Journal of the American Society of Brewing Chemists

The Science of Beer

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ujbc20

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To cite this article: Toru Kishimoto, Satoko Teramoto, Akiko Fujita & Osamu Yamada (2021): Evaluation of Components Contributing to the International Bitterness Unit of Wort and Beer, Journal of the American Society of Brewing Chemists, DOI: 10.1080/03610470.2021.1878684

To link to this article: https://doi.org/10.1080/03610470.2021.1878684

Published online: 01 Mar 2021.

JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS
https://doi.org/10.1080/03610470.2021.1878684

Evaluation of Components Contributing to the International Bitterness Unit of Wort and Beer

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ABSTRACT

This study evaluated the components that contribute to the International Bitterness Unit (IBU) of wort and beer. A range of samples were analyzed in this study, including beer with 47.5 IBU containing 39.9 ppm iso-α-acid, and one with 49.9 IBU containing only 2.2 ppm iso-alpha acid. The IBU value of wort and beer is mainly contributed by iso-α-acid, non-isomerized α-acid, oxidative polar compounds, and malt-derived polar components. The isomerization of α-acid to iso-α-acid is initiated between 60 °C and 70 °C and the isomerization ratios accelerate rapidly at higher temperature. In the wort boiled below 60 °C, over 50% of the IBU value was contributed by non-isomerized α-acid. Most of the non-isomerized α-acid disappeared during fermentation, whereas the concentration of iso-α-acid was stable. The disappearance of α-acid resulted in a large decrease in the IBU value during fermentation, and the isomerization ratio influenced the degree of the decrease. Over 80% of the IBU value was contributed by the oxidative polar compounds in the beer hopped with the pellet stored at 40 °C for 90 days. Bitterness intensity was less in the beers with deteriorated hops than in those with non-deteriorated hops, despite both having the same IBU values.

KEYWORDS

International bitterness unit; isomerization temperature; oxidative polar compounds; aged hop

Introduction

Hops (*Humulus lupulus L.*) are used in the brewing process to add a characteristic bitterness and distinctive aroma to beer. The bitterness of beer is ascribed to the iso α-acid (IAA) produced by the isomerization of α-acid (AA) during wort boiling and the whirlpool process of brewing. AA is composed of homologues, normal-humulone, co-humulone, and ad-humulone, and IAA is also composed of its corresponding homologues, iso-normal-humulone, iso-co-humulone, and iso-ad-humulone.

Hops are susceptible to oxygen degradation during storage, and oxidative polar compounds (OPCs) are present in freshly baled, aged, or improperly stored hops in varying levels.[1,2] It had been reported that OPCs are composed of many kinds of hop-derived compounds, such as oxidative degradation products of bitter acids, polyphenols, fatty acids and aroma components.[1,3–9] Hao et al. reviewed the structures and suggested the formation mechanism and distribution profiles of a total of 39 oxidative substances, including 15 derived from AA, 15 from IAA, and 9 from β-acid.[10] These components have impacts on the sensory bitterness of beer,[9,11–14] though their contributions vary. It was reported that the bitterness intensity of OPCs were lower,[9,15–18] and Algazzali and Shellhammer reported that oxidized hop acids, hulupones, and humulinones are 84% and 66% as bitter as isohumulones, respectively.[9]

The IBU value is used to express bitterness intensity in beers and is measured by the method defined by the American Society of Brewing Chemists (ASBC)[19] and European Brewery Convention (EBC).[20] In the measurement of the IBU value, components of worts and beers are extracted using 2,2,4-trimethylpentane, and the IBU values are calculated from the absorbance values of the 2,2,4-trimethylpentane layer at 275 nm, as measured by a spectrophotometer. The double bond structures of the compounds in the 2,2,4-trimethylpentane layer absorb ultraviolet (UV) light in the 275 nm region.[21] Although the IBU value is believed to reflect the concentration of IAA, this method measures IAA, AA, β-acid, and OPCs, including polyphenols in beer[9,11–14,22] and the sum of these compounds is then incorporated in the IBU calculation. Hahn et al.[11] examined the components that influence sensory bitterness perception in highly hopped beers and concluded that the IBU cannot predict bitterness in a linear response to sensory perception. They proposed an alternative model for predicting bitterness based on isohumulones, humulinones, and ethanol concentrations.

In this study, worts with different compositions, containing normal and highly oxidized hops, and different degrees of isomerization ratio, were prepared and fermented. Then, the impact of the components contributing to the IBU value of the wort and beer were examined.

In the sensory evaluation, the variations among interindividual panelists are the large issue. Humans perceive at least five taste modalities, as sweet, salty, sour, bitter, and umami.

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Individual differences in taste appear to be caused by physiological differences in the gustatory system, cognitive processing of taste signals in the brain, genetics, or environmental influences, and large variations occur in the perception of bitterness and umami.[23–26] To elucidate the issue, a taste sensing system with less variability was adopted in this study. This particular sensor does not simply respond to a concentration of a substance but to the intensity of the bitterness, and the higher correlation with organoleptic evaluation and the taste sensing system has been previously studied in detail.[27] The bitterness intensity of the finished beers were compared using this instrumental sensory method.

Experimental

Reagents and materials for brewing

HPLC analysis standards of IAA (DCHA - ISO Standard) and AA (ICE-4 Hops Extract Standard) were purchased from the American Society of Brewing Chemists (Minnesota, U.S.A.). The chemicals 2,2,4-trimethylpentane, toluene, 6 mol/L hydrochloric acid, and methanol for HPLC analysis, phosphoric acid, and 10% tetraethylammonium hydroxide solution were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The malt extract was obtained from Coopers Brewery (Australia). Bottom-fermenting sludgy yeast was purchased from the Chuetsu Yeast Co., Ltd. (Niigata, Japan). Hop pellets of Hallertau Magnum, containing 14% α-acid, was purchased from MoreFlavor, Inc. (Pittsburg, CA, U.S.A.). Forcibly deteriorated hops were prepared by storing the Hallertau Magnum hop pellets at 40 °C in opened aluminum bags and sampled at the 3rd, 5th, 7th, 10th, 14th, 21st, 30th, and 90th day from the start of storage.

Small scale brewing processes

Malt extract (250 g) was dissolved in 1.5 L of distilled water. Boiling processes were conducted on a two-liter scale after the adjustment of the pH to 5.1 and the concentration of extract to 11.6%. At the beginning of the wort boiling processes, 2.1 g of hop pellet was added to 1.5 L of wort. The wort was boiled for 60 min with an evaporation rate of 8.0% per hour. Apart from the above boiling experiment, the wort temperatures were kept at 20, 30, 40, 50, 60, 70, 80, and 90 °C for 60 min to examine the isomerization ratio. The worts were then cooled immediately to 4 °C to prevent further isomerization, and the water to replenish the evaporated amount was added after the boiling process.

The wort (850 mL) was fermented on a one-liter scale. The sludgy yeast was washed two times by suspending it in 1 L of unhopped wort and centrifuging at 4700 × *g*. After centrifugation, 4 g of the precipitated yeast was added to 850 mL of hopped wort and fermented for 7 days at 10 °C with stirring at a speed of 1000 rpm. After 7 days of fermentation, the beer was centrifuged at 10,000 × *g* for 30 min. The supernatants were subjected to further analysis. The alcohol content and apparent extracts were analyzed using Alcolyzer Beer ME equipped with DMA4500M density meter (Anton Paar® GmbH, Graz, Austria).

Measurements of IBU values

The IBU values of wort and beers were analyzed according to the method defined by the American Society of Brewing Chemists (Method Beer-23A)[19] and the European Brewery Convention (Method 9.6).[20] To 10 mL of wort or beer, 0.5 mL of 6 mol/L hydrochloric acid and 20 mL of 2,2,4-trimethylpentane were added. The mixture was shaken vigorously for 15 min on a wrist action shaker, and then centrifuged at 1580 × *g* for 5 min at room temperature. The 2,2,4-trimethylpentane layer was subjected to an IBU measurement, followed by HPLC analysis.

The absorbance of the 2,2,4-trimethylpentane layer at 275 nm was measured by a spectrophotometer, using pure 2,2,4-trimethylpentane as a control, and then the IBU values were calculated by multiplying the absorbance by 50.

HPLC analysis of bitter components in beer

The 2,2,4-trimethylpentane layer, filtered with a 0.5 μm PTFE membrane filter, was subjected to HPLC analysis. The HPLC (Shimadzu, Kyoto, Japan) was connected to a UV detector set at 275 nm using a previously described method with slight modifications.[7] The mobile phase was prepared as follows: 2800 mL of methanol was added to 1200 mL of pure distilled water containing 150 mL of 10% tetraethylammonium hydroxide solution and 150 g of phosphoric acid. Twenty microliters of sample volume was injected, and the separation was performed on a Sim-pack CLC-ODS/H column (250 mm length x 4.6 mm i.d.; Shimadzu) set at 40 °C with a flow rate of 1.0 mL/min. The analytical HPLC column was washed using pure distilled water and 0.05% phosphoric acid for every analysis. The concentrations of AA and IAA were calculated with the absolute calibration curve method using the areas of each analytical peak and those of the authentic standards, DCHA-ISO Standard and ICE-4 Hops Extract Standard.

HPLC analysis of bitter components in hop pellets

The bitter components were extracted from 5 g of milled hop pellet using 100 mL of toluene for 2 h at room temperature. Thereafter, 50 μL of each toluene extract was evaporated and dried under the flow of N_2 gas in a tube. Then 2500 μL of methanol was added to the tube to dissolve the bitter compounds.

The methanol layer, filtered with a 0.5 μm PTFE membrane filter, was analyzed using an HPLC system (Shimadzu, Kyoto, Japan) connected to a UV detector set at 302 nm. The mobile phase was prepared as follows: 800 mL of methanol was added to 200 mL of pure distilled water, containing 29.5 mL of 10% tetraethylammonium hydroxide solution and 17 g of phosphoric acid. Ten μL of

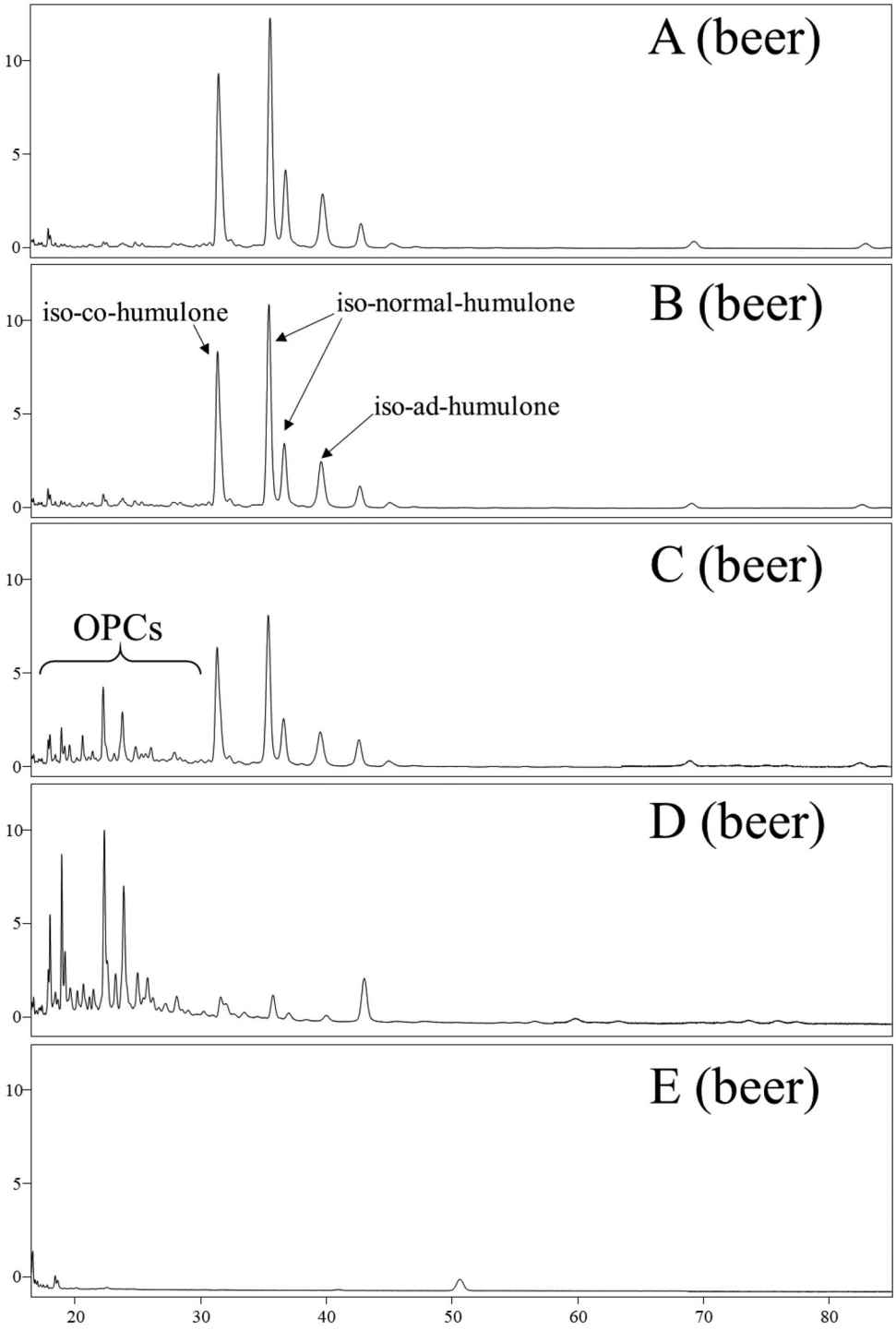

Figure 1. HPLC chromatograms of the beer extracts with (A) normally stored hop pellet at 4 °C, (B) hop pellets stored at 40 °C for 10 days, (C) 21 days, (D) 90 days, and (E) unhopped beer.

sample was injected and separation was performed on a Sim-pack CLC-ODS/H column (250 mm length x 4.6 mm i.d.; Shimadzu) set at 40 °C with a flow rate of 1.0 mL/min. The analytical HPLC column was washed using pure distilled water and 0.05% of phosphoric acid for every analysis. The concentrations of AA and IAA were calculated by the absolute calibration curve method using the areas of each analytical peak and those of the authentic standards, ICE-4 Hops Extract Standard.

Measurement of bitterness intensity using a taste sensing system

Prior to analysis by the taste sensing system, the IBU values of all the sample beers were adjusted to 40 IBU using unhopped beer. Bitterness intensity values were measured by the SA402 taste sensing system (Intelligent Sensor Technology, Inc., Kanagawa, Japan), fitted with a sensor probe for bitter taste (SB2C00) and a reference probe.[28] The taste sensor probe consisted of a polymer membrane, an Ag/AgCl electrode, and

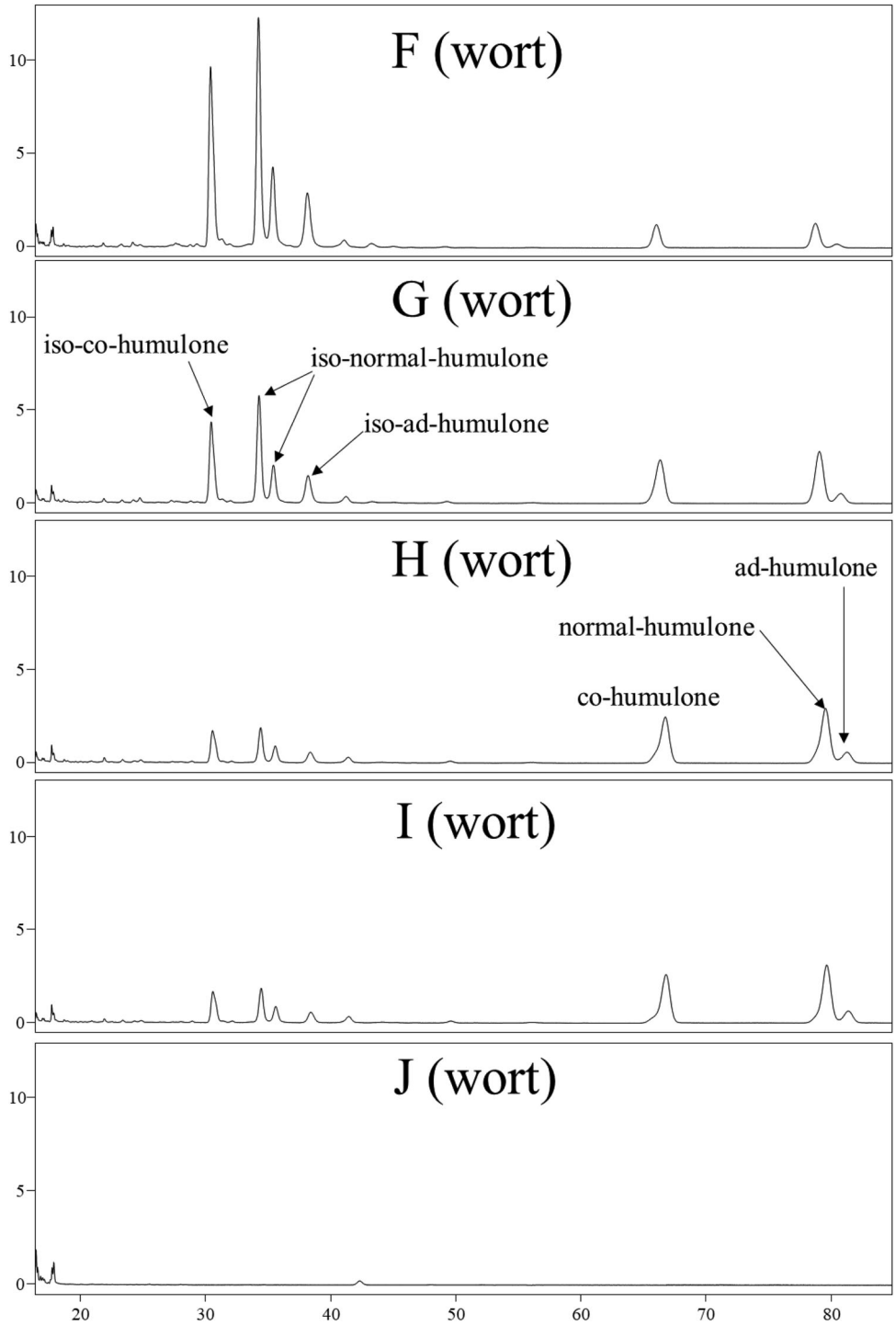

Figure 2. HPLC chromatograms of the wort extracts (F) boiled at 100 °C for 60 min, (G) kept at 80 °C, (H) 50 °C, (I) 20 °C for 60 min after the addition of normally stored hop pellet, and (J) unhopped wort.

an internal cavity filled with 3.3 M KCl aqueous solution saturated with AgCl. The polymer membrane of the bitter taste sensor was composed of tetradodecylammonium bromide, 2-nitrophenyl octyl ether, and poly vinyl chloride. The reference probe consisted of a liquid junction made with ceramics, an Ag/AgCl electrode, and an internal cavity filled with 3.3 M KCl aq. solution, saturated with AgCl.

The sensor measurement was automatically carried out at 25 °C. The taste sensor probe and the reference probe were dipped into sample solutions or standard substance solutions for 30 s to detect the change in membrane potential. The bitter taste intensity of the sample solution was defined as the difference between the change in membrane potential in the sample solution and that in the reference solution, generated by taste substances adsorbed on the polymer membrane of the sensor probe. The value of each sample was obtained from the average of three measurements.

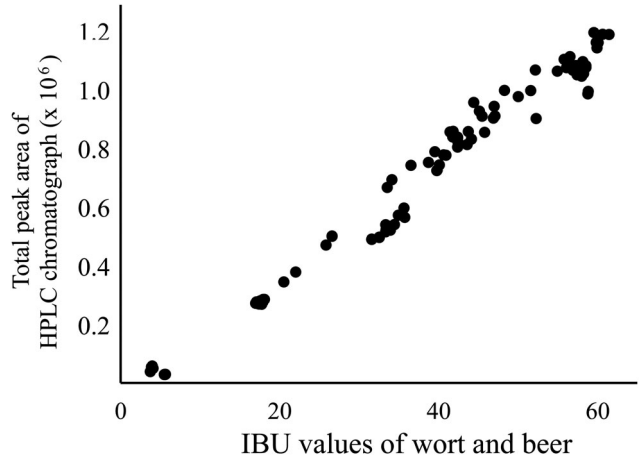

Figure 3. Correlations between the total peak areas of HPLC chromatograms and IBU values of wort and beer extracts.

Results and discussion

Components contributing to the IBU value

Figure 1 and Figure 2 show the HPLC chromatograms of the components in 2,2,4-trimethylpentane layers that contribute to the IBU value of beer. No chromatographic peaks were detected outside the chromatographic time. Figure 3 shows the correlation between the total peak areas of the HPLC chromatograms and the IBU values of wort and beer. The total peak areas were proportional to the IBU value of wort and beer as shown in Figure 3, indicating that the IBU values were attributed to the components included in the chromatogram in Figure 1 and 2.

The peaks of IAA (iso-co-humulone, iso-normal-humulone, and iso-ad-humulone) and AA (co-humulone, normal and ad-humulone) were identified by comparing those of authentic compounds. As shown in Figure 1(C and D), the fractions of polar components that appeared before the peak of iso-co-humulone increased as hop deterioration progressed and were identified as OPCs in previous reports.[7,8] As shown in Table 1, unhopped beer and wort have IBU values of 3.7 and 5.6, respectively, and slight amounts of malt-derived polar components contributed to the IBU value, as depicted in Figure 1(E) and 2(J). Thus, the IBU values of wort and beer were mainly attributed to IAA, AA, OPCs, and malt-derived polar components. Almost all the AA disappeared during fermentation (Figures 1 and 2).

The effect of non-isomerized α-acid during fermentation

The contribution of non-isomerized AA to the IBU value of wort and beers has been described above. Worts with different isomerization ratios were prepared by changing the isomerization temperature. The hopped wort was kept at 20, 30, 40, 50, 60, 70, 80, 90, and 100 °C for 60 min.

Table 1 shows the concentrations of IAA and AA in wort and beer produced at different boiling temperatures. Figure 4 shows the correlation between the boiling temperature and isomerization ratio of AA in worts. The isomerization ratio was calculated by the formula; IAA (mg)/(AA (mg) + IAA (mg)), as the ratio of the homologues (normal, co, ad-form)

Table 1. Concentrations of iso-α-acid and α-acid in wort and beer produced at different boiling temperatures.

	Wort				Beer						
Boiling temperature (°C)	IBU	IAA (ppm)	AA (ppm)	Contribution (%) of AA to IBU value	IBU	IBU decrease during fermentation (%)	IAA (ppm)	AA (ppm)	Contribution (%) of AA to IBU value	Alcohol (%)	Apparent extract (%)	pH
20	33.7	6.9	27.3	56.5	19.1	43.5	7.2	3.3	14.6	4.6	2.4	4.2
30	33.4	7.0	27.5	56.2	19.1	42.7	6.9	3.2	14.6	4.6	2.3	4.2
40	33.4	6.6	26.9	56.8	18.7	44.2	7.1	3.2	14.3	4.6	2.3	4.2
50	34.5	7.3	26.8	55.1	19.5	43.5	7.4	3.3	14.2	4.6	2.3	4.2
60	35.8	8.7	26.2	51.6	20.0	44.2	7.3	3.4	15.6	4.6	2.4	4.2
70	35.7	11.8	23.6	44.0	22.6	36.7	11.1	4.2	14.0	4.5	2.4	4.2
80	39.9	18.7	23.2	35.8	27.8	30.2	17.2	4.1	10.0	4.6	2.4	4.2
90	50.1	34.4	18.1	21.2	38.4	23.3	28.5	2.8	4.8	4.5	2.3	4.2
100	55.8	40.1	10.3	11.9	47.5	14.9	39.9	2.5	3.1	4.7	2.4	4.2
unhopped	5.6	0	0	-	3.7		0	0	-	4.8	2.4	4.1

Wort was kept at each temperature for 60 min, after the adjustment of pH to 5.1 and apparent extract to 11.6.
The contribution (%) of AA to IBU value was calculated from the peak area of HPLC chromatograms.

and the molecular weight of the AA does not change after the isomerization. As shown in Figure 4 and Table 1, the isomerization ratio and IAA concentration of beer increased from the boiling temperature of 60 °C to 70 °C and the increase in ratio is accelerated at higher temperatures. The wort kept at 20 °C already contained 6.9 ppm of IAA, which was assumed to have been derived from the drying process or pelletizing process of hop cones as no IAA was detected from the undried fresh hop cone (data not shown).

As shown in Table 1, for the wort boiled at 20 °C to 60 °C, over 50% of the IBU value was contributed by the AA content. Larger decreases in IBU values were observed during fermentation in the wort with the lower isomerization ratio, as depicted in Figure 1(A, F), and Table 1. The concentration of AA in each wort dramatically decreased during fermentation as in Table 1, although those of IAA were stable before and after fermentation. Thus, the decrease in the IBU value during fermentation was mainly caused by the disappearance of AA, and the isomerization ratio influenced the decrease in the IBU value during fermentation. The reasons for the disappearance of hydrophobic components are discussed in the literature.[29–32] As the pH decreases during fermentation, hydrophobic components such as AA become insoluble in the beer. These hydrophobic components also adhere to yeast cell walls; moreover, the degree of adsorption to the cell surface depends on the degree of hydrophobicity of the cell surface.[31]

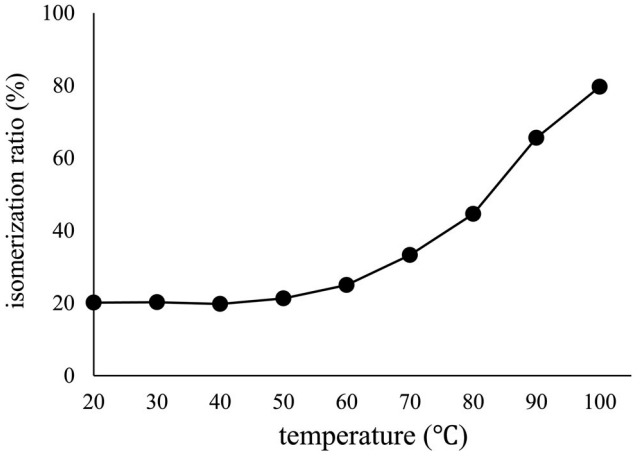

Figure 4. Correlations between boiling temperatures and isomerization ratio of α-acid. The wort was kept at each temperature for 60 min after the addition of hops. Isomerization ratios were calculated by the formula; iso-α-acid (ppm)/(iso-α-acid (ppm) + α-acid (ppm)).

Figure 4 shows that isomerization started at a temperature between 60 °C and 70 °C. This indicates that isomerization progresses not only in the wort boiling process, but also in the subsequent whirlpool process, wherein the wort is exposed to higher temperatures. In order to control the bitterness value of the final beer product, the time spent in the whirlpool rest process should also be considered.

Content of OPCs in beer depends on the degradation of used hops

The contribution of OPCs to the IBU values of wort and beers are as mentioned earlier. Worts with different ratios of OPCs were prepared using hops with different degrees of oxidization. Hop pellets were stored at 40 °C in order to prepare forcibly deteriorated hops, and they were sampled at the 3rd, 5th, 7th, 10th, 14th, 21st, 30th, and 90th day from the start of storage.

Table 2 shows the concentration of the bitter components in beer hopped with forcibly deteriorated pellet. As shown in Table 2 and Table 3, as the storage period of the hopped pellet became longer, the AA content in the hop pellet, and the corresponding IAA in beer, decreased, and

Table 2. Content of bitter components of beer hopped with forcibly deteriorated hop pellet.

Storage periods of hop pellet at 40 °C	α-acid (%) content in hopped pellet	IBU of finished beer	iso-co-humulone (ppm)	iso-normal-humulone (ppm)	iso-ad-humulone (ppm)	co-humulone (ppm)	normal, ad-humulone (ppm)
0 day	14.0	47.5	12.9	21.6	5.3	1.3	1.2
3 days	13.4	48.2	11.4	19.1	4.6	0.8	0.8
5 days	12.8	48.8	11.8	19.3	4.6	1.0	0.9
7 days	12.1	47.1	12.4	20.4	4.9	0.9	0.8
10 days	10.9	49.9	11.4	18.6	4.6	0.9	0.9
14 days	9.8	48.9	11.0	17.9	4.4	0.8	0.7
21 days	4.4	54.7	6.6	10.5	2.6	0.4	0.4
30 days	1.2	51.1	3.4	5.1	1.3	0.1	0.1
90 days	0.0	49.9	1.0	1.0	0.2	0.0	0.0

The wort, including 2.1 g of each stored hop pellet, was boiled for 60 min and fermented for 7 days.

Table 3. Contributions (%) to IBU value of beer calculated from peak area of HPLC chromatograms.

Storage periods of hop pellet at 40 °C	Malt-derived compounds (%)	Oxidative polar components (%)	iso-α-acid (%)	α-acid (%)
0 day	7.7	8.4	80.7	3.3
3 days	7.6	9.3	80.7	2.4
5 days	7.5	9.5	80.3	2.8
7 days	7.8	10.7	79.2	2.3
10 days	7.3	12.2	78.0	2.5
14 days	7.5	13.6	76.7	2.3
21 days	6.7	36.9	55.0	1.4
30 days	7.1	59.1	33.5	0.3
90 days	7.3	83.4	9.3	0.0

The contribution was calculated from the ratio of the area of each peak to the total peaks.

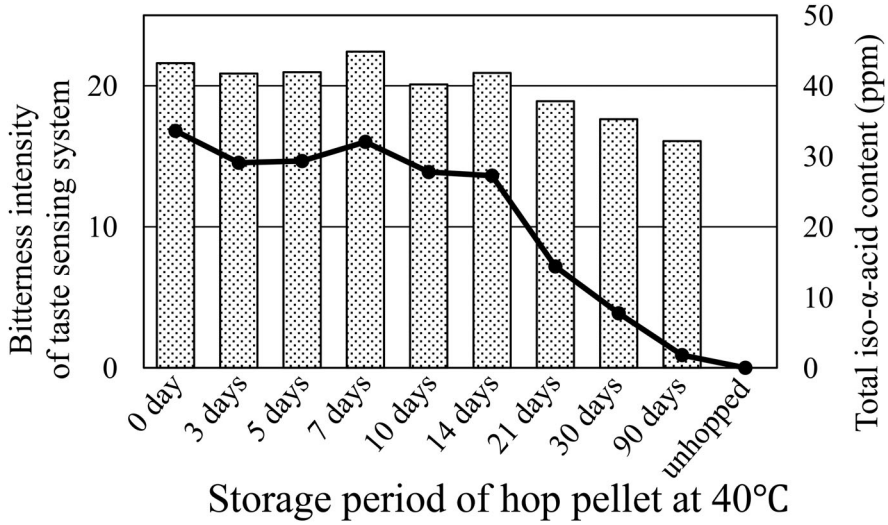

Figure 5. The bitterness intensity evaluated by taste sensing system and total iso-α-acid content. The IBU values of all beers were adjusted to the same 40 IBU using unhopped beer and were subjected to taste sensing analysis. The bar graph shows the bitterness intensity by the taste sensing system, and the line graph shows the iso-alpha-acid content (ppm) after adjusting the bitterness value to 40 IBU.

the ratio of the OPC (%) in the total peak area of the chromatogram of beer, increased. However, the IBU values of the beer did not vary, as it depended on the storage periods of the hopped pellet, and the contents of IAA and OPCs did not decrease during fermentation to contribute to the IBU values of beer. It should be noted that the beer derived from the hop, which was forcibly deteriorated at 40 °C for 90 days, contained only 2.2 ppm of IAA and an IBU value of 49.9. The beer with normally stored hop had almost the same IBU value and contained 39.9 ppm of IAA.

Evaluation of bitterness intensity of beers with forcibly deteriorated hop pellets using a taste sensing system

The intensity of bitterness is expected to vary, even in beers with the same IBU values: while some beers have IBU values mainly composed of IAA, the others have IBU values mainly composed of OPCs. The bitterness intensity of the beers hopped with oxidized hops was evaluated using an electronic tongue, a taste sensing system, after the adjustment of their IBU value to 40 using unhopped beer.

The taste sensing system is an instrumental method for evaluating the taste attributes of food and beverages, such as beer.[27,28,33–37] The electronic tongue of the taste sensing system consists of sensor electrodes with lipid/polymer membranes prepared for each primary taste.[28] The taste intensities are recorded as membrane potential changes caused by the interaction between the lipid/polymer membranes and the taste substances. A difference of 0.5 in the response value between samples, from the taste sensing system, can be recognized by a panelist who is accustomed to drinking beer. If there is a difference of 1.0 in the response value between samples, ordinary people can recognize the difference in bitterness intensity.[27]

Figure 5 shows the bitterness intensity evaluated by the taste sensing system and the total IAA content. The bitterness intensity largely decreased in the beer with hops stored for 21, 30, and 90 days, where the total IAA also decreased markedly. It was found that the bitterness intensities of the beer composed of larger ratios of OPCs were lower. Our observations are consistent with previous reports that beers made with deteriorated hops have less bitterness than that expected from the IBU values.[9,22] This is because the bitterness intensities derived from OPCs are lower as the threshold levels of OPCs are generally higher.[9,15–18]

Conclusions

The IBU value had been believed to reflect the concentration of iso-α-acid, however, in the current study, samples with disparate iso-α-acid content were analyzed: one was a beer sample with 47.5 IBU containing 39.9 ppm iso-α-acid, and the other was a beer sample with 49.9 IBU containing only 2.2 ppm iso-alpha acid. The IBU value of the wort and beer is mainly contributed by iso-α-acid, non-isomerized α-acid, OPCs, and malt-derived polar components. It was observed that the isomerization of α-acid to iso-α-acid was initiated between 60 °C to 70 °C and the isomerization ratios accelerated rapidly at higher temperatures. In the wort boiled at temperatures below 60 °C, over 50% of the IBU value was contributed by non-isomerized α-acid, and most of the non-isomerized α-acid disappeared during fermentation. However, the concentration of iso-α-acid was stable. Thus, the disappearance of the α-acid resulted in the larger decrease in IBU value during fermentation, and the isomerization ratio influenced the degree of the decrease.

The OPCs that increase with the deterioration of hop contribute to the IBU value of beer. When non-deteriorated hop with small amounts of OPCs were used in brewing, the IBU was mainly contributed by IAA and only 8.4% was contributed by the OPCs. However, in the beer hopped with the pellet stored at 40 °C for 90 days, over 80% of the IBU value was contributed by the OPCs. The bitterness intensity of beers hopped with deteriorated hops were examined using the taste sensing system. The beers with the deteriorated

hop had lesser bitterness intensity, despite having the same IBU values as the beer with non-deteriorated hop.

Disclosure statement

No potential competing interest was reported by the authors.

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Literature cited

[1] Maye, J. P.; Smith, R.; Leker, J. Humulinone Formation in Hops and Hop Pellets and Its Implications for Dry Hopped Beers. *Tech. Q. Master Brew. Assoc. Am.* **2016**, *53*, 23–27.

[2] Oladokun, O.; Cowley, S. J.; Smart, K.; Hort, J.; Cook, D. Dry-Hopping: The Effects of Temperature and Hop Variety on the Bittering Profiles and Properties of Resultant Beers. *BrewingScience.* **2017**, *70*, 187–196.

[3] Haseleu, G.; Lagemann, A.; Stephan, A.; Intelmann, D.; Dunkel, A.; Hofmann, T. Quantitative Sensomics Profiling of Hop-Derived Bitter Compounds throughout a Full-Scale Beer Manufacturing Process. *J Agric Food Chem.* **2010**, *58*, 7930–7939. DOI: 10.1021/jf101326v.

[4] Dresel, M.; Dunkel, A.; Hofmann, T. Sensomics Analysis of Key Bitter Compounds in the Hard Resin of Hops (*Humulus lupulus L.*) and Their Contribution to the Bitter Profile of Pilsner-Type Beer. *J Agric Food Chem*. **2015**, *63*, 3402–3418. DOI: 10.1021/acs.jafc.5b00239.

[5] 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. In *Proc. Eur. Brew. Conv. Congr.Venice, Italy*. CD Fachverlag Hans Carl: Nürenberg, **2007**; pp 226–235.

[6] Taniguchi, Y.; Matsukura, Y.; Ozaki, H.; Nishimura, K.; Shindo, K. Identification and Quantification of the Oxidation Products Derived from α-Acids and β-Acids during Storage of Hops (*Humulus lupulus* L.). *J Agric Food Chem*. **2013**, *61*, 3121–3130. DOI: 10.1021/jf3047187.

[7] Ono, M.; Kakudo, Y.; Nagami, K.; Kumada, J.; Ueda, S. Simultaneous Analysis of Hop Bittering Components by High-Performance Liquid Chromatography. III. Development of on-Line Sample Enrichment by Precolumn Switching. *J. Am. Soc. Brew. Chem*. **1987**, *45*, 70–76. DOI: 10.1094/ASBCJ-45-0070.

[8] Kowaka, M.; Fukuoka, Y.; Kawasaki, H.; Asano, K. The True Value of Aroma Hops in Brewing. In *Proc. Eur. Brew. Conv. Congr., London UK*. IRL Press: Oxford, **1983**; pp 71–78.

[9] Algazzali, V. A.; Shellhammer, T. H. Bitterness Intensity of Oxidized Hop Acid: Humulinones and Hulupones. *J. Am. Soc. Brew. Chem*. **2016**, *74*, 36–43.

[10] Hao, J.; Speers, R. A.; Fan, H.; Deng, Y.; Dai, Z. A Review of Cyclic and Oxidative Bitter Derivatives of Alpha, Iso-Alpha and Beta-Hop Acid. *J. Am. Soc. Brew. Chem*. **2020**, *78*, 89–102. DOI: 10.1080/03610470.2020.1712641.

[11] Hahn, C. D.; Lafontaine, S. R.; Pereira, C. B.; Shellhammer, T. H. Evaluation of Nonvolatile Chemistry Affecting Sensory Bitterness Intensity of Highly Hopped Beers. *J. Agric Food Chem*. **2018**, *66*, 3505–3513. DOI: 10.1021/acs.jafc.7b05784.

[12] Malowicki, M. G.; Shellhammer, T. H. Isomerization and Degradation Kinetics of Hop (*Humulus lupulus*) acids in a model wort-boiling system. *J. Agric Food Chem*. **2005**, *53*, 4434–4439. DOI: 10.1021/jf0481296.

[13] Maye, J. P.; Smith, R. Dry Hopping and Its Effects on the International Bitter Unit Test and Beer Bitterness. *Tech. Q. Master Brew. Assoc. Am*. **2016**, *53*, 134–136.

[14] Shellhammer, T. H.; McLaughlin, I. R.; Lederer, C. Bitterness Modifying Properties of Hop Polyphenols Extracted from Spent Hop Material. *J. Am. Soc. Brew. Chem*. **2008**, *66*, 174–183. DOI: 10.1094/ASBCJ-2008-0619-01.

[15] Intelmann, D.; Kummerlowe, G.; Haseleu, G.; Desmer, N.; Schulze, K.; Frohlich, R.; Frank, O.; Luy, B.; Hofmann, T. Structures of Storage-Induced Transformation Products of the Beer's Bitter Principles, Revealed by Sophisticated NMR Spectroscopic and LC-MS Techniques. *Chemistry*. **2009**, *15*, 13047–13058. DOI: 10.1002/chem.200902058.

[16] Haseleu, G.; Intelmann, D.; Hofmann, T. Identification and RP-HPLC-ESI-MS/MS Quantitation of Bitter-Tasting Beta-Acid Transformation Products in Beer. *J. Agric. Food Chem*. **2009**, *57*, 7480–7489. DOI: 10.1021/jf901759y.

[17] Intelmann, D.; Batram, C.; Kuhn, C.; Haseleu, G.; Meyerhof, M.; Hofmann, T. Three TAS2R Bitter Taste Receptors Mediate the Psychophysical Responses to Bitter Compounds of Hops (*Humulus lupulus L.*) and Beer. *Chem. Percept.* **2009**, *2*, 118–132. DOI: 10.1007/s12078-009-9049-1.

[18] Haseleu, G.; Intelmann, D.; Hofmann, T. Structure Determination and Sensory Evaluation of Novel Bitter Compounds Formed from b-Acid of Hop (*Humulus lupulus L.*) upon Wort Boiling. *Food Chem*. **2009**, *116*, 71–81. DOI: 10.1016/j.foodchem.2009.02.008.

[19] American Society of Brewing Chemists. *Method Beer-23A, Methods of Analysis of the ASBC*, 8th ed.; Minnesota: American Society of Brewing Chemists, **1992**.

[20] European Brewery Convention. *Analytica-EBC Method 9.6*, 4th ed.; Brauerei- und Getränke-Rundschau: Zurich, Switzerland. **1987**; E155.

[21] University of Toronto Scarborough. Interpreting UV-Vis Spectra, In *Chemistry Online*, https://www.utsc.utoronto.ca/webapps/chemistryonline/production/uv/uv_spectra.php.

[22] Peacock, V. The International Bitterness Unit, Its Creation and What It Measures. In *Hop Flavor and Aroma, Proceedings of the 1st International Brewers Symposium,* Master Brewers Association of the Americas: Minnesota, **2009**; pp 157–166.

[23] Puputti, S.; Aisala, H.; Hoppu, U.; Sandell, M. Factors Explaining Individual Differences in Taste Sensitivity and Taste Modality Recognition among Finnish Adults. *J. Sens. Stud*. **2019**, *34*, e12506. DOI: 10.1111/joss.12506.

[24] Knaapila, A.; Hwang, L. D.; Lysenko, A.; Duke, F. F.; Fesi, B.; Khoshnevisan, A.; James, R. S.; Wysocki, C. J.; Rhyu, M.; Tordoff, M. G.; et al. Genetic Analysis of Chemosensory Traits in Human Twins. *Chem. Senses*. **2012**, *37*, 869–881. DOI: 10.1093/chemse/bjs070.

[25] Lugaz, O.; Pillias, A.-M.; Faurion, A. A. A New Specific Ageusia: Some Humans Cannot Taste L-Glutamate. *Chem. Senses*. **2002**, *27*, 105–115. DOI: 10.1093/chemse/27.2.105.

[26] Puputti, S.; Aisala, H.; Hoppu, U.; Sandell, M. Multidimensional Measurement of Individual Differences in Taste Perception. *Food Qual. Pref*. **2018**, *65*, 10–17. DOI: 10.1016/j.foodqual.2017.12.006.

[27] Kobayashi, Y.; Habara, M.; Ikezazki, H.; Chen, R.; Naito, Y.; Toko, K. Advanced Taste Sensors Based on Artificial Lipids with Global Selectivity to Basic Taste Qualities and High Correlation to Sensory Scores. *Sensors (Basel)*. **2010**, *10*, 3411–3443. DOI: 10.3390/s100403411.

[28] Toko, K. Taste Sensors. In *Biomimetic Sensor Technology*; Cambridge University Press: Cambridge, UK, **2000**; pp 113–180.

[29] Popescu, V.; Soceanu, A.; Dobrinas, S.; Stanciu, G. A Study of Beer Bitterness Loss during the Various Stages of the Romanian Beer Production Process. *J. Inst. Brew.* **2013**, *119*, 111–115. DOI: 10.1002/jib.82.

[30] 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.

[31] Kishimoto, T.; Uemura, K.; Aizawa, M. A New Insight for Controlling the Hop Aroma Using Hydrophobicity of Yeast Cell Surface. In *Proc. Eur. Brew. Conv. Congr., Luxembourg*, CD Fachverlag Hans Carl: Nürenberg, **2013**. DOI: 10.13140/RG.2.2.34844.64643.

[32] Haslbeck, K.; Bub, S.; Schönberger, C.; Zarnkow, M.; Jacob, F.; Coelhan, M. On the Fate of β-Myrcene during Fermentation – The Role of Stripping and Uptake of Hop Oil Components by Brewer's Yeast in Dry-Hopped Wort and Beer. *Brew. Sci.* **2017**, *70*, 159–169.

[33] Ezaki, S.; Yuki, T.; Toko, K.; Tsuda, Y.; Nakatani, K. Analysis of Taste Qualities and Ingredients of Beer by Taste Sensing System. *IEEJ Transsm.* **1997**, *117*, 449–455. DOI: 10.1541/ieejsmas.117.449.

[34] Kobayashi, Y.; Hamada, H.; Yamaguchi, Y.; Ikezaki, H.; Toko, K. Development of an Artificial Lipid-Based Membrane Sensor with High Selectivity and Sensitivity to the Bitterness of Drugs and with High Correlation with Sensory Score. *IEEJ Trans. Elec. Electron. Eng.* **2009**, *4*, 710–719. DOI: 10.1002/tee.20469.

[35] Takagi, S.; Toko, K.; Wada, K.; Ohki, T. Quantification of Suppression of Bitterness Using an Electronic Tongue. *J. Pharm. Sci.* **2001**, *90*, 2042–2048. DOI: 10.1002/jps.1155.abs.

[36] Hayashi, N.; Ujihara, T.; Chen, R.; Irie, K.; Ikezaki, H. Objective Evaluation Methods for the Bitter and Astringent Taste Intensities of Black and Oolong Teas by a Taste Sensor. *Food Res. Int* **2013**, *53*, 816–821. DOI: 10.1016/j.foodres.2013.01.017.

[37] Hayashi, N.; Chen, R.; Hiraoka, M.; Ujihara, T.; Ikezaki, H. Beta-Cyclodextrin/Surface Plasmon Resonance Detection System for Sensing Bitter-Astringent Taste Intensity of Green Tea Catechins. *J Agric Food Chem.* **2010**, *58*, 8351–8356. DOI: 10.1021/jf1012693.