JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 8855–8861 **8855**

Comparison of the Odor-Active Compounds in Unhopped Beer and Beers Hopped with Different Hop Varieties

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Odorants comprising the hop aromas of beers were examined. Strongly hopped beers with Saazer, Hersbrucker, and Cascade hops were compared with unhopped beer by gas chromatography−olfactometry (CharmAnalysis) and sensory evaluation. Twenty-seven odorants were revealed as hop-derived, which derived either directly from hops or via metabolization, and 19 components were identified. Of the components, linalool, geraniol, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and ethyl 2-methylpropanoate were determined as odor-active components from their Charm values and aroma values. The muscat-like aroma of Cascade beer and the spicy aroma of Hersbrucker beer were predominant in sensory evaluation, and the contributors to these characteristics were investigated.

KEYWORDS: GC−olfactometry; hop aroma; muscat-like; sensory evaluation; spicy

INTRODUCTION

In the brewing process, hops are added during or after the wort-boiling process to provide a bitter taste and characteristic aroma. Characteristics of hop aroma for hop varieties were described by Kaltner and Mitter (*1*), who observed that beers brewed with the variety Hallertauer have flowery and fruity notes and those with Styrian Golding have fruity, flowery, pine-resin notes with high linalool content. The aroma qualities of the beers significantly differ from those of the hop pellets or cones themselves used in the brewing process. Many volatile compounds are evaporated during boiling or washed out by carbon dioxide during fermentation. Hydrophobic or high molecular weight substances are absorbed during the hot/cold break or by yeast (*1*). Some components are metabolized, particularly through ester hydrolysis and esterification by yeast (*1*, *2*). Controlling the aroma characteristics of beers therefore requires detailed knowledge of the hop-derived odor-active components.

Investigators have attempted to assess the impact of hops on beer flavor. Most studies have used quantitative analysis employing gas chromatography (GC)−flame ionization detection (FID) or GC−mass spectrometry (MS) to investigate hopped beer or raw hops. We have previously reported (*3*) that hop-derived terpenes and terpenoids could be evaluated by using the highly sensitive and quantitative stir bar sorptive extraction (SBSE) method with GC-MS. Using this technique, we showed that most hop-derived terpenes and terpenoids are lost during fermentation, possibly due to their highly hydrophobic properties. Moreover, we showed that these components have high threshold values and, thus, make relatively little contribution to the hop aroma.

Recently, the use of GC−olfactometry (GC-O) techniques, such as aroma extract dilution analysis (AEDA) (*4*), CharmAnalysis (*5*), and Osme (*6*), in combination with quantitative analysis has been reported in the determination of the odor-active components. Steinhaus and Schieberle (*7*) have described a method for characterizing the most odor-active compounds in both fresh and dried hop cones utilizing AEDA. Twenty-three components including *trans*-4,5-epoxy-(*E*)-2-decenal, linalool, and myrcene were identified as odor-active components in hop cones. Fritsch and Schieberle (*8*) revealed odor-active constituents responsible for the aroma of a Pilsner-type beer by AEDA in combination with aroma simulation experiments. Of the hop-specific compounds, linalool (ethyl 4-methylpentanoate) was found as a contributor to Pilsner flavor.

In the current study, to reveal the hop-derived odor-active components that persist even after fermentation and comprise the hop aromas in beers, the comparison of beers hopped with different varieties and unhopped beer was performed using CharmAnalysis in combination with the sensory evaluation and quantification.

MATERIALS AND METHODS

Chemicals. Ethyl 2-methylpropanoate [Chemical Abstracts Service (CAS) No. 97-62-1], *o*-aminoacetophenone (CAS No. 551-93-9), 2-methyl-3-furanthiol (CAS No. 28588-74-1), 3-methylindole (CAS No. 83-34-1), 2-methoxy-4-vinylphenol (CAS No. 7786-61-0), decanoic acid (CAS No. 334-48-5), ethyl butyrate (CAS No. 105-54-4), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (CAS No. 3658-77-3), 2-furanmethanethiol (CAS No. 98-02-2), γ-nonalactone (CAS No. 104-61-0), *o*-

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10.1021/jf061342c CCC: $33.50
Published on Web 10/17/2006

methoxyphenol (CAS No. 90-05-1), hexanoic acid (CAS No. 142-62-1), 1-hexanol (CAS No. 111-27-3), indole (CAS No. 120-72-9), isoamyl acetate (CAS No. 123-92-2), isoamyl alcohol (CAS No. 123-51-3), 2-methylpropanoic acid (CAS No.79-31-2), 3-hydroxy-2-methyl-4-pyrone (CAS No. 118-71-8), 3-methylthiopropionaldehyde (CAS No. 3268-49-3), 3-methylthiopropanol (CAS No. 505-10-2), octanoic acid (CAS No. 124-07-2), ethyl hexanoate (CAS No. 123-66-0), n-butyric acid (CAS No. 107-92-6), 3-methylbutanoic acid (CAS No. 503-74-2), β-phenylethyl alcohol (CAS No. 60-12-8), and vanillin (CAS No. 121-33-5) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). β-Ionone (CAS No. 79-77-6), (*Z*)-3-hexen-1-ol (CAS No. 928-96-1), 3-methyl-2-butenal (CAS No. 107-86-8), (*Z*)-3-hexenal (CAS No. 6789-80-6), 2,3-butanedione (CAS No. 431-03-8), β-damascenone (CAS No. 23696-85-7), and (*E*,*Z*)-2,6-nonadienal (CAS No. 557-48-2) were purchased from Sigma-Aldrich (St. Louis, MO). (±)-Ethyl 2-methylbutanoate (CAS No. 7452-79-1), 2-phenylethyl 3-methylbutanoate (CAS No. 140-26-1), 4-(4-hydroxyphenyl)-2-butanone (CAS No. 5471-51-2), and (*Z*)-3-hexenoic acid (CAS No. 4219-24-3) were purchased from Acros Organics (Fair Lawn, NJ). (*Z*)-1,5-octadien-3-one (CAS No. 65767-22-8), 4-mercapto-4-methylpentan-2-one (CAS No. 19872-52-7), 2-acetyl-1-pyrroline (CAS No.85213-22-5), 2-mercapto-3-methyl-1-butanol, and 2-propionyl-1-pyrroline (CAS No. 133447-37-7), and 3-mercapto-2-methyl-1-butanol were obtained from San-Ei Gen FFI, Inc. (Osaka, Japan). (*R*/*S*)-Linalool (CAS No. 78-70-6), (*R*-linalool (CAS No. 126-91-0), geraniol (CAS No. 106-24-1), β-damascone (CAS No. 23726-91-2), ethyl 3-methylbutanoate (CAS No. 108-64-5), ethyl 4-methylpentanoate (CAS No. 25415-67-2), (−)-borneol (CAS No. 464-45-9), and (*Z*)-3-hepten-1-ol (CAS No. 1708-81-2) were purchased from Fluka (Buchs, Switzerland). 3-Mercaptohexan-1-ol (CAS No. 51755-83-0) was purchased from Avocado Research Chemicals Ltd. (Lancashire, U.K.). 1-Hexanal (CAS No. 66-25-1) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 3-Methyl-2-butene-1-thiol (CAS No. 5287-45-6) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). *trans*-4,5-Epoxy-2(*E*)-decenal (CAS No. 134454-31-2) was purchased from Cayman Chemical Co. (Ann Arbor, MI).

Brewing Processes. Saazer (5.6% α-acid pellets; Czech Republic), Cascade (5.5% α-acid pellets; USA), and Hersbrucker (3.8% α-acid pellets; Germany) were used in the brewing processes. Beers hopped with different varieties and unhopped beer were brewed independently at a 20-L volume scale. When appropriate, 30 g of hops was added at the beginning of the boiling process and a further 60 g after the end of the process.

Static fermentations were carried out in 5-L stainless-steel tall tubes, which are the modified model of 2-L EBC tall tubes (*9*), equipped with the strict controller for pressure and temperature. Yeast used for the fermentation was cultured in unhopped wort for 3 days to wash out the hop-derived components. The yeasts were recovered by centrifugation and pitched at the rate of 20 million cells per milliliter. A 4.5-L sample of each type of wort (11.5 °P) was fermented at 12.0 °C for 8 days, and yeasts settled in the tank bottom were eliminated. After the maturation step at 12.0 °C for 4 days, the beers were cooled to 0 °C for 5 days and then centrifuged to obtain the finished beer.

Isolation of the Volatiles for GC-O. A 2-L sample of each beer was extracted with 1 L of dichloromethane by stirring gently, without making an emulsion, for 12 h at 4 °C. The dichloromethane and aqueous layers were separated, and the former was dried over anhydrous sodium sulfate for 30 min. Each extract was then carefully concentrated to 10 mL using a Kuderna−Danish evaporative concentrator.

GC-O. CharmAnalysis was conducted on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA), which was modified by DATU Inc. (Geneva, NY) and equipped with a DB-Wax capillary column (Agilent Technologies; length = 15 m; i.d. = 0.32 mm; film thickness = 0.25 μm), using helium (1 mL/min) as a carrier gas. The rate of humidified air flow into the sniff port was set at 30 mL/min at 60 °C. The inlet temperature was set at 225 °C in the splitless mode, and the oven temperature was programmed to rise from 40 to 230 °C (held for 20 min) at a rate of 6 °C/min. Samples of 1 μL were injected into the testing apparatus.

The original odor extract of each beer was stepwise diluted with dichloromethane to 3^n (where $n = 0-3$). A dilution series was analyzed for each extract, ranging from undiluted concentrate to a 1:27 dilution. Quantitative responses to the eluting aromas were generated using Charmware (DATU Inc.). A series of alkanes (C10−C32) was also analyzed using FID to establish the Kovats retention indices (RIs).

Sensory Evaluation. Flavor-profile analyses were performed for Saazer, Hersbrucker, and Cascade beers compared to the unhopped beer by seven trained sensory panelists. The five attributes that best expressed the hop aroma characteristics were selected by tasting commercial and pilot test beers; these were identified as “green”, “citrus”, “floral”, “spicy”, and “muscat-like”. The responses to each description were shown to be consistent between the seven trained sensory panelists using a matching test (*10*). The members of the sensory panel were then asked to evaluate the total hop aroma intensity and the intensity of the five odor attributes for the beers hopped with different varieties by setting the intensity of each attribute for unhopped beer as control. The respective odor intensities were rated on the following scale (using 0.5-interval steps): 0 = not perceivable; 1 = weak; 2 = normal; 3 = strong; 4 = very strong. The characteristics between the varieties were compared following Scheffé’s method (*11*) by calculating the mean intensity value of the score and the paired *t*-test value for the characteristics.

Determination of Difference Threshold Values. The orthonasal difference threshold values of ethyl 2-methylpropanoate, (±)-ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 4-methylpentanoate, 4-mercapto-4-methylpentan-2-one, (*Z*)-1,5-octadien-3-one, (*Z*)-3-hexen-1-ol, (*R*)-linalool, 3-mercaptohexan-1-ol, geraniol, β-ionone, 2-phenylethyl 3-methylbutanoate, and 4-(4-hydroxyphenyl)-2-butanone were determined using the method of the American Society of Brewing Chemists (*12*, *13*). The threshold values were established by a triangle test using a series of six concentrations. An ethanol solution of the chemical was added to a light-tasting Japanese beer. During the test, the members of a panel comprising nine trained individuals were asked to taste three samples and to identify the odd one. The best estimate threshold was calculated for each assessor 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 assessors.

Identification of Odorants. Identifications of the hop-derived components were attempted by GC-MS and CharmAnalysis equipped with DB-Wax and DB-1 columns comparing their RIs, mass spectra, and odor qualities with those of the authentic compounds.

The separation of each extract in GC-MS was performed with an Agilent 6890 gas chromatograph coupled to an Agilent MSD5973N quadrupole mass spectrometer equipped with a DB-Wax and a DB-1 capillary column (Agilent Technologies; length = 60 m; i.d. = 0.25 mm; film thickness = 0.25 μm), respectively, using pulsed splitless injection with helium (1 mL/min) as a carrier gas. The inlet temperature was set at 250 °C, and the oven temperature was programmed to rise from 40 °C (held for 5 min) to 240 °C (held for 20 min) at a rate of 3 °C/min. A 1-μL sample of concentrated volatile was injected into the GC-MS apparatus, which was set to detect ions with a mass-to-charge ratio (*m*/*z*) of 30−350 and was operated in the electron-impact mode at 70 eV.

Quantification of Volatiles. The quantification of linalool, geraniol, and β-ionone in wort and beer was carried out by the SBSE method using β-damascone as an internal standard, as described in our previous paper (*3*). The amounts of ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 1-hexanal, (*Z*)-3-hexen-1-ol, 2-phenylethyl 3-methylbutanoate, and 4-(4-hydroxyphenyl)-2-butanone in wort and beer were measured by using the liquid extraction method with dichloromethane using (−)-borneol and (*Z*)-3-hepten-1-ol as an internal standard, as described previously (*3*). The data are shown as the mean values of duplicated analysis. The aroma value is expressed as the concentration of the compound divided by the difference threshold value (*14*).

RESULTS AND DISCUSSION

Unhopped beer and strongly hopped beers, using approximately 5-fold the amount of hops than normal, were brewed to distinguish clearly the characteristics of the aromas and the

Table 1. Charm Value for the Volatile Fraction of Unhopped, Saazer, Hersbrucker, and Cascade Beers

RI on DB-Wax	odor quality	Charm value				compound
		unhopped	Saazer	Hersbrucker	Cascade	
962	solvent	0	32	58	24	
976	citrus	49	22	14	0	
996	diacetyl	211	36	372	238	2,3-butanedione[d]
1004	citrus, pineapple, sweet	348	1125	1582	1252	ethyl 2-methylpropanoate[a]
1048	solvent	2685	1016	1584	2132	
1050	citrus	1981	1368	1090	1664	ethyl butyrate[a]
1068	citrus, apple-like	114	952	1370	968	(±)-ethyl 2-methylbutanoate[a]
1084	citrus, sweet, apple-like	0	1601	765	564	ethyl 3-methylbutanoate[a]
1103	green, leafy	0	493	568	712	1-hexanal[a]
1111	almond, roasted	88	426	490	410	3-methyl-2-butene-1-thiol[b]
1129	solvent	1060	1202	711	1095	isoamyl acetate[a]
1148	green, leafy	72	1081	1111	1002	(*Z*)-3-hexenal[a]
1180	citrus, pineapple	0	1286	646	647	ethyl 4-methylpentanoate[a]
1199	almond, roasted	0	247	618	205	3-methyl-2-butenal[a]
1211	floral	1844	1936	1682	1830	isoamyl alcohol[a]
1235	citrus, estery	705	479	165	567	ethyl hexanoate[a]
1298	roasted meat, vitamin B	1744	1273	1059	1824	2-methyl-3-furanthiol[d]
1326	popcorn-like	728	1096	729	809	2-acetyl-1-pyrroline[d]
1338	fruity, catty, thiol-like	43	241	486	302	
1350	green, leafy	524	444	974	539	1-hexanol[a]
1363	muscat-like, fruity	0	612	479	1083	4-mercapto-4-methyl-pentan-2-one[b]
1373	green, metallic	41	1016	1073	450	(*Z*)-1,5-octadien-3-one[b]
1383	muscat-like, green	133	566	415	1186	(*Z*)-3-hexen-1-ol[a]
1413	cracker-like	284	89	8	26	2-propionyl-1-pyrroline[d]
1422	roasted meat	983	502	572	684	2-furanmethanethiol[d]
1424	solvent	799	738	680	883	
1430	potato, soy-sauce	1102	1029	416	214	3-methylthiopropionaldehyde[d]
1454	musty	207	224	213	251	
1495	solvent	1372	1150	1101	746	
1508	sweet	473	178	164	20	
1516	cracker-like	763	574	599	337	
1540	green	582	112	443	142	
1548	floral, citrus, terpenic	28	1066	1329	1011	(*R*/*S*)-linalool[a]
1561	cheesy	353	227	130	411	2-methylpropanoic acid[c]
1571	green, cucumber	0	178	370	237	(*E*,*Z*)-2,6-nonadienal[a]
1590	green, metallic	0	612	1304	871	
1600	roasted meat	536	641	394	311	
1614	cheesy	1368	938	714	738	n-butyric acid[a]
1647	meat, onion-like	94	0	122	223	2-mercapto-3-methyl-1-butanol[d]
1652	cheesy	1304	1330	1115	1197	3-methylbutanoic acid[a]
1682	fatty	218	750	653	633	
1704	potato, soy-sauce	1078	296	423	1131	3-methylthiopropanol[c]
1721	meat, onion-like	427	69	194	285	3-mercapto-2-methyl-1-butanol[d]
1730	cracker-like	735	924	15	787	
1789	citrus	1292	697	505	1386	β-damascenone[a]
1810	roasted, chocolate	778	988	644	781	
1825	fruity, catty, thiol-like	151	1165	1010	1226	3-mercapto-hexan-1-ol[b]
1834	sweet	833	737	501	606	*o*-methoxyphenol[c]
1838	rancid, sweaty	1858	1415	1373	670	hexanoic acid[a]
1850	floral, rose-like	0	1046	820	1460	geraniol[a]
1886	floral	2946	2415	1801	2027	phenethyl alcohol[a]
1915	floral, violet-like, berry	25	827	870	1003	β-ionone[a]
1942	floral, sweet	870	1058	1912	1042	
1945	rancid, sweaty	195	741	322	514	(*Z*)-3-hexenoic acid[a]
1977	sweet	12	127	40	23	3-hydroxy-2-methyl-4-pyrone[c]
1980	floral, minty	120	711	422	498	2-phenylethyl 3-methylbutanoate[a]
1987	green, metallic	0	111	165	14	*trans*-4,5-epoxy-(*E*)-2-decenal[d]
1995	sweet	1616	1043	1338	1692	γ-nonalactone[c]
2019	strawberry, citrus	1531	1534	1649	1514	
2029	roasted	282	137	345	160	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[d]
2034	rancid, sweaty	66	75	43	32	
2049	rancid, sweaty	2733	1527	2140	2621	octanoic acid[c]
2071	phenolic	1304	924	967	1399	
2102	strawberry, sweet	39	58	22	121	
2114	chocolate, roasted	292	794	909	776	
2168	roasted, caramel	3164	2117	3121	3086	2-methoxy-4-vinylphenol[c]
2187	muscat, grape	1340	1117	1038	1299	*o*-aminoacetophenone[c]
2236	spicy	0	194	979	344	
2250	phenolic	348	88	20	29	
2255	roasted	44	185	215	58	
2272	rancid, sweaty	2514	2645	2207	512	decanoic acid[c]
2330	musty, cucumber	1161	513	281	331	
2370	floral, acid	1336	1267	831	1465	
2380	spicy	0	0	1134	0	

Table 1. (Continued)

RI on DB-Wax	odor quality	Charm value unhopped	Saazer	Hersbrucker	Cascade	compound
2414	feces	78	171	41	72	indole[c]
2459	feces	1784	1547	1389	1470	3-methylindole[c]
2512	vanilla, chocolate	2176	1891	1710	2298	vanillin[c]
2557	roasted	1210	1144	1142	1293	
2570	roasted	1322	339	1052	905	
2603	acid	1829	699	1224	724	
2648	spicy	0	0	1398	0	
2839	acid	192	257	166	335	
2970	citrus, raspberry	96	1293	1309	1257	4-(4-hydroxyphenyl)-2-butanone[a]

[a] Identified by matching RIs and mass spectra and odor qualities with the authentic compounds in DB-Wax and DB-1 columns. Tentatively identified by matching [b] RIs and odor qualities in DB-Wax and DB-1 columns, [c] RIs and mass spectra and odor qualities in DB-Wax column, and [d] RIs and odor qualities in DB-Wax column, with the authentic compounds.

Table 2. Concentrations (Micrograms per Liter) of Quantified Hop-Derived Potent Odorants in Beer[a]

component	unhopped wort	unhopped beer	Saazer wort	Saazer beer (aroma value)	Hersbrucker wort	Hersbrucker beer (aroma value)	Cascade wort	Cascade beer (aroma value)	CV[b] (%)	detection limit[c] (μg/L)
ethyl 2-methylpropanoate	ND[d]	0.27	0.15	3.98 (0.63)	0.45	8.01 (1.27)	0.42	6.39 (1.01)	7.8	0.07
(±)-ethyl 2-methylbutanoate	ND	0.02	0.11	1.67 (1.52)	0.16	1.83 (1.66)	0.11	1.20 (1.09)	7.5	0.04
ethyl 3-methylbutanoate	ND	0.01	0.23	5.32 (2.66)	0.23	2.66 (1.33)	0.24	2.13 (1.07)	7.4	0.02
1-hexanal	12.5	6.79	40.2	16.9 (0.05)	33.5	14.2 (0.04)	31.6	14.3 (0.04)	10.3	0.02
(*Z*)-3-hexen-1-ol	0.01	0.02	7.14	17.6 (0.02)	7.11	18.6 (0.02)	19.1	27.7 (0.03)	3.7	0.01
(*R*/*S*)-linalool	ND	ND	27.9	30.3 (15.8<)	71.3	70.5 (36.7<)	52.4	53.9 (28.0<)	4.3	0.05
geraniol	ND	ND	19.8	8.15 (2.04)	14.5	7.37 (1.84)	26.2	12.4 (3.09)	5.7	0.10
β-ionone	ND	ND	0.05	0.16 (0.27)	0.05	0.18 (0.30)	0.06	0.15 (0.25)	2.0	0.005
2-phenylethyl 3-methylbutanoate	ND	0.02	ND	3.05 (0.03)	ND	1.53 (0.02)	ND	2.46 (0.03)	7.1	0.02
4-(4-hydroxyphenyl)-2-butanone	ND	0.11	ND	2.29 (0.11)	ND	1.88 (0.09)	ND	1.37 (0.06)	14.3	0.05

[a] The mean value of duplicated analyses. [b] Coefficients of variance were calculated from 12 analyses of the same beer lot. [c] Detection limits are the concentration when the height of the signal was 3-fold that of noise. [d] The concentration was under the detection limit.

compounds contributing to the characteristics. Each extract was carefully concentrated using a Kuderna−Danish evaporative concentrator to reduce the loss of highly volatile odorants.

The odorants observed in this GC-O analysis are shown in **Table 1** along with the identified components. Beer extracts contain numerous aroma components and matrices that derive from malts, hops, and the process of fermentation; thus, many odorants are presented simultaneously during GC-O. In this study, a wide range of aromas comprising 83 odorants was detected because CharmAnalysis allows aroma components to be carried on air flowing at 30 mL/min (*15*), the flow of the odorants does not stay at the sniff port, and the boundaries between the aroma components are clearly defined. Chromatographic peaks for each extract are generated in CharmAnalysis, and the peak areas were integrated to yield the Charm values (*16*) shown in **Table 1**.

Hop-Derived Odorants. The GC-O comparison between unhopped and hopped beers revealed 27 components to be hop-derived odorants in beer **(Table 3)**. Most of these hop-derived substances were common to all three of the beers tested, and some are detected in slight amounts even in unhopped beer. Among them, 15 hop-derived odorants were identified utilizing their Kovats RIs, mass spectra, odor quality agreement with the standard compounds in DB-Wax and DB-1 columns, and their absence or rarity in unhopped beer. The mass spectra signals of four odorants, 3-methyl-2-butene-1-thiol (MBT), 4-mercapto-4-methyl-pentan-2-one (4-MMP), 3-mercapto-hexan-1-ol (3-MH), and (*Z*)-1,5-octadien-3-one, were too weak to give an unequivocal identification by using the method employed in this study. These odorants were therefore tentatively identified by matching their RIs and odor qualities with those of standard compounds in both DB-Wax and DB-1 columns. The remaining eight odorants could not be identified because they were complex mixtures and/or were present in insufficient quantities.

Some components are assumed to derive from the metabolism of degraded or isomerized products of hop-derived substances including α-acids, β-acids, polyphenols, and hydrocarbons during fermentation (*1*, *2*). Thus, in the current study, one-third of the total amount of hops was added at the beginning of the boiling process, to allow any substances generated during wort boiling to persist. Ethyl 2-methylpropanoate, (±)-ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 2-phenylethyl 3-methylbutanoate, and 4-(4-hydroxyphenyl)-2-butanone were either detected in small amounts or not detected in the wort. The concentrations of these odorants increased after fermentation and, moreover, these components were not detected in the unhopped beer **(Table 2)**. Our results therefore indicate that these compounds were mainly produced by the esterification of hop-derived short-chain acids or by equilibrium reactions with ethanol. Many short-chain acids result from the deterioration of α-acid in hops (*17*). This may be partly consistent with the contributions of deteriorated hops to the increased hop aroma in beer (*18*).

Hop-Derived Odor-Active Components. Among the identified hop-derived odorants, the most intense odor-active components with aroma values of >1.0 **(Table 2)** and Charm values of >1000 **(Table 1)** were as follows: linalool, geraniol, ethyl 3-methylbutanoate, (±)-ethyl 2-methylbutanoate, and ethyl 2-methylpropanoate.

Higher Charm values of >1000 for β-ionone (which had an aroma value of >0.3), 4-(4-hydroxyphenyl)-2-butanone, and ethyl 4-methylpentanoate, and 3-MH, 4-MMP, (*Z*)-1,5-octadien-

Table 3. Twenty-Seven Identified Hop-Derived Potent Odorants and Threshold Values in Beer[a]

RI on DB-Wax	odor qualities detected by GC-O	component	difference threshold value (μg/L) (*12*, *13*)
1004	citrus, pineapple, sweet	ethyl 2-methylpropanoate	6.3
1068	citrus, apple-like	(±)-ethyl 2-methylbutanoate	1.1[b]
1084	citrus, sweet, apple-like	ethyl 3-methylbutanoate	2.0
1103	green, leafy	1-hexanal	350 (*31*)
1111	almond, roasted	3-methyl-2-butene-1-thiol[d]	0.002 (*30*)
1148	green, leafy	(*Z*)-3-hexenal	20.0 (*31*)
1180	citrus, pineapple	ethyl 4-methylpentanoate	1.0
1199	almond, roasted	3-methyl-2-butenal	500 (*26*)
1338	fruity, catty, thiol-like	unknown	
1363	muscat-like, fruity	4-mercapto-4-methyl-pentan-2-one[d]	0.0015
1373	green, metallic	(*Z*)-1,5-octadien-3-one[d]	0.0034
1383	green	(*Z*)-3-hexen-1-ol	884
1383	muscat-like	unknown	
1548	floral, citrus, terpenic	(*R*/*S*)-linalool	1.0[c]
1571	green, cucumber	(*E*,*Z*)-2,6-nonadienal	0.5 (*31*)
1590	green, metallic	unknown	
1682	fatty	unknown	
1825	fruity, catty, thiol-like	3-mercapto-hexan-1-ol[d]	0.055
1850	floral, rose-like	Geraniol	4.0
1915	floral, violet-like, berry	β-ionone	0.6
1945	rancid, sweaty	(*Z*)-3-hexenoic acid	1300 (*31*)
1980	floral, minty	2-phenylethyl 3-methylbutanoate	88.5
2114	chocolate, roasted	unknown	
2236	spicy	unknown	
2380	spicy	unknown	
2648	spicy	unknown	
2970	citrus, raspberry	4-(4-hydroxyphenyl)-2-butanone	21.2

[a] Values are shown relative to the thresholds in beer (*30*, *31*) and water (*26*). [b] The value was determined by using the racemate. [c] The value was determined by using the (*R*)-isomer. [d] Tentatively identified by matching their RIs and odor qualities with the authentic compounds in DB-Wax and DB-1 column.

3-one, (*Z*)-3-hexenal, and unknown components at RIs of 1383, 1590, 2380, and 2648 were also observed and taken to be the odor-active components for the hop aroma in beer. In addition, extremely low threshold values were determined for 3-MH, 4-MMP, and (*Z*)-1,5-octadien-3-one **(Table 3)**, which were thus supposed to have effects on the hop aroma, although we failed to quantify the amount comparable to the threshold value. In support of this contention, Vermeulen (*19*) detected 3-MH and 4-MMP in fresh lager and reported that they had an influence on beer aroma.

Sensory Evaluation. The sensory evaluation examined the intensity of the green, citrus, floral, spicy, and muscat-like characteristics, along with the total hop aroma intensity of the beers. **Figure 1** shows the characteristics of each variety as an average intensity of the individual panelists' scores, and the detailed data for the figure are shown in **Table 4**.

The results show that citrus and floral notes characterized the hop aroma of Saazer beer. Hersbrucker beer was characterized by spicy, green, and floral notes, and its score for spicy characteristic was significantly higher than those of the other two varieties with the *t*-test value below 0.003, whereas that of citrus characteristics was lower.

Cascade beer was characterized by muscat-like and citrus notes. The significantly higher intensity of the muscat-like characteristic than for the other two varieties was observed with the *t*-test value below 0.001. The sensory score for total intensity of aroma was highest for Cascade with the *t*-test value below 0.02, followed by Saazer and then Hersbrucker.

Contributors to each of these characteristics are discussed in detail, along with the associated Charm value data, in the following sections.

Green Characteristic. Green odorants were observed for 1-hexanal, (*Z*)-3-hexenal, (*E*,*Z*)-2,6-nonadienal, (*Z*)-3-hexen-1-ol, (*Z*)-1,5-octadien-3-one, and an unknown odorant at RI 1590 by the GC-O analysis. The aldehydes and alcohol listed above have been described as odorants present in green leaves (*20*). In addition, (*Z*)-3-hexenal and (*Z*)-1,5-octadien-3-one (*7*) and 1-hexanal (*21*) have previously been reported in hops. The (*Z*)-3-hexen-1-ol content in beer increased after fermentation and so is assumed to be generated from compounds such as (*Z*)-3-hexenal (*20*). The total of the Charm values derived from green odorants present in Hersbrucker beer was greater than that for Saazer and Cascade, which was consistent with the results of the sensory evaluation. The concentrations of (*Z*)-3-hexen-1-ol and 1-hexanal themselves were lower than the threshold values as shown in **Table 2**; however, the relationship between the result of total Charm value and sensory evaluation indicates that the sum of these odorants comprises the green aroma of over the threshold (*14*).

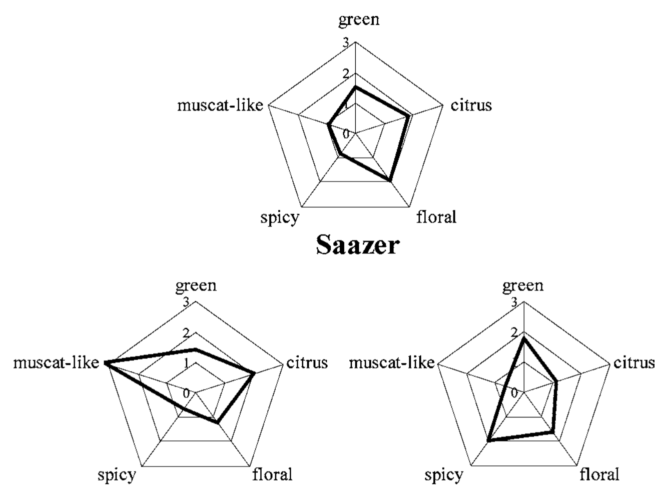

Figure 1. Aroma profile of beers hopped with Saazer, Cascade, and Hersbrucker.

Table 4. Intensity and *t* Test Value for the Characteristics of Beers Brewed with Different Hop Varieties

	intensity[a]			*t*-test value[b]		
	Saazer	Hersbrucker	Cascade	Saazer:Hersbrucker	Hersbrucker:Cascade	Cascade:Saazer
green	1.50 ± 0.52	1.79 ± 0.80	1.43 ± 0.71	0.41	0.38	0.85
citrus	1.79 ± 0.75	1.14 ± 0.41	2.00 ± 0.71	0.08	0.04	0.45
floral	1.93 ± 0.49	1.64 ± 0.66	1.21 ± 0.42	0.32	0.25	0.02
spicy	0.86 ± 0.49	2.00 ± 0.92	0.64 ± 0.26	0.003	0.002	0.08
muscat-like	0.93 ± 0.38	0.64 ± 0.52	3.14 ± 0.68	0.23	0.00001	0.0002
total hop aroma intensity	2.07 ± 0.45	1.79 ± 0.76	3.07 ± 0.66	0.28	0.01	0.02

[a] Mean intensity value of the scores from seven panelists ± standard deviation of the mean value. [b] Paired *t* test value comparing Saazer and Hersbrucker beers, Hersbrucker and Cascade beers, Cascade and Saazer beers.

Muscat-like Characteristic. Cascade beer was identified as having a muscat-like characteristic according to the results of the sensory analysis. Intense muscat-like odorants, which had Charm values of >1000, were detected at RIs of 1363 and 1383 by GC-O. The former odorant was tentatively identified as 4-MMP, which was revealed to have an extremely low threshold value in beer **(Table 3)**, and recently 4-MMP was detected in Cascade hops (*23*). It was assumed to have an effect on the aroma as it does in wine (*22*), although we were unable to quantify the threshold amounts of the compound.

The second muscat-like flavor was identified at RI 1383, where (*Z*)-3-hexen-1-ol was identified by GC-MS. (*Z*)-3-Hexen-1-ol itself was confirmed as the odor-active and green odorant by the Charm analysis employing a DB-1 column. It has also been described as one of the major volatiles in muscat grape flavor (*24*, *25*), and the higher concentration was observed in Cascade beer. Thus, (*Z*)-3-hexen-1-ol in combination with the unknown odorant at RI 1383 was assumed to be a contributor to the muscat flavor. The sum of the Charm values of these two RIs in the Cascade-hopped beer was higher than that of the other two beers tested: this indicated that these components were the main contributors to the muscat-like characteristic. Geraniol, which itself has a floral characteristic, has also been described as a major volatile in muscat grape flavor (*24*−*26*). As we found it at a higher Charm value and concentration, it was assumed to be a contributor to this characteristic odor.

Spicy Characteristic. As shown in **Figure 1**, Hersbrucker was also strongly characterized by a spicy aroma. Spicy odorants were detected by CharmAnalysis at RIs of 2236, 2380, and 2648, which is a region where sesquiterpenoids are abundant. Our observations were consistent with a previous paper in which the sesquiterpenoid fraction of Hersbrucker was thought to yield a spicy characteristic (*27*, *28*). Components at RIs of 2380 and 2648 were detected only in Hersbrucker. The sum of the Charm values of the spicy characteristics of Hersbrucker was significantly higher than those of the other two types of beer. Thus, we demonstrate that these components contribute to the spicy characteristic, although they could not be identified in the current study.

Floral Characteristic. Linalool, geraniol, and β-ionone were shown to contribute to the floral note, on the basis of the Charm values and the aroma values. In the current study, the threshold value of linalool, 1.0 mg/L, was determined using the (*R*)-isomer. Although enantiomeric quantification of the beer was not performed here, the reported enantiomeric ratio of the (*R*)-isomer in beer is >52% (*29*). As for Saazer beer, which was observed to have the lowest concentration of linalool, 30.3 mg/L, among the three varieties, the calculated concentration of the (*R*)-isomer and the aroma value of linalool were greater than 15.8 mg/L and 15.8, respectively **(Table 2)**.

In addition to these three terpenoids, 2-phenylethyl 3-methylbutanoate was also associated with the floral characteristic. The component was not detected in wort and so is assumed to be generated during fermentation from compounds such as 3-methylbutanoic acid and 2-phenylethanol (*26*). The sensory score for floral attributes was highest for Saazer, followed by Hersbrucker and then Cascade, and thus was not consistent with the total Charm values of these components. This indicated that additional components from hops and other raw materials might contribute synergistically or antagonistically to the floral characteristic. Further investigations will be required to clarify this issue.

Citrus Characteristic. Linalool, ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, 4-(4-hydroxyphenyl)-2-butanone, ethyl 4-methylpentanoate, 3-MH (which was revealed to have an extremely low threshold value), and an unknown component at RI 1338 were identified as odorants contributing to the citrus flavor. Remarkably higher Charm values of ethyl 3-methylbutanoate and ethyl 4-methylpentanoate **(Table 1)** and the aroma value of ethyl 3-methylbutanoate **(Table 2)** were observed in Saazer beer than in the other two varieties. The citrus score for Cascade according to the organoleptic estimation was higher than that for the other beers, which could not be explained by the sum of the Charm values. Additional components, both from hops and from other raw materials, are therefore likely to contribute to this characteristic, either synergistically or antagonistically.

To reveal the contributions of these components in more detail, identification of the unknown components mentioned above and quantification of the components with extremely low threshold value as well as investigation from an enantiomeric viewpoint followed by aroma simulations recombining the odorants will be required. This might also allow the characterization of novel odorants.

ABBREVIATIONS USED

3-MH, 3-mercaptohexan-1-ol; 4-MMP, 4-mercapto-4-methylpentan-2-one; AEDA, aroma extract dilution analysis; CAS, Chemical Abstracts Service; FID, flame ionization detector; GC-O, GC−olfactometry; MS, mass spectrometer; ND, not detected; RI, retention index; SBSE, stir bar sorptive extraction.

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Received for review May 12, 2006. Revised manuscript received August 30, 2006. Accepted September 3, 2006.

JF061342C