2 (Fig. 5), which was late-hopped with Sorachi Ace and dry-hopped with Mosaic, the average scores of 'lemon' and 'green' increased, when compared to test-beer B2-1, which dry-hopped only with Mosaic.

In the series B2-1 to B2-2, both test-beers were dry-hopped with Mosaic. In other words, an effect of Sorachi Ace-specific compounds, especially geranic acid, would be compared in a presence of Mosaic-derived compounds including 4MSP. There was no difference in the scores of 'tropical' between B2-1 and B2-2 as well as B1-1 and B1-6. The tropical character might not be affected by geranic acid. On the other hand, the 'lemon' and 'green' characters in B2-2 increased in comparison with those in B2-1. Especially, the 'green' characters drastically increased under the presence of geranic acid. From these results, it is assumed that 4MSP containing hops could be effective choice for blending with Sorachi Ace.

4 Conclusions

In this study, the mechanism of the varietal aroma of Sorachi Ace was further investigated in detail using sensory omission test. The selected 16 compounds were separated to five groups as follows: 'carboxylic acids', 'alcohols', 'aldehydes', 'esters', and 'hydrocarbons'. In the omission test, each group of compounds was omitted. From this test, it was shown that all groups could affect to flavour characters of model beers. Of all groups, 'aldehydes' could affect both 'flowery' and 'fruity' characters, 'esters' could affect 'flowery'

character, and 'hydrocarbons' could affect 'flowery', 'fruity', and 'lemon' characters, despite their very low concentrations, which might be regarded as below their threshold levels. As a result, it was concluded that all groups could affect to the varietal aroma of Sorachi Ace.

In the field of flavour science, it is well-known that there is an additive effect between classes of compounds with similar structures at sub-threshold levels [33–34]. For example, 3-sulfanyhexan-1-ol (C6) can enhance the odour intensities of 3-sulfanypentan-1-ol (C5) and 3-sulfanyheptan-1-ol (C7) [34]. In addition, the intensities of several coffee aroma compounds could be enhanced by certain carboxylic acids, such as acetic acid (C2) and butyric acid (C4), at sub-threshold levels [35–36]. From these studies [33–36] and our study [19], it was assumed that geranic acid could enhance the intensities of hop-derived terpenoids below subthreshold levels because geranic acid have similar structure to such terpenoids, and it is also one of the carboxylic acids.

In addition, an effect of blend-hopping with Sorachi Ace and other hop varieties containing various flavour compounds was investigated. It was surprisingly found that blend-hopping between Sorachi Ace and certain hop varieties (Kazbek, Hallertau Blanc, and Cascade) could negatively affect to the flavour impression of test-beers. On the other hand, blend-hopping between Sorachi Ace and Citra or Mosaic could positively affect to the flavour impression of test-beers. Because of high concentrations of 4MSP derived from these hops, it is assumed that 4MSP containing hops could be effective choice for blending with Sorachi Ace. It was thought that more experiments would be necessary to confirm this positive effect of 4MSP in future.

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Supplemental information

2.5.1 Determination of the Ratio of Isomers in the Reference Compounds by Gas Chromatography–Flame Ionization Detection (GC-FID)

The reference compounds of citral, geranic acid, and methyl geranate contained isomers. Citral is a mixture of geranial and neral. Commercial geranic acid and methyl geranate contained small amounts of neric acid and methyl nerolate, respectively. All the reference compounds contained small amounts of contaminants and/or degradation products. For isomer analysis, GC-FID measurements were conducted on a 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) [19]. The carrier gas was helium with a flow rate of 1.7 mL/min in the constant-flow mode. The detector used was a flame-ionization detector at 250 °C. Hydrogen gas at a flow rate of 40 mL/min and air at 450 mL/min were used for the FID. Aliquots (1 µL) of each chemical (500 mg/L) were injected into a split injector (250 °C; split rate, 100:1; purge flow, 168.5 mL/min) at an oven temperature of 50 °C onto a type HP-INNOWax capillary column (30 m × 0.25 mm internal diameter (i.d.); 0.25 µm film thickness; Agilent Technologies). For all measurements, the temperature program was as follows: 50 °C for 2.5 min, heated at 10 °C/min to 240 °C, and a 5 min isotherm. The ratio of isomers and contaminants was calculated on the basis of the areas of all the peaks obtained using the FID detector. The calculated isomer ratios are listed in Table S1.

2.5.2 Quantitation of Hop-Derived Compounds by HS-SPME-GC-MS

2.5.2.1 Quantitation of Terpene Alcohols, Aldehydes, and Esters.

As described in our previous paper [19], the quantitation of terpene alcohols, aldehydes, and esters was performed on a 6890N GC with a 5973N MS (Agilent Technologies). An SPME fibre (PDMS/DVB, 65 µm film thickness, Supelco, Bellefonte, PA, USA) was used for extraction of volatile compounds. Eight milliliters of each

beer sample was placed into a 20-mL glass vial with 3 g of sodium chloride, followed by spiking with 40 µL of 10 mg/L benzyl acetate as an internal standard. The vial was hermetically sealed with a magnetic cap and agitated at 40 °C for 15 min on a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland). The SPME fibre was inserted into the head space of the vial and held for 15 min for adsorption. After adsorption, the SPME fibre was immediately inserted into a GC injector for thermal desorption for 3 min at 270 °C. The volatiles were injected in splitless mode. The separation of volatiles was performed on a DB-WAX column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies) with a helium carrier gas at a constant flow rate of 1.2 mL/min. The oven temperature setting was as follows: 40 °C for 3 min, raised to 250 °C at a rate of 5 °C/min, followed by a 5 min iso-therm. The mass spectrometer functioned in the EI mode (70 eV) and was operated in selected ion monitoring (SIM) mode. Calibration curves were constructed using the beer samples spiked with reference compounds. An appropriate range of the calibration curves was determined for calculating the concentrations of the compounds in sample beers. The monitored ions, range of the calibration curve, and the ratios of the isomers in the reference compounds are listed in Table S1. All calibrations produced a linear correlation with an R² value > 0.99, across the entire concentration range analysed. All analyses were repeated in duplicate.

2.5.2.2 Quantitation of Geranic Acid and Neric Acid.

Geranic acid and neric acid were quantitated on a 6890N GC with a 5973N MS (Agilent Technologies) [19]. Four milliliters of each test-brewed beer sample and 4 mL of 0.1 % (v/v) aqueous phosphoric acid were added to a 20-mL glass vial containing 3 g of sodium chloride, followed by spiking with 20 µL of 10 mg/L benzyl acetate as the internal standard. The vial was hermetically sealed using a magnetic cap and agitated at 60 °C for 15 min on a Combi-PAL autosampler (CTC Analytics). The SPME fibre (PDMS/DVB, 65 µm film thickness, Supelco) was inserted into the head space of the vial and held for 15 min for adsorption. After adsorption, the SPME

Table S1 GC-MS parameters for quantitation of selected flavor compounds (this Table was previously published in ref 19)

group	compound	isomers	ratio of isomers	quantifier ion (m/z)	qualifier ion (m/z)	column	R ²	slope	linear range (µg/L)	LOD (µg/L)	LOQ (µg/L)
alcohols	linalool	–	–	136	121	DB-WAX	1.000	867	1-100	0.3	1
	α-terpineol	–	–	136	121	DB-WAX	1.000	823	1-100	0.3	1
	β-citronellol	–	–	138	109	DB-WAX	1.000	2964	1-100	0.3	1
	geraniol	–	–	136	121	DB-WAX	1.000	4088	1-100	0.3	1
	nerol	–	–	121	93, 69	DB-WAX	1.000	3650	1-100	0.3	1
aldehydes	citral	geranial	0.53	152	137	DB-WAX	0.999	6535	0.5-50	0.3	1
		neral	0.27	137	119	DB-WAX	1.000	16794	0.3-30	0.3	1
carboxylic acids	geranic acid	geranic acid	0.7	123	168, 100	DB-FFAP	1.000	7082	7-700	10	25
		neric acid	0.2	123	168, 100	DB-FFAP	0.999	8636	10-200	10	25
esters	methyl geranate	methyl geranate	0.715	114	182	DB-WAX	1.000	2335	1-70	0.3	1
		methyl nerolate	0.285	114	182	DB-WAX	1.000	243	0.3-30	0.3	1
hydrocarbons	α-terpinene	–	–	136	121	DB-WAX	0.996	118	1-5	0.1	0.3
	γ-terpinene	–	–	136	121	DB-WAX	0.996	91	1-5	0.1	0.3
	(+)-limonene	–	–	136	121	DB-WAX	0.995	265	1-5	0.1	0.3
	terpinolene	–	–	136	121	DB-WAX	0.997	56	1-5	0.1	0.3
	myrcene	–	–	93	69	DB-WAX	0.996	67	1-100	0.3	1
–	"benzyl acetate (ISTD)"	–	–	108	91	–	–	–	–	–	–

LOD, limit of detection; LOQ, limit of quantitation

fibre was immediately inserted into a GC injector for thermal desorption for 3 min at 270 °C. The volatiles were injected in the splitless mode. The separation of volatiles was performed on a DB-FFAP (30 m × 0.25 mm i.d., 0.25 µm thickness; Agilent Technologies) with a helium carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was increased from 40 °C (held for 3 min) to 250 °C at a rate of 5 °C/min, followed by a 5 min isotherm. The mass spectrometer functioned in the EI mode (70 eV) and was operated in the SIM mode. Calibration curves were constructed using test-brewed beers containing standard substances at concentrations of 10, 25, 50, 100, 250, 500, and 1000 µg/L. The monitored ions, range of the calibration curves, and ratios of the isomers are listed in Table 2. All calibrations indicated a linear correlation with an R² value of > 0.99 over the entire concentration range analyzed. All tests were run twice to reduce error.

2.5.3 Analysis of Volatile Thiols by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS)

2.5.3.1 Specific Extraction of Volatile Thiols from Beer.

The specific extraction method of volatile thiols from hops and beers was performed according to the method reported by Takazumi et al. [5], as follows: In a 50 mL glass centrifuge tube, 6 g of sodium chloride, 20 mL of beer, 20 mL of dichloromethane, and 20 µL of internal standard solution (10 mg/L 3SPH in ethanol) were added and shaken for 15 min. After centrifugation at 1800g for 15 min, the organic phase was obtained. The organic phase was dried on anhydrous sodium sulfate and concentrated to

8 mL under nitrogen flow. Solid phase extraction using silver ion was carried out with an ASPEC GX-274 (Gilson, Middleton, WI, USA). First, a solid phase extraction cartridge (Meta-Sep IC-Ag; GL Sciences, Tokyo, Japan) was conditioned with 6 mL of dichloromethane. Then, 10 mL of each beer extract was loaded onto the cartridge at a flow rate of 2 mL/min. The cartridge was then rinsed with 10 mL of dichloromethane and 20 mL of acetonitrile in succession. The cartridge was reversed and washed with 10 mL of dichloromethane. Volatile thiols were eluted with 6 mL of 10 g/L thioglycerol in dichloromethane at a flow rate of 0.66 mL/min. The eluate and 30 mL of saturated salt solution were added to a 50 mL glass centrifuge tube and shaken for 15 min. After centrifugation at 1800g for 15 min, the organic phase was obtained and dried on anhydrous sodium sulfate. Finally, 1 mL of ethyl acetate was added to the eluate, and the mixture was concentrated to 20 µL under nitrogen flow.

2.5.3.2 Quantitation of Volatile Thiols by GC-MS/MS.

The method of GC-MS/MS analysis for volatile thiols was performed according to the method reported by Takazumi et al. [5]. The analysis was performed with an Agilent 7890A gas chromatograph coupled to a 7000B triple quadrupole mass spectrometer (Agilent Technologies). An InertCap PureWAX capillary column (30 m × 0.25 mm internal diameter and 0.25 µm film thickness; GL Sciences) was used for separation. Here, 3 µL of extract was injected using a CombiPAL system (CTC Analytics). The inlet was operated in a pulsed splitless mode (30 psi, 1 min) at 250 °C. The flow rate of the helium carrier gas was 1 mL/min. The oven temperature was

increased from 70 °C (held for 1 min) to 250 °C at a rate of 5 °C/min and was held at 250 °C for 10 min. The triple quadrupole mass spectrometer was operated in the selected reaction monitoring (SRM) mode. Target and qualification transitions and the corresponding collision energy values are described in previous paper [5]. Calibration curves were obtained by analyzing standard mixture solutions. The concentration of each volatile thiol stock

solution was determined according to Ellmans's method using 5,5-dithiobis (2-nitrobenzoic acid) before mixing. Standard mixture solutions were prepared by mixing five volatile thiol stock solutions and diluting them with ethyl acetate. To all standard mixture solutions, 3SPH (final concentration, 10 mg/L) and thioglycerol (final concentration, 10 g/L) were added as an internal standard and an analyte protectant, respectively.

Table S2 SRM Conditions for Volatile Thiols

compound	target			qualification		
	precursor ion	product ion	collision energy (V)	precursor ion	product ion	collision energy (V)
4MSP	132	89	6	132	75	2
3S4MPA	116	88	4	116	101	4
3SHA	116	88	4	116	101	4
3S4MP	134	100	0	134	57	8
3SH	134	82	2	100	82	0
3SPH	106	60	4	106	88	0

4MSP, 4-methyl-4-sulfanylpentan-2-on; 3S4MPA, 3-sulfanyl-4-methylpentyl acetate;

3SHA, 3-sulfanylhexyl acetate; 3S4MP, 3-sulfanyl-4-methylpentan-1-ol; 3SH, 3-sulfanylhexan-1-ol; 3SPH, 3-Sulfanylpropyl hexanoate