JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Comparison of 4-Mercapto-4-methylpentan-2-one Contents in Hop Cultivars from Different Growing Regions

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Contributions of hop-derived thiols were examined. Extremely strong fruity, black currant-like aromas were detected in beers hopped with some U.S. cultivars. 4-Mercapto-4-methylpentan-2-one (4MMP) was supposed to be the main contributor to the fruity aroma, and the contents between cultivars were investigated. In hop pellets, a negative correlation between 4MMP concentration and copper ion content in hops was observed. 4MMP was detected only in U.S., Australian, and New Zealand cultivars, but no European ones, which are treated with copper-containing fungicides (Bordeaux mixture) and therefore have a high content of copper ions. The 4MMP content was highest in Simcoe cultivars, followed by Summit, Apollo, Topaz, Cascade pellets, and also differed between crop years. It was indicated that most 4MMP exists freely in wort or in hop pellets with only small amounts formed from precursors and that the amounts increased during the fermentation process.

**KEYWORDS: Brewing; CharmAnalysis; gas chromatography–mass spectrometry; hops; thiols**

## INTRODUCTION

Hops are used in the brewing process to add a characteristic bitterness and aroma to beer. Terpenoids are the main components in oils of hop cones or pellets. Of the components, myrcene, caryophyllene, and humulene make up the majority, and, especially, myrcene characterizes the aroma of hop cones or pellets (*1*). Previous studies (*2, 3*), however, revealed that only a few terpenoids contributed to beer hop aroma, and the qualities of hop aroma in beers differed remarkably from those of the hop cones or pellets. Most hydrophobic odor-active terpenoids either evaporated or metabolized into different odorants in the brewing process, and then only a few hydrophobic odor-active terpenoids were found in the final beer products (*2, 4*).

Hop-derived potent odorants that persisted even after fermentation were also examined in our previous study (*3*) to determine the components which contribute to the sensory hop aroma characteristics in beer. The black currant, muscat-like aroma and floral aroma were predominant in sensory evaluation of strongly hopped Cascade beer, and 4-mercapto-4-methylpentan-2-one (4MMP) and geraniol were identified as contributors to its character (*3*).

It is well-known that each variety has a characteristic aroma (*5*), and contributors to the characteristics are different between varieties. The contribution of 4MMP to the characteristic of Cascade cultivar was reported by Steinhaus et al. (*6*). By comparison of the most odor-active volatiles in different hop cultivars, they found that the odor profile of Cascade hop pellet was dominated by a black currant-like, berry-like note and that 4MMP was the contributor to its character (*6*). Vermeulen et al. (*7*) described the influences of 4MMP on the total flavor of commercial lager beer. They revealed that a 10 ng/L spiking of 4MMP in fresh beer emphasized the fresh and fruity aroma in sensory evaluation.

The occurrence of 4MMP and its content were reported only for Cascade before (*3, 6*) and had not been investigated for other cultivars. Furthermore, why 4MMP exists at higher content in the Cascade cultivar had not been studied in previous studies. In the current study, we examined the 4MMP content in hops depending on cultivars and their growing regions to establish a distinctive hop aroma characteristic in beer.

## MATERIALS AND METHODS

**Chemicals.** (+/−)-Ethyl 2-methylbutanoate (99.0%) and *p*-hydroxymercuribenzoic acid sodium salt (98.0%) were purchased from Acros Organics (Morris Plains, NJ). Dowex (1 × 2, Cl(−) form, strongly basic, 50–100 mesh) was obtained from Sigma-Aldrich (St. Louis, MO). 4MMP [stored at 1% (w/w) in triacetin], (*S*)-(+)-linalool (87.0%), and 4-methoxy-2-methyl-2-mercaptobutane (99.0%) were obtained from San-Ei Gen FFI, Inc. (Osaka, Japan). *tert*-Butyl-4-methoxyphenol (98.0%) and L-cysteine hydrochloride monohydrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). (*R*/*S*)-Linalool (95.0%), (*R*)-(−)-linalool (98.5%), geraniol (99.0%), *β*-damascone (90.0%), ethyl 4-methylpentanoate (97.0%), and (*Z*)-3-hepten-1-ol (95.0%) were purchased from Fluka (Buchs, Swit-

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10.1021/jf072173e CCC: $40.75 
Published on Web 01/04/2008

**Table 1.** Abbreviations, Origins, Crop Years, and Lead Conduct Values of α Acids of Hops Cultivars

| variety | country | abbreviation | crop year | lead conduct value of α acids (%) |
|---|---|---|---|---|
| Simcoe | USA | US-SIM | 2005 | 10.4 |
| | USA | US-SIM | 2006 | 10.4 |
| Summit | USA | US-SUM | 2006 | 16.3 |
| Apollo | USA | US-APO | 2006 | 17.1 |
| Millenium | USA | US-MIL | 2006 | 15.1 |
| Cascade | USA | US-CAS | 2006 | 5.5 |
| Willamette | USA | US-WIL | 2006 | 3.4 |
| Topaz | Australia | AU-TPZ | 2007 | 13.6 |
| Pacific Gem | New Zealand | NZ-PGM | 2006 | 14.0 |
| Perle | USA | US-PEL | 2006 | 7.0 |
| Perle | Germany | GE-PEL | 2006 | 10.7 |
| Nugget | USA | US-NUG | 2005 | 11.9 |
| Nugget | Germany | GE-NUG | 2006 | 11.3 |
| Magnum | Germany | GE-MAG | 2006 | 12.5 |
| Taurus | Germany | GE-TAU | 2006 | 13.7 |
| Hersbrucker | Germany | GE-HER | 2005 | 2.9 |
| Saazer | Czech Republic | CZ-SAZ | 2005 | 5.4 |
| Fuggle | United Kingdom | UK-FGL | 2005 | 4.6 |

zerland). Tris(hydroxymethyl)aminomethane (99.0%), $HNO_3$ for inductively coupled plasma mass spectroscopy (ICP-MS) analysis, yttrium and indium for atomic absorption analysis, and granular copper (99.9%) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). (*S*)-(+)-Ethyl 2-methylbutanoate (85.0%) was obtained from T. Hasegawa Co., Ltd. (Tokyo, Japan). 3-Mercaptohexan-1-ol (97.0%) was purchased from Avocado Research Chemicals Ltd. (Heysham, U.K.). 3-Mercaptohexyl acetate (95.0%) was purchased from Atlantic Research Chemicals Ltd. (Bude, U.K.).

**Hops.** The hop cultivars Simcoe (2005 and 2006 crops, United States) and Topaz (2007 crop, Australia) were purchased from Yakima Chief (Sunnyside, WA) and Hop Products Australia (Tasmania, Australia), respectively. Hersbrucker (2005 crop, Germany), Saazer (2005 crop, Czech Republic), Fuggle (2005 crop, United Kingdom), and Perle (2006 crop, Germany) were purchased from Joh. Barth & Sohn GmbH & Co. (Nuremberg, Germany). Summit (2006 crop, United States), Millennium (2006 crop, Unites States), and Nugget (2005 crop, United States) were purchased from John I. Haas, Inc. (Yakima, WA). Apollo (2006 crop, United States), Cascade (2006 crops, Unite States), Willamette (2006 crop, United States), and Perle (2006 crop, United States) were purchased from S. S. Steiner, Inc. (New York, NY). Magnum (2006 crop, Germany), Taurus (2006 crop, Germany), Nugget (2006 crop, Germany), and Pacific Gem (2006 crop, New Zealand) were purchased from Simon H. Steiner, Hopfen, GmbH (Mainburg, Germany).

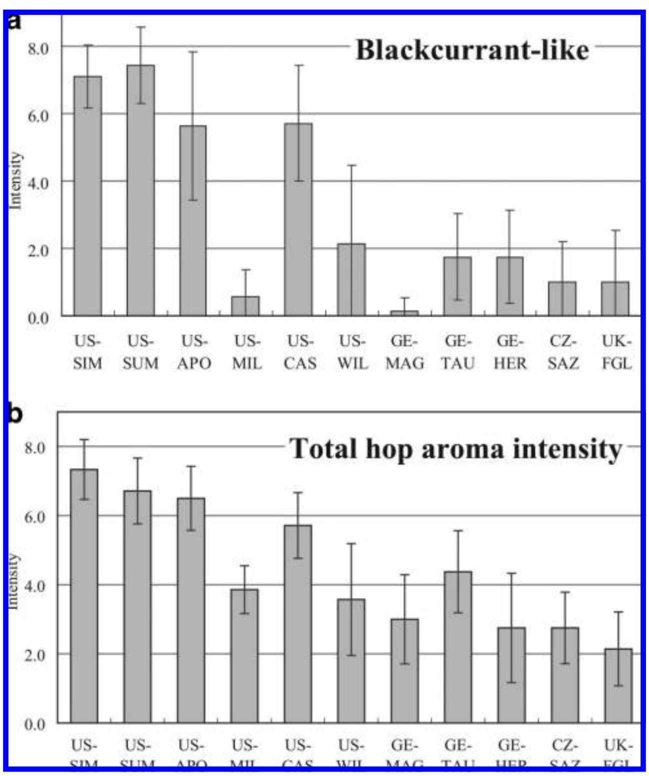


**Figure 1.** Intensities of the black currant-like aroma (**a**) and total hop aroma (**b**) evaluated in sensory analysis.

**Brewing Processes.** Pellets of the hop cultivars Simcoe (2005 crop), Summit, Apollo, Millennium, Cascade, Willamette, Magnum, Taurus, Hersbrucker, Saazer, and Fuggle were used in the brewing process. Unhopped beer and beers hopped with each cultivar were brewed independently. For the evaluation of cultivars, a 20 L volume of wort without hops was boiled for 60 min in a wort kettle; a 40 g sample of hops was then added after the wort-cooling process, in order to examine the properties of the hop components and the aroma characteristics independent of any temperature effect.

Static fermentations were carried out in 5 L stainless steel tall tubes, modified from 2 L European Brewery Convention (EBC) tall tubes (*8*), which were equipped with precise pressure and temperature controls. The yeast used in the fermentations was washed twice (for 5 days each time) in unhopped wort. The washed yeast was pitched at a rate of 20 × $10^6$ cells/mL into the wort. A 5 L sample of each wort (11.5 °P) was fermented at 12.0 °C for 6 days, under an inner-tank pressure of 0.05 MPa. Then any yeast that settled on the tank bottom was eliminated. Each beer was then allowed to mature at 12.0 °C for 5 days. The finished beer was obtained by cooling to −1 °C for 5 days and then centrifuging at 7000 rpm for 30 min.

**Sensory Evaluation.** Sensory evaluation of the hopped beers was performed by nine trained panelists, using the intensity of unhopped beer as a control. The panelists were asked to describe the hop aroma characteristics and to evaluate the intensities of the "black currant-like" aroma and the total hop aroma. The responses to each description were shown to be consistent among the panelists using a matching test (*9*). The odor intensities were rated on the following scale (with 1.0 interval steps): 0 = not perceivable; 2 = weak; 4 = normal; 6 = strong; 8 = very strong. The cultivar characteristics were compared using the mean intensity values of the scores.

**Solvent-Assisted Flavor Evaporation (SAFE).** A SAFE apparatus (Kiriyama Glass Works Co., Tokyo, Japan) designed to the specifications reported by Engel et al. (*10*) was used to prepare extracts for gas chromatography−olfactometry (GC-O) analysis. The volatiles were isolated from 150 mL samples of unhopped, Magnum, Summit, Simcoe, and Apollo beers. The SAFE apparatus was connected to a 500 mL distillation flask and a 500 mL receiving flask. The distillation flask was warmed to 35 °C by pumping water from a heated reservoir through the jacketed body of the apparatus. The receiving vessel and cold trap were cooled by liquid nitrogen, and the apparatus was evacuated under a high vacuum (1.5 × $10^{-5}$ Torr) by a VPC-250F diffusion pump (Ulvac Kiko, Inc., Yokohama, Japan). Each sample was added dropwise via the sample reservoir into the distillation flask to prevent excessive foaming. After distillation, the vacuum was released, and the surface of the cold trap was washed with 10 mL of ethanol. The distillate was then applied to isolate thiols using resin and sodium *p*-hydroxymercuribenzoate (*p*HMB) as described below.

**Isolation of Volatile Thiols.** The volatile thiols were isolated using a strongly basic anion-exchanger resin (Dowex 1) with elution columns (Kiriyama Glass Works Co.), which were designed according to the description given by Tominaga et al. (*11*).

For the GC-O analysis, 0.02 mM *tert*-butyl-4-methoxyphenol was added as an antioxidant to 150 mL of SAFE distillate, which was employed for the subsequent extraction with *p*HMB. For the quantification of 4MMP in beer by GC-MS, 100 mL beer samples containing 1.0 μg/L 4-methoxy-2-methyl-2-mercaptobutane as an internal standard and 0.02 mM *tert*-butyl-4-methoxyphenol were subjected to degassing by sonication for 20 min and then were applied for the following extraction with *p*HMB. For the quantification of 4MMP in hop pellets by gas chromatography–mass spectrometry (GC-MS), aroma components were extracted from 2 g of hop pellets with 100 mL of 40 °C

**Table 2.** Charm Values of Black Currant-like Odorants Extracted from Beer

| retention indices | | | Charm values | | | | | |
|---|---|---|---|---|---|---|---|---|
| DB-Wax | HP-5 | characters detected by GC-O | US-SIM | US-APO | US-SUM | GE-MAG | unhopped | identified compound |
| 1208 | | black currant-like, passion fruit-like | 414 | 360 | 444 | 0 | 0 | |
| 1342 | | black currant-like, passion fruit-like | 400 | 306 | 452 | 303 | 0 | |
| 1377 | 933 | fruity, black currant-like | 990 | 765 | 777 | 65 | 0 | 4-mercapto-4-methyl-pentan-2-one |
| 1442 | | black currant-like, passion fruit-like | 143 | 180 | 162 | 0 | 0 | |
| 1515 | | black currant-like | 123 | 131 | 144 | 177 | 48 | |
| 1719 | 1249 | black currant-like, grapefruit-like | 371 | 411 | 401 | 387 | 456 | 3-mercaptohexyl acetate |
| 1840 | 1121 | black currant-like, grapefruit-like | 364 | 406 | 389 | 316 | 153 | 3-mercaptohexan-1-ol |

water for 30 min. The water extracts with 0.02 mM *tert*-butyl-4-methoxyphenol were applied for the following extraction with *p*HMB. The extractions using the *p*HMB solution and Dowex 1 columns were performed according to the method described previously by Tominaga et al. (*11*). In the release of thiols from thiol–*p*HMB conjugate, cysteine was used in place of cysteamine.

**GC-O Analysis.** GC-O analysis of the extracts prepared using the SAFE apparatus, Dowex 1, and *p*HMB from unhopped, Magnum, Summit, Simcoe, and Apollo beers was performed by CharmAnalysis (Datu Inc., Geneva, NY), as described previously (*3*).

Identification of the hop-derived thiols was attempted by comparing their odor qualities, Kovats retention indices (RIs), and mass spectra with those of authentic compounds on both DB-Wax and HP-5 capillary columns (Agilent Technologies, Santa Clara, CA; length = 30 m; i.d. = 0.25 mm; film thickness = 0.25 $\mu$m) by GC-MS [Agilent 6890 gas chromatograph coupled to an Agilent MSD5973N quadrupole (QP) mass spectrometer].

**Quantification of Thiols by Multidimensional (MD)-GC-MS.** 4MMP quantification was performed by MD-GC-MS using an Agilent 6890 gas chromatograph (Agilent Technologies) equipped with a first column, a multicolumn switching system (MCS2; Gerstel, Mulheim a/d Ruhr, Germany), and an Agilent 6890 GC coupled to an MSD5973N QP mass spectrometer equipped with a second column.

A 1 $\mu$L sample was injected, and extract separation was performed on the first column (DB-5MS capillary column; 30 m length × 0.25 mm i.d.; film thickness = 0.25 $\mu$m; Agilent Technologies). The inlet temperature was set at 250 °C with splitless injection. The oven temperature was programmed to rise from 40 °C (held for 1 min) to 160 °C (held for 16 min) at a rate of 10 °C/min and then to 300 °C (held for 10 min) at a rate of 10 °C/min with a constant carrier helium gas flow (1 mL/min).

At the elution of 4MMP, the effluent was transferred to a cold trap at −100 °C using the MCS2 cooled by liquid nitrogen. After cooling, the trapped material was further separated by the second column (DB-WAX capillary column; 30 m length × 0.25 mm i.d.; film thickness = 0.25 $\mu$m; Agilent Technologies). The oven temperature was programmed to rise from 40 °C (held for 15 min) to 160 °C (held for 16 min) at a rate of 5 °C/min and then to 230 °C (held for 7 min) at a rate of 10 °C/min with a constant carrier helium gas flow (1 mL/min). The GC-MS system was operated in the electron-impact mode at 70 eV, with the select ion monitoring (SIM) mode at *m*/*z* 132 for 4MMP and at *m*/*z* 100 for 4-methoxy-2-methyl-2-mercaptobutane.

**Quantification of Esters and Terpenoids.** The quantification of myrcene, (*R*/*S*)-linalool, and geraniol in the beer samples was carried out by the stir-bar sorptive extraction (SBSE) method using $\beta$-damascone as an internal standard, as described previously (*2, 12*). The amount of (+/−)-ethyl 2-methylbutanoate in the beer samples was measured according to the liquid extraction method with dichloromethane using (*Z*)-3-hepten-1-ol as an internal standard, as described previously (*2*). The data are shown as the mean values of duplicate analyses.

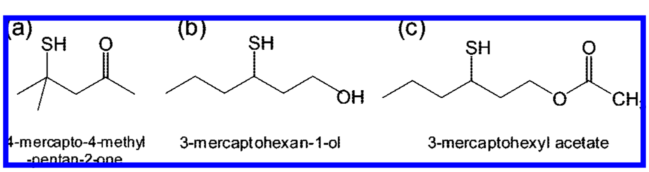


**Figure 2.** Structures of 4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol, and 3-mercaptohexyl acetate.

**Quantification of Ethyl 4-Methylpentanoate.** Ethyl 4-methylpentanoate was quantified using the large-volume dynamic headspace method with GC-MS and the Entech 7100A system (Entech, Simi Valley, CA). Methyl propionate was added to each beer sample at a final concentration of 1.25 mg/L as an internal standard. Then, 2 mL of the beer sample including the internal standard was diluted with 98 mL of distilled water. The 100 mL diluted sample was then transferred into a 250 mL jar containing a glass bubbling tube. Helium gas (750 mL) was bubbled into the jar at a temperature of 40 °C and a flow rate of 60 mL/min. A three-stage concentration method was used to remove excess water and carbon dioxide from the stream of volatiles from the jar, which was subsequently introduced into the preconcentration system. The flow was initially concentrated in a cryogenic trap consisting of glass beads and Tenax (module 1) at 20 °C. The trap was then heated to 180 °C, and the concentrated volatiles were transferred by passing helium gas into a secondary Tenax trap (module 2) that was held at 20 °C. The trap was then heated to 180 °C for 3.5 min, and the concentrated volatiles were transferred into an inert empty glass tube (module 3) that was held at −150 °C. Separation of the volatiles was performed with an Agilent 6890 gas chromatograph coupled to an MSD5973N QP mass spectrometer (Agilent Technologies) equipped with a DB-1 capillary column (60 m length × 0.32 mm i.d.; film thickness = 1.0 $\mu$m; Agilent Technologies) with a helium carrier gas (1.2 mL/min). The third trap was heated to 150 °C for 4 min to inject the volatiles into the GC-MS apparatus. The volatiles were injected using a pulsed split mode with a split ratio of 15:1. The oven temperature was programmed to rise from 40 °C (held for 5 min) to 300 °C (held for 5 min) at a rate of 10 °C/min. The GC-MS system was operated in the electron-impact mode at 70 eV, with the SIM mode at *m*/*z* 88.

**Determination of Enantiomeric Excess (ee) Values.** The ee values of linalool and ethyl 2-methylbutanoate were investigated using MD-GC-MS as described above, employing a DB-Wax capillary column (60 m length × 0.32 mm i.d.; film thickness = 0.25 $\mu$m; Agilent Technologies) as the first column and an RT-BetaDEXse chiral column (30 m length × 0.32 mm i.d.; film thickness = 0.25 $\mu$m; Restek, Bellefonte, PA) as the second column. The inlet temperature was set at 250 °C with splitless injection, and 1 $\mu$lL samples were injected.

The oven temperature of the first column was programmed to rise from 40 °C (held for 2 min) to 220 °C at a rate of 3 °C/min, with a constant carrier helium gas flow (1 mL/min). On the second (chiral) columns, separation of ethyl 2-methylbutanoate and linalool was performed by isothermally maintaining temperatures of 55 and 90 °C, respectively. The GC-MS system was operated in the SIM mode at *m*/*z* 102 for ethyl 2-methylbutanoate and at *m*/*z* 93 for linalool. The ee value was calculated on the basis of the integrated peak area of the *R*-isomer and the *S*-isomer using the following formula: ee (%) = ([R] − [S])/([R] + [S]) × 100.

**Quantification of Divalent Metal Ions.** Divalent metal ions were quantified using ICP-MS (Agilent 7500c). Prior to analysis, samples were digested in closed vessels made of polytetrafluoroethylene (PTFE) using the Multiwave 3000 microwave sample digestion system (Perkin-Elmer Life and Analytical Sciences, Inc., Waltham, MA).

The PTFE vessels were washed with 6 mL of $HNO_3$ and subjected to microwave digestion programmed at 1250 W (held for 10 min) and then at 0 W (held for 20 min). The vessels were then rinsed with ultrapure water and filled with 1 N $HNO_3$. The polypropylene tubes

**Table 3.** Contents[a] of Hop-Derived Main Odorants in Beer Hopped with 11 Cultivars

| | difference threshold value in beer[b] | US-SIM (2005) | US-SUM (2006) | US-APO (2006) | US-MIL (2006) | US-CAS (2006) | US-WIL (2006) | GE-MAG (2006) | GE-TAU (2006) | GE-HER (2005) | CZ-SAZ (2005) | UK-FGL (2005) | unhopped | CV[c] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4MMP (ng/L) | 1.5 | 183.8 | 116.4 | 109.2 | nd | 16.9 | nd | nd | nd | nd | nd | nd | nd[d] | 4.0 |
| myrcene (μg/L) | 9.5 | 28.3 | 57.7 | 37.5 | 37.3 | 21.4 | 11.9 | 41.6 | 89.2 | 11.4 | 12.9 | 5.47 | nd | 7.4 |
| linalool (μg/L) | 1.0[e], 1.7[f] | 95.5 | 87.9 | 73.4 | 84.7 | 56.9 | 64.6 | 43.1 | 167.0 | 80.8 | 62.5 | 35.6 | nd | 4.3 |
| (ee %) | | (85.2) | (85.9) | (85.4) | (89.5) | (87.6) | (89.2) | (87.1) | (92.2) | (91.9) | (87.4) | (90.1) | | |
| geraniol (μg/L) | 4.0 | 58.5 | 59.1 | 33.3 | 23.2 | 82.8 | 11.7 | 22.3 | 9.49 | 6.14 | 11.5 | 4.68 | nd | 5.7 |
| ethyl 2-methylbutanoate (μg/L) | 1.2[f] | 2.04 | 5.38 | 9.78 | 2.75 | 0.80 | 1.01 | 3.80 | 2.61 | 0.72 | 0.80 | 0.75 | 0.09 | 7.5 |
| (ee %) | | (−93.9) | (−97.5) | (−97.7) | (−97.5) | (−92.2) | (−91.8) | (−97.1) | (−96.7) | (−89.1) | (−85.5) | (−91.1) | | |
| ethyl 4-methylpentanoate (μg/L) | 1.0 | 0.60 | 2.39 | 2.23 | 1.93 | 0.41 | 0.35 | 1.97 | 1.52 | 1.07 | 1.16 | 0.43 | 0.09 | 4.3 |

[a] Mean values of duplicated analyses. nd, not detected. [b] Difference threshold value (μg/L) in beer (3). [c] Coefficient of variation. [d] Concentration beneath the detection limit in beer (4MMP, <4.0 ng/L; myrcene, <0.001 μg/L; linalool, <0.05 μg/L; geraniol, <0.10 μg/L), where the height of the signal was 3-fold that of noise. [e] The value was determined by using the (*R*)-isomer. [f] The value was determined by using the racemate.

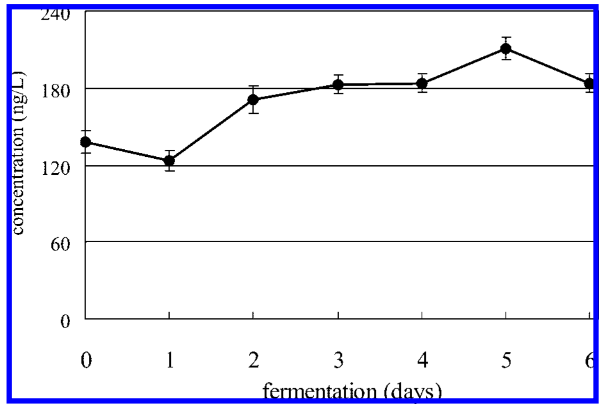


**Figure 3.** Changes in 4-mercapto-4-methylpentan-2-one concentrations during fermentation of US-SIM wort.

were washed by filling with alkaline detergent overnight, followed by 1 N $HNO_3$ overnight, and then rinsing with ultrapure water.

Samples of hop pellets (200 mg) with 5 mL of $HNO_3$ and internal standards (yttrium or indium) were hermetically sealed in the PTFE vessels and subjected to microwave digestion (Multiwave 3000). The microwave was programmed at 0 W (held for 10 min), at 1400 W (held for 40 min), and then at 0 W (held for 20 min). The PTFE vessels were left to cool, and the digested samples were transferred to polypropylene tubes by washing with ultrapure water and adjusting the volume to 20 mL. The samples were then filtered with cellulose nitrate 0.2 μm filters (Advantec, Tokyo, Japan).

The samples were subjected to ICP-MS analysis with an Agilent 7500c under the indicated plasma conditions [radio frequency (RF) output = 1500 W; RF matching = 1.75 V; carrier gas flow = 0.8 L/min; makeup gas flow = 0.2 L/min; microflow as nebulizer at 100 μL/min; scotchamber temperature = 2 °C], ion lens conditions (pull-out voltage = 3.4 V; einzel1.3 = −100 V; einzel2 = 15 V; incidence into the cell = −22 V; output from the cell = −15 V; plate bias = −45 V), octapole conditions (RF = 200 V with a bias of −8 V), QP parameters (atomic mass unit (AMU) gain = 129; AMU offset = 124; QP bias = −7 V), and detector conditions [−8 mV discriminator, 1670 V analog high voltage (HV), 1100 V pulse HV]. Helium (2 mL/min) was used as the reaction gas for copper and zinc, 3 mL/min helium was used for iron, and 2 mL/min hydrogen was used for manganese.

**Lead Conductance of Hop Pellets.** The lead conductance values of the hops were determined using the EBC 7.4 method (*13*).

## RESULTS AND DISCUSSION

The 17 hop cultivars examined in the current study, their origins, crop year, abbreviations, and lead conductance values are listed in **Table 1**. Of the 17 cultivars, 11 were evaluated in beer in addition to the analysis of hop pellets.

**Black Currant-like Aroma of Beers.** The intensities of black currant-like aroma and total hop aromas were evaluated in beers hopped with the 11 hop cultivars, as shown in **Figure 1**. Hops were added after the wort-cooling process to investigate the hop-derived aroma qualities that remain even after fermentation and to obtain objective analytical data without the effect of temperature.

The fruity black currant-like aroma was highest in the beer hopped with U.S. cultivars, Summit and Simcoe, followed by Apollo and Cascade. In addition, a pungent green onion-like, sulfur-like aroma was also detected only in Summit and Apollo beers (data not shown). The black currant-like characteristic is often attributed to the presence of thiols in beer and wine (*7, 14–16*). The black currant-like characteristics, as well as the pungent green onion-like aroma, extremely decreased in perception when granular copper was added to the beers, suggesting that thiols were possible contributors to these characteristics (*7*).

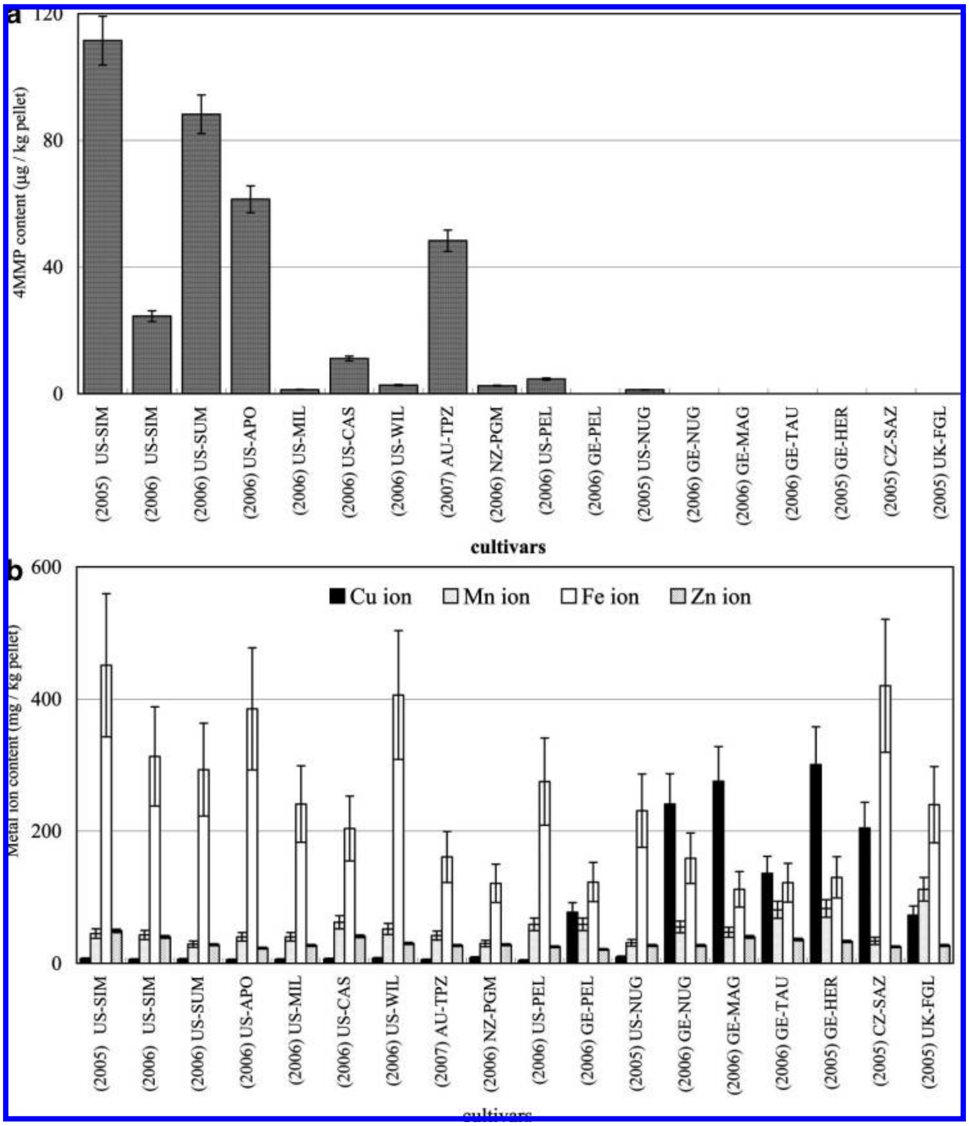


**Figure 4.** Contents of (**a**) 4-mercapto-4-methylpentan-2-one and (**b**) divalent metal ions in hop pellets.

Therefore, we examined black currant-like thiols in beers and contributors to the characteristics of Simcoe, Summit, and Apollo by GC-O analysis.

**GC-O Analysis of Beers.** The extracts from beers hopped with Summit, Apollo, Simcoe, and Magnum and unhopped beer were prepared according to a method that extracts thiols specifically. In the preparation of the beer extracts for GC-O analysis, the SAFE apparatus was used to prevent the formation of artifacts from nonvolatile compounds in the GC-O inlets, and then the extraction was performed using a strongly basic anion exchanger resin (Dowex 1) and *p*HMB.

CharmAnalysis was used in GC-O analysis because it allows aroma components to be carried on air flowing at 30 mL/min (*3*), 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 (**Table 2**) (*17*).

The black currant-like odorants in beer extracts detected by GC-O analysis are shown in **Table 2**. Of the seven components listed, four odorants (RIs at 1208, 1442, 1515, and 1719) were novel to the current study, and the other three were described in our previous study (*3*). The components at RIs of 1377, 1719, and 1840 were identified as 4MMP (**Figure 2a**), 3-mercaptohexyl acetate (3MHA; **Figure 2c**), and 3-mercaptohexan-1-ol (3MH; **Figure 2b**), respectively, by comparing odor qualities, RIs, and mass spectra with those of authentic compounds on both DB-Wax and HP-5 capillary columns by GC-MS.

With the exception of 3MHA, the remaining six odorants were detected with little or no Charm value in unhopped beer but were increased by the addition of hops, indicating that they were hop-derived components. Of the six black currant-like odorants derived from hops, three components including 4MMP were not detected in the extract from Magnum, for which black currant-like aroma was not detected in the sensory evaluation. Furthermore, an extremely higher Charm value was detected at 4MMP, and therefore 4MMP was supposed as the main contributor to the character.

**4MMP Contents of Beers.** The concentrations of 4MMP in beers hopped with 11 cultivars (**Table 3**) were determined according to the method using Dowex 1, *p*HMB, and MD-GC-MS. In beers, as shown in **Table 3**, extremely high contents of 4MMP were observed in beers hopped with Simcoe (183.8 ng/L), followed by Summit (116.4 ng/L), Apollo (109.2 ng/L), and Cascade (16.9 ng/L), for which the black currant-like characteristic was strong in the sensory evaluation (**Figure 1**).

In wine, 4MMP is believed to exist as cysteine conjugate (*18*) and to be released from the precursor during fermentation processes. Changes in 4MMP concentrations during fermentation were observed for Simcoe beer as depicted in **Figure 3**. The 4MMP content increased by 33% during fermentation, and this indicates that most 4MMP exists freely in wort or in hop pellets and that only a small amount is formed during the fermentation process from precursors. The 4MMP content peaked during the early stages of fermentation (**Figure 3**), as was also observed in wine (*19*). Cysteine conjugate precursors present in hops or wort are thought to be transported into yeast cells along with other amino acids and then undergo enzymatic activity, as suggested by a previous paper on wine aroma (*20*), considering the distinguishability from other amino acids, optimal pH, and concentration of the enzyme activity in wort.

In the current study, we investigated the contributions of 4MMP to both the black currant characteristic and the total hop aroma intensity. On the basis of the extremely low threshold value in beer (1.5 ng/L) determined in our previous study (*3*), 4MMP was expected to be the main contributor to the black currant-like characteristic. The 4MMP contents of the beers also appeared to affect the overall hop aroma intensity, as shown in **Figure 1b**; the highest intensity was observed in Simcoe, followed by Summit, Apollo, and then Cascade. We investigated the components previously reported to contribute to hop aroma, as shown in **Table 3**, and found no associations with total hop aroma intensity. For instance, myrcene, which is reportedly one of the most odor-active components in hop cones (*1*) with a threshold value in beer of 9.5 $\mu$g/L (*21*), was present at the highest levels in Taurus and Summit beers. Linalool, which is reportedly a key odorant in hops (*1, 22, 23*) that is also found in beers (*22*), was present at the highest level in Taurus beer followed by Simcoe beer; its ee value (percent) (*22*), which influences the threshold value and therefore the aroma impact, did not differ significantly between varieties (**Table 3**). Geraniol, which is a major contributor to hop aroma (*24*), was found at the highest levels in Cascade beer; its varietal specificity and contributions to the characteristics of Cascade have been reported previously (*3, 6*). Ethyl 4-methylpentanoate, which influences hoppy and citrus aromas in beer (*3, 4*), was present at the lowest levels in Simcoe. These results also support the hypothesis that 4MMP content affects the total hop aroma, although aroma simulations recombining the odorants will be required in future research to reveal the contributions to the aroma impact.

**Comparison of 4MMP Content of Hop Pellets Grown in Different Regions.** The concentrations of 4MMP in 17 hop pellet cultivars (**Figure 4a**) were determined using Dowex 1, *p*HMB, and MD-GC-MS with a detection limit of 1.19 $\mu$g/kg pellet; the height of the signal was 3-fold greater than that of noise. The mean values of duplicate analyses are shown in **Figure 4a**.

4MMP was detected only in the hop pellets of U.S., Australian, and New Zealand cultivars. The highest content of 4MMP was observed in the cultivars 2005 Simcoe (111.5 $\mu$g/kg) and Summit (88.2 $\mu$g/kg), followed by Apollo (61.4 $\mu$g/kg), Topaz (48.3 $\mu$g/kg), Cascade (11.1 $\mu$g/kg), U.S. Perle (4.61 $\mu$g/kg), and Pacific Gem (2.51 $\mu$g/kg). Genetic effects on the high concentration of 4MMP were, by necessity, considered as a reason for the difference in the content. Generally the higher contents of 4MMP were observed in the pellets with higher lead conduct value of $\alpha$ acids (**Table 1**). However, even within the same cultivar such as Perle or Nugget, 4MMP was detected only in U.S. rather than German hop pellets. This is likely to be caused by the European use of copper sulfate (Bordeaux mixture) for protection against downy mildew. Thiols, which contain a sulfhydryl group, have been reported to conjugate with copper ion (*7, 25*), and the wine made from grapes treated with the Bordeaux mixture was found to lose its rich aroma (*26*).

In the divalent metal, copper ion is termed as soft metal ion (*25*), which has large acceptor atoms of relatively low positive charge and low electronegativity and contain unshared pairs of electrons in their valence shells. The sulfhydryl group is termed as soft ligands with donor atoms of low electronegativity and high polarizability holding their valence electrons loosely. Soft metal ions preferentially bond to soft ligands (*25*). **Figure 4b** shows the divalent metal ions in hop pellets as measured by ICP-MS. The European cultivars had extremely high copper ion contents, whereas those of the other divalent metal ions were similar among the different varieties. These results imply that high concentrations of copper ions decrease the 4MMP content. Furthermore, it is likely that an undesirable pungent onion-like aroma in specific cultivars, which was recognized in sensory evaluation, is also controlled by the use of copper sulfate.

Even within an identical cultivar with similar copper ion and $\alpha$-acid contents, the 4MMP content could vary between harvest year as in Simcoe hop pellets; the value for the 2006 crop was only one-fifth of that for the 2005 crop (**Figure 4a**). Further research is planned to investigate the reasons for this variation.

In conclusion, it was indicated that the copper ion content could be of particular relevance to the 4MMP content and that there could be a genetic effect on the high content. Future work will include field tests to reveal the effects of copper on thiol contents in hop cones.

## ABBREVIATIONS USED

4MMP, 4-mercapto-4-methylpentan-2-one; AMU, atomic mass unit; EBC, European Brewery Convention; ee, enantiomeric excess; GC, gas chromatography; HV, high voltage; ICP-MS, inductively coupled plasma mass spectroscopy; MD, multidimensional; *p*HMB, *p*-hydroxymercuribenzoate; PTFE, polytetrafluoroethylene; QP, quadrupole; RF, radio frequency; RI, retention index; SBSE, stir-bar sorptive extraction; SIM, select ion monitoring.

## LITERATURE CITED

(1) Steinhaus, M.; Schieberle, P. Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. cultivar spalter select) based on GC-olfactometry and odor dilution techniques. *J. Agric. Food Chem.* **2000**, *48*, 1776–1783.

(2) Kishimoto, T.; Wanikawa, A.; Kagami, N.; Kawatsura, K. Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar-sorptive extraction method with GC-MS. *J. Agric. Food Chem.* **2005**, *53*, 4701–4707.

(3) Kishimoto, T.; Wanikawa, A.; Kono, K.; Shibata, K. Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *J. Agric. Food Chem.* **2006**, *54*, 8855–8861.

(4) Fritsch, H. T.; Schieberle, P. Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian Pilsner-type beer. *J. Agric. Food Chem.* **2005**, *53*, 7544–7551.

(5) Kaltner, D.; Mitter, W. The influence of hops upon the aroma and taste of beer. *Scand. Brew. Rev.* **2003**, *60*, 8–16.

(6) Steinhaus, M.; Wilhelm, W.; Schieberle, P. Comparison of the most odour-active volatiles in different hop varieties by application of a comparative aroma extract dilution analysis. *Eur. Food Res. Technol.* **2007**, *226*, 45–55.

(7) Vermeulen, C.; Lecocq, S.; Collin, S. Polyfunctional thiols and drinkability of beer. *Proc. Eur. Brew. Conv.* **2003**, 91/1–91/11.

(8) EBC−Analytica Microbiologica *J. Inst. Brew.* **1977**, *83*, 109–118.

(9) Meillgard, M. C.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Technique*, 4th ed.; CRC Press: Boca Raton, FL, 1999.
(10) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation − a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.
(11) Tominaga, T.; Dubourdieu, D. A novel method for quantification of 2-methyl-3-furanthiol and 2-furanmethanethiol in wines made from *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **2006**, *54*, 29–33.
(12) Beer flavor analysis with a twist. *GERSTEL Solution Worldwide* **2007**, *7*, 3–7; http://www.gerstel.de/gerstel_magazine.htm (accessed July 10, 2007).
(13) European Brewery Convention. Lead conductance value of hops, powders and pellets, *Analytica−EBC*, 4th ed.; EBC: Zurich, Switzerland, 1987; Hops-7.4.
(14) Tominaga, T.; Baltenweck-Guyot, R.; Peyrot de Gachons, C.; Dubourdieu, D. Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape cultivars. *Am. J. Enol. Vitic.* **2000**, *51*, 178–181.
(15) Tominaga, T.; Masneuf, I.; Dubourdieu, D. Powerful aromatic volatile thiols in wines made from several *Vitis vinifera* grape varieties and their releasing mechanism. *ACS Symposium Series 871*; Shahidi, F., Weerasinghe, D. K., Eds.; American Chemical Society: Washington, DC, 2003; pp 314–337.
(16) Vermeulen, C.; Guyot-Declerck, C.; Collin, S. Combinatorial synthesis and sensorial properties of mercapto primary alcohols and analogues. *J. Agric. Food Chem.* **2003**, *51*, 3623–3628.
(17) Acree T. E. Bioassays in flavor. In *Flavor Science. Sensible Principles and Techniques*; ACS Professional Reference Book; Acree, T. E., Teranishi, R., Eds.; American Chemical Society: Washington, DC, 1993; pp 1–22.
(18) Tominaga, T.; Masneuf, I.; Dubourdieu, D. A *S*-cysteine conjugate, precursor of aroma of white Sauvignon. *J. Int. Sci. Vigne Vin* **1995**, *29*, 227–232.
(19) Swiegers, J. H.; Francis, I. L.; Herderich, M. J.; Pretorius, I. S. Meeting consumer expectations through management in vineyard and winery–the choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon Blanc wine. *Aust. N. Z. Wine Ind. J.* **2006**, *21*, 34–42.
(20) Swiegers, J. H.; Capone, D. L.; Pardon, K. H.; Elsey, G. M.; Sefton, M. A.; Francis, I. L.; Pretorius, I. S. Engineering volatile thiol release in *Saccharomyces cerevisiae* for improved wine aroma. *Yeast* **2007**, *24*, 561–574.
(21) Kishimoto, T.; Wanikawa, A.; Kono, K.; Aoki, K. Odorants comprising hop aroma of beer: hop-derived odorants increased in the beer hopped with aged hops. *Proc. Eur. Brew. Conv.* **2007**, in press.
(22) Steinhaus, M.; Fritsch, H. T.; Schieberle, P. Quantitation of (*R*)- and (*S*)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA). *J. Agric. Food Chem.* **2003**, *51*, 7100–7105.
(23) Schieberle, P.; Steinhaus, M. Characterization of the odor-active constituents in fresh and processed hops (variety: Spalter select). In *Gas Chromatography−Olfactometry: The State of the Art*; ACS Symposium Series 782; Leland, J., Schieberle, P., Buettner, A., Acree, T. E., Eds.; American Chemical Society: Washington, DC, 1999; pp 23–32.
(24) Lam, K. C.; Foster, R. T.; Deinzer, M. L. Aging of hops and their contribution to beer flavor. *J. Agric. Food Chem.* **1986**, *34*, 763–779.
(25) Walker, M. D. The influence of metal ions on concentrations of flavour-active sulphur compounds measured in beer using dynamic headspace sampling. *J. Sci. Food Agric.* **1995**, *67*, 25–28.
(26) Kobayashi, H.; Tominaga, T. New style of Koshu wine -Kiiro-ka-. *J. ASEV Jpn.* **2005**, *16*, 150–151.

**Received for review July 20, 2007. Revised manuscript received November 13, 2007. Accepted November 17, 2007.**

JF072173E