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Different Beers with Different Hops. Relevant Compounds for Their Aroma Characteristics

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ABSTRACT: Hop-derived aroma characteristics in beer are very important for the quality of beer. This study compared the differences of hop aroma characteristics and the compounds contained in beer by changing the variety of hops applying the idea of "food metabolomics" on the GC×GC/TOF-MS analysis data, to clarify which aroma compounds contribute to the differences of hop aroma profiles indicated by sensory descriptors. As a result, by focusing only on hop-derived compounds, 67 compounds were strongly correlated with one or more of the sensory descriptors. Furthermore, the odor descriptions of each key compound corresponded well to each sensory descriptor. Thus, these compounds are likely to be the key compounds explaining the differences of hop aroma characteristics in beer. This study led to the suggestion that understanding the relationship between the comprehensive nontarget analysis by GC×GC-TOF/MS and organoleptic evaluation using PCA is effective in estimating the key compounds.

KEYWORDS: *hop aroma, hop varieties, beer, GC×GC/TOF-MS, PCA, linalool*

INTRODUCTION

Aroma characteristics of beer derived from hop is very important for the quality of beer. Many studies have been performed mainly focusing on the essential oils of hops such as terpenoids.[1−3] It is true that these essential oils of hops themselves give strong sensory impacts on beer. However, these compounds are not brought directly into beer products in the same balance as was in hop. This means when the compounds in hop go through beer processing steps, by thermal reaction or by biotransformation by yeast,[4−7] such chemical conversions as oxidation/reduction, hydrolysis,[8,9] isomerization, ester exchange,[10−12] and so on can occur, and some of these compounds should even be evaporated. After such conversions, odor active compounds are destined in the final beer. Although the terpene glycosides and acids in original hop itself are odorless or show low impact on beer aroma, these compounds can also play important roles for hop aroma characteristics in beer through these chemical changes.

These observations allow us to predict that the profile of key aroma compounds in beer can be different from those in hops. Therefore, we need to focus not only on the aroma compounds in hop itself but also on the aroma compounds actually present in beer to understand the key hop aroma compounds for beer processing.

It is obvious from preceding studies, except for a few compounds such as β-farnescene and bergamotene, which are present only in some specific aroma hop varieties,[10,13−15] almost all components are common in all hops; thus, the differences of hop aroma characteristics are represented only from the differences in the balance among the hop aroma components.

In this study, we tried to clarify the aroma compounds affecting the various hop aroma characteristics, using beer samples prepared with different hop varieties. To visualize the key compounds, we employed the concept of "food metabolomics"[16−19] to mine data obtained by GC×GC-TOF-MS[20] analysis and quantitative descriptive analysis (QDA), by performing multivariate analysis of both data for the interpretation, followed by validation of the results (Figure 1).

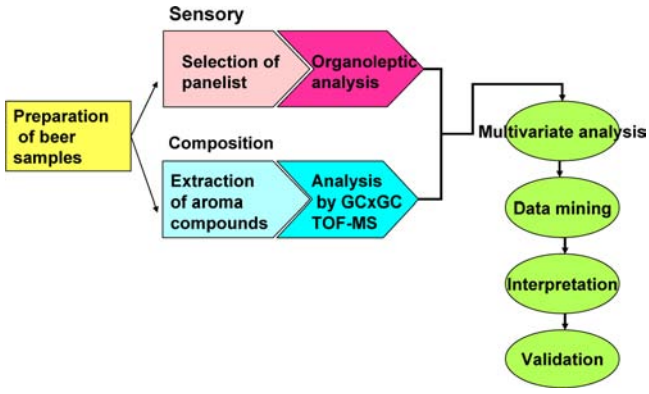

Figure 1. Schematic procedure to correlate the components and the sensory attributes of hop aroma characteristics in beer by "food metabolomics" approach using GC×GC/TOF-MS data.

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All of the compounds including compounds generated during the brewing process are included to correlate the differences of hop aroma characteristics and the compounds in beer. However, the interactions or biosynthetic pathways, which are generally considered in metabolomics studies, were disregarded.

MATERIALS AND METHODS

Chemicals. Linalool (>98%, racemic mixture) and methyl octanoate (>99.0%(GC)) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Dichloromethane (PRA grade > 99.9%) and anhydrous sodium sulfate (guaranteed reagent grade) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Hop Raw Materials. Five hop varieties, Hallertauer Mittelfrüh, Saazer, Tradition, Perle, and Cascade, were commercial hop pellets and were delivered by the Barth-Haas Group (Nuremberg, Germany). Each hop pellets was harvested in 2009 and stored refrigerated to prevent deterioration after being pelletized until brewing trial. Thirty percent iso-α acid extract was purchased from Steiner Hops Ltd. (Mainburg, Germany).

Brewing Trials of Five Hopped Beers and Unhopped Beer. Five hopped beers and one unhopped beer were prepared as follows using the same wort under the same boiling and fermentation conditions in a 100 L pilot scale. The 100% malts wort was prepared and boiled at 100 °C for 90 min.

The timing and amount of hop addition were determined to perceive the differences of hop aroma characteristics in beer adequately with the same hop aroma intensity in each beer. Hops were added twice, at the beginning and at the end of boiling (late-hopping) just before whirlpool treatment to give enough hop aroma in the finished beer. As linalool is well-known to contribute to the floral note of hopped beer and can be used as a quantity indicator for hopped beer,[26−28] the amount of hop added for the late-hopping was determined to adjust the concentration of linalool in each beer to be the same, which is 33 μg/L. Furthermore, the amount of first hop addition was determined to be the same level of bitterness in the finished beer in consideration of bitterness derived from hop for last-hopping. To adjust the bitterness, iso-α acid extract (24 mg/L) was added to unhopped beer. Beer fermentation was performed using lager yeast at 10 °C for 14 days. After the fermentation, each fermented wort was stored at 0 °C for 3 days. Filtration and bottling were done on a pilot scale after adjustment of carbon dioxide pressure.

Organoleptic Evaluation. Organoleptic evaluation was implemented by five well-trained panelists. Six generic hop aroma characteristics, 'floral', 'herbal', 'citrussy', 'spicy', 'ester', and 'sylvan' (woody), were used as sensory descriptors. The references obtained by physically separated fractions from hop oil (pure hop aroma, PHA) provided from BOTANIX Ltd. (The Barth-Haas Group, Kent, UK),[29] were scored from 0 to 3. Scores were normalized to remove the bias of each panelist's score.

Extraction of Hop Aroma Compounds in Beer. Other researcher's methods of aroma extraction were referred.[30−32]

Three hundred and fifty grams of each beer sample was added to 300 g of dichloromethane after addition of the internal standard (methyl octanoate 10 mg/L in 99.5% methanolic solution), 100 μL, and was stirred with a magnetic stirrer at 100 rpm at room temperature for 60 min. The sample was then left to separate into two phases for 15 min. The aqueous phase was removed using a pipet, and the organic phase was dried over 70 g of anhydrous sodium sulfate and was concentrated to 500 μL using a vacuum rotary evaporator.

Analytical Instrument. The GC×GC-TOF-MS system consisted of an HP 6890 gas chromatograph (Agilent Technologies), and a LECO Pegasus 4D GC×GC (LECO Corp., St. Josephs, MI, USA) in electron impact (EI) mode at 70 eV was used for the analysis of the aroma compounds. The HP 6890 GC system was equipped with a secondary oven for independent temperature program for the second-dimension column and a quad-jet dual-stage cryomodulator using liquid nitrogen for GC×GC modulation. The analytical conditions are summarized in Table 1.

Table 1. Analytical Conditions of GC×GC/TOF-MS and Data Processing

detector	Pegasus 4D time-of-flight mass spectrometer
acquisition rate	200 spectra/s
acquisition delay	2 min
stored mass range	m/z 33−400
transfer line temperature	250 °C
source temperature	250 °C
detector voltage	−1800 V
mass defect	0 units
first column	Rtx-1, 30 m × 0.25 mm i.d., 0.25 μm film thickness
second column	InartCap 17, 1.6 m × 0.10 mm i.d., 0.1 μm film thickness
first column oven	40 °C for 2 min, to 250 °C at 4 °C/min, hold for 5 min
second column oven	60 °C for 2 min, to 370 °C at 4 °C/min hold for 5 mm
modulation period	7 s
modulator temperature offset	20 °C
inlet	splitless 50:1 at 250 °C
injection	1 μL
carrier gas	helium, 1.5 mL/min corrected constant flow
	Data Processing
software	ChromaTOF 4.33 from LECO Corp.
peak finding	True Signal Deconvolution from LECO Corp.
peak identification	Wiley 08

Data Processing and Multivariate Statistical Analysis. At the first data processing step, spectral deconvolution[33] was conducted to extract pure spectrum component from contaminated spectra by data processing software (Chroma TOF 4.33 from LECO Corp.). This software was used for peak apex finding, mass spectral deconvolution, library searching, and integration. The combination of slices corresponding to a compound was performed by comparing the mass spectra under pre-established match criteria. Wiley 08 and NIST databases were utilized for spectral identifications with a match factors threshold of >700. Subsequently, one-way analysis of variance (ANOVA) was conducted for the screened data to select the compounds that show significant difference between hopped and unhopped beer samples. At the third stage, principal component analysis (PCA) of the screened compounds and organoleptic scores were performed to understand the key aroma compounds explaining the differences of six aroma characteristics in each beer samples. All statistical analyses including multivariate analysis were conducted using JMP version 8.02 (SAS Institute Inc.). After processing, 2400 peaks for data obtained were narrowed down to 67 key compounds to explain the differences of hop aroma characteristics.

Finally, we have examined the odor descriptions of each compound to validate whether the selected compounds are certainly related to each hop aroma characteristic.

RESULTS AND DISCUSSION

Organoleptic Evaluation and Preference of Beer Samples. Five hop varieties that represent the differences of hop aroma characteristics to some extent (Table 2) were selected to compare the organoleptic differences of beer when they are used for brewing. Among these, Hallertauer Mittelfrüh (Hallertauer Mfr.), Saazer, Traditio,n and Perle have European parentage. Among them, Hallertauer Mfr. and Saazer are landraces and Tradition and Perle are of mixed parentage. On the other hand, Cascade is a hybrid of the European and wild American varieties. Seefelder et al. have reported the genetic similarity of 90 hop cultivars based on polymorphisms of their

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Table 2. Genotypes of Five Hop Varieties Used for Brewing in This Study

variety	parentage	origin
Hallertauer Mittelfrüh	landrace	Germany
Saazer	landrace	Czech Republic
Tradition	Hallertauer Gold × 75/15/106M4[a]	Germany
Perle	Northern Brewer × 63/5/27M[b]	Germany
Cascade	(Fuggle × [Serebrianca × Fuggle − seedling) × open-pollinated	USA

[a]7% Hallertauer, 15% Saazer, 9% Spalter, 28% wild hops, 1% Northern Brewer. [b]Hallertauer Mfr., Spalter, Saazer. The percentages of mixed breed are not known.

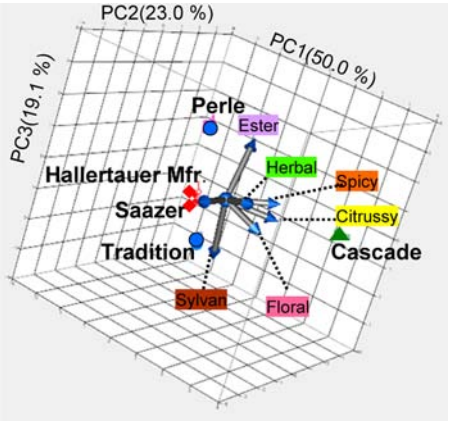

Figure 3. Principal component biplot of six generic hop aroma characteristics and five hopped beers.

genes[21] as indicated partially in Figure 4b. Patzak et al. have characterized European wild hops by chemical and molecular genetic analyses.[22] Many studies on other agricultural crops about the relationship between genetic and "taste and flavor" similarities have been conducted[23−25] and are used for cultivar improvement by breeding. These genetic and sensory evaluation areas of information were utilized for hop cultivar improvement. In contrast to such a viewpoint, we are interested not only in the differences of hop aroma characteristics in beer attributable to hop varieties but also in the relationship between sensory and genetic similarities.

In this study, unhopped beer is essential as the control either for the comparative sensory evaluation of each beer or for the comparative studies of the components that are derived from the unhopped materials. Although the amount of linalool in beer influences the floral notes[26−28] as mentioned under Materials and Methods, it is not valid to interpret the differences of each hop aroma characteristic in beer by a single compound and a single character such as 'floral'. Therefore, understanding which compound has relevance to which hop

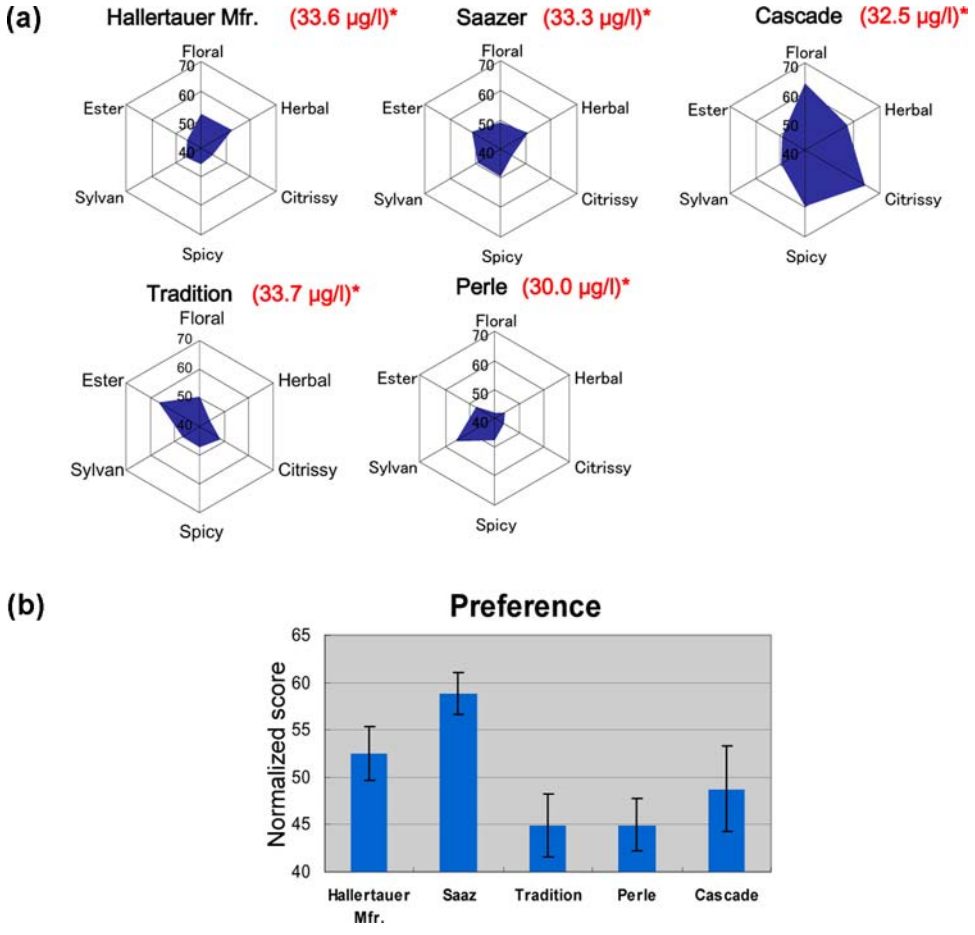

Figure 2. (a) Aroma profiles of beers brewed with five hop varieties. The numbers in parentheses indicate the concentrations of linalool in each beer. (b) Preference of beers brewed with five hop varieties. The numbers are the averages of the normalized panel's scores.

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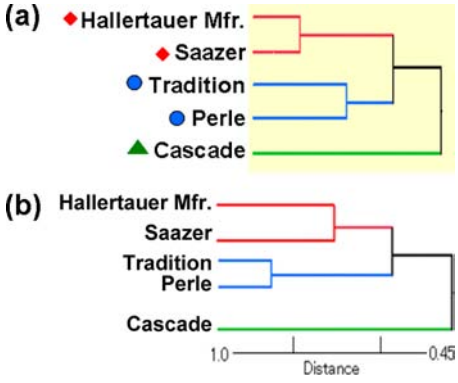

Figure 4. (a) Sensory hierarchical cluster of the beers brewed with five hop varieties on the sensory scores of six generic hop aroma characteristics. (b) Genetic hierarchical cluster of five hop varieties based on the polymorphisms of their genes.[21]

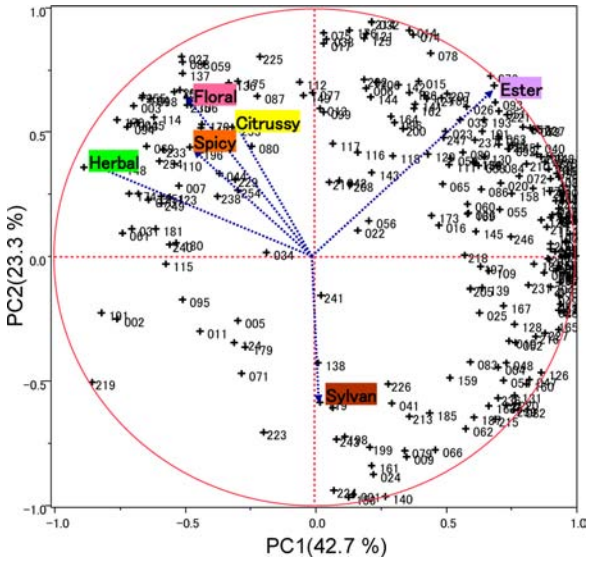

Figure 5. Principal component loadings of the organoleptic scores and selected aroma.

aroma characteristic in beer is important for selection of hop varieties, hop qualities, and controlling hop boiling conditions to achieve the target hop aroma characters in beer.

Organoleptic evaluation data of fresh beer samples with five different hop varieties showed significant differences of sensory profiles on six generic hop aroma characteristics, despite almost the same concentration of linalool is present in each beer (Figure 2). Hallertauer Mfr. and Saazer beers represent relatively similar sensory profiles. Among the other three beers, Tradition showed a high score in 'ester' character, Perle was high in 'sylvan' character, and Cascade beer showed the highest intensity, especially in 'floral', 'citrussy', and 'spicy'.

To speculate which variety is in tune with consumer taste in Japan, preference was also assessed using Japanese panelists. Preference score was also significantly different (Figure 2b); the Saazer beer was the highest followed by Hallertauer Mfr. with small variance among the panel. There could be several reasons why Saazer was preferred most. One possibility might be the balance of the characteristics. However, the six characteristics considered in this study might not be sufficient, and some other characteristics should have been considered. This problem should be clarified by analyzing the comments made by the panelists during the descriptive analysis.

Table 3. Key Aroma Compounds Related to the Hop Aroma Characteristics in Beer Selected by PCA and Their Odor Descriptions[33]

character	composition	odor description
floral, herbal	8-acetoxylinalool	warm, fruity, woody
	8-hydroxylinalool	honey citrus and dill herb
	3-nopinenone	minty, medium
citrussy, spicy	(*E*)-geraniol	floral, rose, green
	1,3,5-trimethylcyclohexane	
	7-hydroxy-α-terpineol	intensively fruity, floral-rose, slightly citrus
	trans-shisool	
	acetic acid 2-pentyl ester	ripe, fruity, apple
	β-citronellol	floral, leather waxy, rose bud, citrus
	acetic acid citronellyl ester	floral, rose, fruity, sweet
	nerol	citrus, rose, fresh
	trans-*Z*-α-bisabolene epoxide	
	α-calacorene	woody
sylvan	methyl (*E*)-geranate	waxy, green, fruity, flower
	pentanoic acid ethyl ester	sweet, fruity, apple, pineapple, green,tropical
	acetic acid heptyl ester	woody, citrus, pear, apricot
	α-terpineol	fresh, clean, woody, pine, floral, lime
	myrcene	peppery, terpene, spicy, balsam, plastic
	terpinen-4-ol	woody, ceding, mentholic, citrus terpy, spicy
	caryophyllene oxide	sweet, fresh, dry, woody, spicy
ester	2-methyl-2-propenoic acid pentyl ester	
	propionic acid propyl ester	sharp, chemical, pungent with sweet fruity lift notes
	sorelon	sherry aroma, pleasant wine-like odor
	benzenemethanol	sweet, floral, fruity with chemical nuances
	benzoic acid, ethyl ester	fruity, dry musty, sweet, wintergreen
	cyclohexanecarboxylic acid ethenyl ester	fruity, cheese, winey
	(*E*)-2-methyl-2-pentenoic acid	sour, acidic, sweaty, fruity with a jammy, woody nuance
	3-hydroxy-α-damascone	
	dihydrojasmone	fruity, sweet, floral, woody with a powdery nuance
	1-hydroxy-3-methyl-2-butanone	
	β-ionone epoxide	fruity, sweet, berry, woody, violet, orris
preference	humulene oxide II	herbal
	β-eudesmol	wood, green
	toluene	sweet

Multivariate Analysis of Organoleptic Scores. The principal component biplot indicates the positional relationship among six hop aroma characteristics and five hopped beers

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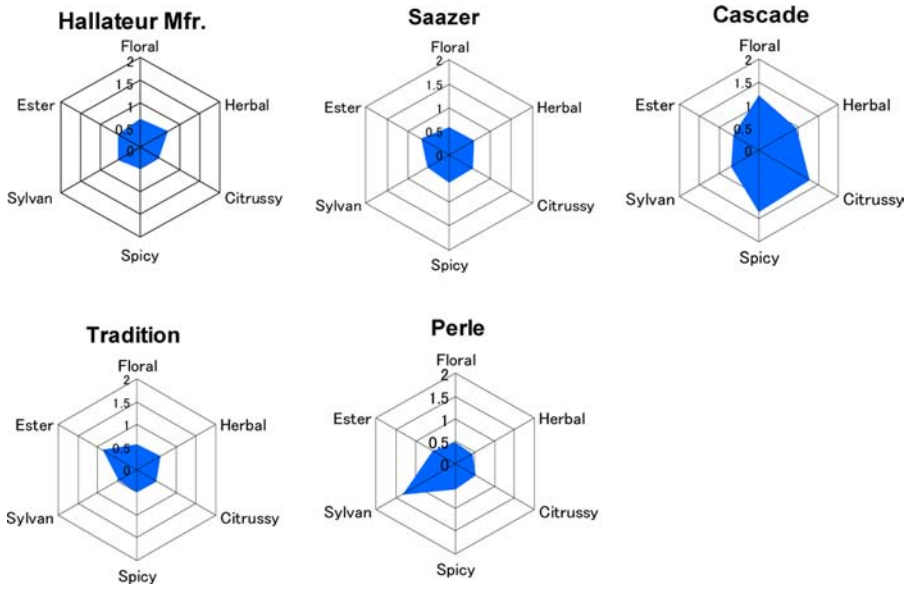

Figure 6. Simulated aroma profiles of beers brewed with five hop varieties. The scores on each character were obtained from the total peak intensities of the components for each character in Table 3.

(Figure 3). Each characteristic vector showed individual directions. Among them, vectors for 'floral', 'herbal', 'spicy', and 'citrusy' were comparatively in similar direction, whereas 'ester' and 'sylvan' went in different directions. Particularly, the third component distinguished 'ester' and 'sylvan' directed opposite to one another. The similarities of each characteristic represented by the spider-web charts (Figure 2a) corresponded well with the three-dimensional plot (Figure 3), in which Hallertauer Mfr. and Saazer were located close together, whereas the other three were spread.

In the hierarchical cluster analysis shown in Figure 4a, Hallertauer Mfr. and Saazer beers fell into the same cluster, Tradition and Perle into another, and Cascade was separated as the third cluster. It is interesting that the cluster obtained on the sensory result coincided well with the clusters obtained with their genetic data shown in Figure 4b.

Key Compounds Influencing the Differences of Hop Aroma Characteristics in Beer. Hop varieties fall into two categories based on the amount of bitter compounds, which is 'bitter hop' and 'aroma hop'. However, as there are no big differences of structural components of hop aroma among all hop varieties, the differences of hop aroma composition balance are supposed to be a main reason for differences of hop aroma characteristics in each hop variety. Therefore, hop aroma characteristics in beer were broken down into six generic hop aroma characteristics, and the differences of their profile, meaning the differences of hop aroma characteristics, were assessed. The relevant components to six generic hop aroma characteristics were verified by multivariate analysis of organoleptic evaluation together with component analysis results. As a result, the components with significant differences among five hopped beers and one unhopped beer were selected according to the logics mentioned under Materials and Methods. Finally, on the basis of the distances between these 297 selected components and the vectors of each sensory characteristic in Figure 5, the components that influenced each sensory characteristic were narrowed down to 67 compounds (Table 3). Naturally, linalool should not appear as a key compound in this experiment. Instead, other terpenoids and esters surfaced as key compounds. It is interesting that some of them, such as citronellol, citronellyl acetate, 2-phenylethyl 3-methylbutanoate, and 4-(4-hydroxyphenyl)-2-butanone, cannot be found in hop itself; thus, they should be newly generated during the brewing process. King et al. and Kishimoto et al. have already obtained the same results.[4,34]

To validate whether the selected compounds are certainly related to each hop aroma characteristic, the odor descriptions of each compound were examined (Table 3). With regard to their good agreement of the aroma characteristics and the odor description of each compound in consideration, the selected compounds are likely to be the key aroma compounds to explain the differences of hop aroma characteristics in beer.

To confirm the effectiveness of the key compounds by visualizing the profiles of each beer sample, we have compared the sensory profiles and the simulated profiles obtained by summing the peak intensities of selected key compositions (Figure 6) of each characteristic. Obviously, both profiles match reasonably well.

Accordingly, it is quite clear that PCA of the combination of GC×GC-TOF-MS and QDA was effective and reliable in determining the key compounds from numerous unknown components.

In general, gas chromatography–mass spectrometry–olfactometry (GC-MS-O) and aroma extract dilution analysis (AEDA) are performed to determine the key components that are related to each odor descriptor.[35,36] This method is useful to detect odor characters corresponding to each separated compound by GC and identify the compounds that indicate the unique odor character alone. However, hop aroma characteristics in beer are compositive, and although some compounds are contained at less than threshold concentration, these compounds may show influences on aroma or flavor in beer by the effect of other coexisting compounds in beer.

Conclusion. Hop aroma in beer is presumed to consist of more than 100 compounds, and some of these compounds are contained at less than threshold concentration. However, these compounds may show influences on aroma or flavor in beer by the effect of other coexisting compounds in beer products.

Therefore, it is incomplete to estimate the key components to explain the hop aroma characteristics in beer just from the results of GC-O. Therefore, nontargeted analysis is requisite for mining the key hop aroma compounds in beer. Additionally, as beers are composed of thousands of components, it is impossible to separate all of the components by a single one-dimensional GC analysis and detect each peak or each odor peak comprehensively as a single component. From such a viewpoint, multivariate analysis of chemical data obtained by GC×GC-TOF/MS together with quantitative sensory scores such as QDA is thought to be a rational methodology. It was startling that this methodology drew plausible results because odor descriptions of the most deduced key hop aroma compounds corresponded well with each hop aroma characteristic. Thus, the key compounds determined by this analysis can be used as indicators of aroma characteristics for analytical results, but still not be confirmed as the components that influence the impact aroma components in real beers. We have to keep performing studies to prove the results obtained by the analysis are practically correct. One possibility must be to verify the selected key compounds able to affect hop aroma characteristics in beer by the addition of extracted compounds from beer or these individual chemicals prepared independently. Furthermore, information on the hop-derived precursors of these selected key compounds, including generation during brewing process, are extremely important to orchestrate the total hop aroma characteristics in beer throughout the brewing processes. These studies should give brewers useful information on the requirement of the characteristics of hops for designing and improving beer products and also give hop culturists ideas for expected varieties to be developed.

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Notes

The authors declare no competing financial interest.

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