Foam-Stabilizing Effects and Cling Formation Patterns of Iso-α-acids and Reduced Iso-α-acids in Lager Beer

TAKESHI KUNIMUNE AND THOMAS H. SHELLHAMMER*

Department of Food Science and Technology, Oregon State University, 100 Wiegand Hall, Corvallis, Oregon 97331

Foam-stabilizing properties and cling formation patterns of iso-α-acids and reduced iso-α-acids were investigated using an unhopped lager beer. Unhopped beer was dosed with iso-α-acid (Iso), rho-iso-α-acid (Rho), tetrahydro-iso-α-acid (Tetra), and hexahydro-iso-α-acid (Hexa), separately, over a range of concentrations from 2 to 10 ppm. A uniform foam was created by Inpack 2000 Flasher Head and was measured by a NIBEM Foam Stability Tester (NIBEM-TPH) followed by a NIBEM Cling Meter (NIBEM-CLM) to determine the relationship between the concentration and NIBEM-30 and the cling formation ability of each compound. The foam-stabilizing power was determined to be Tetra, Hexa, Iso, and Rho from the strongest to weakest. Linear regression models were created using the NIBEM-TPH data set, and on the basis of 95% confidence intervals, the foam stability of Tetra or Hexa became significantly larger than that of Iso when 2.4 or 4.2 ppm of Tetra or Hexa was used as a replacement for Iso, respectively. Cling formation patterns could be categorized into three groups: "ring", "mesh", and "powdery". The control beer had the lowest foam stability and did not yield any foam cling.

KEYWORDS: Hop acid; iso-α-acid; tetrahydro-iso-α-acid; hexahydro-iso-α-acid; rho-iso-α-acid; beer; foam; cling; foam stability; NIBEM

INTRODUCTION

Foam is an important quality for beer aesthetics. Consumers judge foam quality by comparing attributes such as head retention, quantity, texture (density), cling (adherence to glass), whiteness, bubble size, and viscosity (*1–5*). Furthermore, beer foam preferences vary tremendously by multiple factors such as gender, nationality, and race (*2, 5*). Generally speaking, superior foam can be defined as foam with uniform, small, spherical bubbles that persist for a desirably long period of time and leave a pleasant adherence to glass (*6*). There are multiple variables for brewers to manipulate in order to achieve this type of foam. Factors such as the balance of foam-positive proteins from barley malts, the levels of iso-α-acids (Iso) from hops, alcohol content, divalent metal cations, and minimal existence of lipids are believed to play significant roles in determining the quality of foam (*1, 7–14*).

Isos play an important role for the quality of foam since they not only stabilize the head retention but also create lacing (*11, 14*). The absence of Isos results in a beer where negligible foam adheres to the inner glass sidewalls (*12, 15, 16*).

Isos are photosensitive compounds. In the presence of riboflavin, they can undergo UV photolysis to create a prenyl mercaptan, which is an off-flavor often described as "light-

struck" and/or "skunk(y)" (*10*). To prevent this reaction from occurring in finished beer, chemically reduced Isos were created (**Figure 1**). These materials are produced either via hydrogenation for tetrahydro-iso-α-acid (Tetra), reduction with sodium borohydride rho-iso-α-acid (Rho), or both hydrogenation and reduction for hexahydro-iso-α-acid (Hexa). Chemical reduction
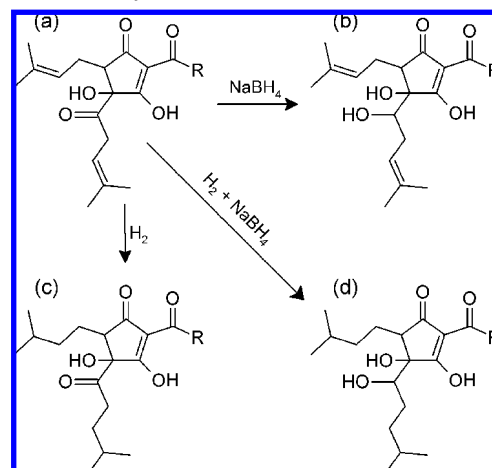

Figure 1. Isos (**a**) and their reduced derivatives, Rhos (**b**), hydrogenated derivatives, Tetras (**c**), and hydrogenated + reduced derivatives, Hexas (**d**). Side groups (R) designated as humulone [$CH_2CH(CH_3)_2$], cohumulone [$CH_2CH_3)_2$], and adhumulone [$CH(CH_3)CH_2CH_3$].

* To whom correspondence should be addressed. Tel: 541-737-9308. Fax: 541-737-1877. E-mail: tom.shellhammer@oregonstate.edu.

8630 *J. Agric. Food Chem.,* Vol. 56, No. 18, 2008

Kunimune and Shellhammer

Table 1. Concentrations of Reduced and Nonreduced Isos in the Commercial Hop Products Used to Prepare Beer Samples

	concentrations (% w/w)	
product	UV photometric method (*26*)	HPLC method (*20, 27*)
Iso	31.1	29.7
Tetra	11.6	10.0
Hexa	12.2	10.3
Rho	35.9	29.9

or hydrogenation effectively alters their photoreactivity such that the "skunky" aroma (3-methyl-2-butene-1-thiol) is not produced when beer made with these compounds is exposed to UV light (*17, 18*). The concentration of Isos in commercial beer is influenced by the beer style and ultimately the hopping rate and regime. Commercial U.S. lager beer will have from 4 to 20 ppm Iso, international lagers range from 10 to 30 ppm, and European Pilsners range from 25 to 40 ppm Iso. U.S. and British ales typically range from 15 to 35 ppm, although some U.S. specialty ales and British Bitters will have greater than 50 ppm. Tetra and Hexa are used at much lower levels, in the range of 2−5 ppm and most often in combination with each other or Rho. Because of its reduced bitterness (*19*), Rho is used at higher levels, up to 55 ppm in European lager beer.

The objective of this study was to determine the effects of Iso and three chemical derivatives, Rho, Tetra, and Hexa, on the foam stability and cling formation over a range of concentrations in lager beer using the NIBEM Foam Stability Tester (NIBEM-TPH) and NIBEM Cling Meter (NIBEM-CLM). Another aim of this investigation was to characterize qualitative differences using digital photography of cling patterns.

MATERIALS AND METHODS

Preparation of Beer. Unhopped lager beer was brewed using a single temperature infusion mash at 68 °C for 45 min of pale two-row malted barley (Great Western Malting Co., Vancouver, WA). Rice syrup solids (California Natural Products) were added during the kettle boil such that they contributed 25% of the soluble extract, while the malted barley contributed 75% of the extract. No hop material was added to the wort during boiling or prior to fermentation. At the conclusion of a hour boil, the wort was cooled to fermentation temperature (14.5 °C), oxygenated, and pitched with hop acid-free lager yeast (Yeast Strain 2007: Pilsner Lager, Wyeast Laboratories, Inc., Odell, OR) at a concentration of 12×10^7 viable cells per mL wort. The original gravity and final gravity were 11.7 and 3.5 °P, respectively. Following fermentation at 14.5 °C for 2 weeks, the temperature was dropped to 1.7 °C, and 35 g/hL polyvinyl polypyrrolidone (Polyclar VT, International Specialty Products) was added to remove polyphenols that could cause haze. The beer was pumped into a sterile, CO_2-charged conical fermenter and held for another week at 1.7 °C before filtration. The beer was filtered through a 1.2 μm nominal filter pad (Cellupore, 1940SD grade, Gusmer Cellulo Co.), dosed with high levels of Iso (57 ppm; John I. Haas, Yakima, WA), Rho (55 ppm; John I. Haas), Tetra (24 ppm; John I. Haas), and Hexa (44 ppm; John I. Haas) and then refiltered to remove insoluble hop acids. The Iso, Rho, and Tetra samples were commercially available products, while Hexa was a purified preparation in an aqueous solution (courtesy of John I. Haas). All products contained only the particular hop acid in question and were not blends of different types of reduced hop acids. The purity of each product was verified by comparing high-performance liquid chromatography (HPLC) and spectrophotometric analyses of the compounds in question (**Table 1**).

Hop Acids Analyses. The hop acid concentrations of filtered samples were analyzed by ASBC method Beer-23C (*20*) using a Supelco (Bellfontane, PA) Discovery C18, 250 mm × 4.6 mm, 5 μm particle size, column and a Hewlett-Packard 1090 HPLC. The oven temperature was set at 40 °C, with a 10 μL injection volume, and a gradient mobile phase was used at a flow rate of 1.4 mL/min. At 13 min, the mobile phase was switched from 100% B (75% MeOH, 24% H_2O, and 1% H_3PO_4) to 50% A (100% MeOH), and at 18 min, it was switched back to 100% mobile phase B. Each sample elution took 22 min. The following hop acids International Calibration Standards (ICS) prepared as dicyclohexylamine salts of Isos (DCHA) were purchased from the American Society of Brewing Chemists (St. Paul, MN): DCHA-Iso ICS-I1, DCHA-Rho ICS-R1, Tetra ICS-T1, and DCHA-Hexa ICS-H1.

Hop Acids Adjustments in the Base Beer. The beer with the highest concentration of each compound was diluted with unhopped beer and mixed gently to obtain five separate samples with a range of concentrations approximately between 2 and 10 ppm. Each sample was labeled as "compound-a, -b, -c, -d, or -e" from the lowest to highest concentration. For instance, the lowest concentration of Iso was labeled as "Iso-a," and the second highest concentration of Hexa was labeled as "Hexa-d." After blending, all samples were carbonated to 5.7 g/L (2.8 vol/vol) and analyzed via HPLC to obtain the final hop acids concentration of each sample (**Table 2**). Prior to foam testing, samples were removed from the cold room and were left at room temperature overnight (20.0 ± 0.5 °C) to equilibrate in temperature.

Foam Stability and Cling Area Analyses. A modified EBC 9.42 method (*21*) was applied to measure the foam stabilities of each sample. A Haffmans standard glass (inside diameter, 60 mm; inside height, 120 mm) was used for all measurements. Because cling can be greatly influenced by the state of glass, the glass was washed with hot water and sodium tetraborate decahydrate (Borax) and rinsed thoroughly with deionized water before every measurement. Immediately before dispensing foam into the glass, it was rinsed again thoroughly with deionized water. Reproducible foam was prepared using an Inpack 2000 Haffmans flasher head, which was directly connected to a 12 or 20 L stainless steel keg. A CO_2 pressure of 30 psi was applied to the keg to push the sample beer through the flasher head and dispense foam into a Haffmans standard glass up to the rim of the glass. Quickly after the foam was dispensed, the glass was placed in a Haffmans Foam Stability Tester (NIBEM-TPH) that had been previously calibrated using a standard glass filled with tap water at room temperature.

NIBEM-30 data were collected and analyzed keeping the analysis environment as close as possible to the standard ambient condition: 20 °C foam temperature, 1013 mbar atmospheric pressure, and 50% relative humidity. NIBEM values and cling area percentages were collected over 5 days under the average conditions of 20.3 °C foam temperature, 1006 mbar atmospheric pressure, and 45.9% relative humidity.

The foam was allowed to decay to 10 mm below the top edge of the standard glass before the NIBEM measurement commenced, and the time required to reach 10, 20, and 30 mm from this distance was recorded as NIBEM-10, NIBEM-20, and NIBEM-30, respectively. As the NIBEM-TPH started, the duration of the foam stability analysis was measured separately by a stopwatch to calculate the wait-time for a Haffmans Cling Meter (NIBEM-CLM). The equation below was developed by interpolating Haffmans' suggested time to find the best wait time (t_{wait}) in seconds for each sample.

$$t_{wait} = 1.5 \times (\text{NIBEM-30}) + 60 \qquad (1)$$

After NIBEM-TPH analysis was performed and the appropriate wait time had elapsed, the sample was moved to NIBEM-CLM to perform an analysis on the cling percentage on the inside of standard glass sidewall. The glass inner sidewall scanned by NIBEM-CLM corresponded to the distance of NIBEM-TPH, that is, NIBEM-CLM scanned the 30 mm band, from 10 to 40 mm below the rim of standard glass. NIBEM-CLM was calibrated using a 77.8% reflectivity calibration column provided by Haffmans. The NIBEM-CLM wait time was set as 0 in its configuration so that the analysis could be started instantly when the calculated wait time ended. Digital photographs of cling were taken soon after the cling area measurement was finished. A sheet of black plastic was inserted halfway into the glass so that the cling of only one side of glass was visible. For each compound and concentration combination, five separate foam measurements were taken. The order of analysis was randomly assigned.

Statistical Analysis. NIBEM-30 results were statistically analyzed with linear regression models using S-Plus (Version 8.0, Insightful

Foam Stability and Cling Formation of Reduced Hop Acids

J. Agric. Food Chem., Vol. 56, No. 18, 2008 **8631**

Table 2. NIBEM-10, -20, and -30 Values (s), Cling Areas (%), and the Environmental Conditions during the Measurements

sample	concentration (mg/L)	NIBEM-10[a] (s)	NIBEM-20[a] (s)	NIBEM-30[a] (s)	foam temperature[a] (°C)	atmospheric pressure[a,c] (mbar)	relative humidity[a] (% RH)	cling area[a] (%)
Iso								
control	0.0	37 (12.2)	62 (9.7)	90 (4.9)	20.3 (0.4)	1004	45 (1.2)	—[b,d]
a	2.7	50 (3.3)	87 (4.0)	116 (4.3)	20.2 (0.4)	1002	41 (2.4)	18.7 (13.0)
b	5.2	54 (2.0)	99 (1.8)	145 (1.3)	20.1 (0.4)	1010	54 (10.9)	67.2 (9.3)
c	7.1	52 (2.8)	100 (1.1)	148 (0.9)	19.9 (0.4)	1006	46 (1.0)	72.0 (4.9)
d	9.5	57 (2.6)	109 (2.4)	163 (1.5)	20.0 (0.5)	1006	44 (2.0)	77.2 (4.9)
e	12.4	59 (3.7)	113 (1.9)	170 (1.1)	20.1 (1.5)	1008	47 (5.8)	71.6 (5.9)
Rho								
a	2.9	34 (2.1)	62 (3.2)	92 (2.9)	19.7 (0.8)	1009	55 (7.8)	0.2 (132.6)
b	5.1	46 (3.9)	85 (2.7)	126 (1.7)	20.1 (0.4)	1006	45 (1.0)	35.0 (4.3)
c	7.0	46 (2.5)	87 (1.1)	130 (1.0)	20.5 (0.8)	1004	51 (8.9)	54.1 (2.4)
d	10.0	55 (1.6)	106 (1.1)	157 (0.8)	20.0 (0.6)	1002	37 (2.4)	72.6 (5.5)
e	12.1	51 (3.0)	99 (1.3)	151 (1.8)	19.7 (0.6)	1009	46 (2.0)	57.3 (5.4)
Hexa								
a	2.0	43 (5.1)	83 (4.1)	125 (2.7)	20.7 (1.9)	1008	47 (3.3)	47.3 (13.1)
b	3.7	57 (2.9)	114 (2.1)	172 (0.5)	20.4 (0.9)	1008	53 (2.9)	70.2 (7.1)
c	6.1	58 (2.8)	112 (2.6)	167 (2.3)	20.8 (2.3)	1006	45 (7.3)	66.0 (9.0)
d	8.8	76 (1.7)	145 (1.4)	214 (1.3)	20.5 (0.6)	1008	45 (1.9)	63.7 (6.3)
e	10.3	95 (2.5)	176 (1.2)	256 (2.0)	20.6 (1.2)	1007	45 (6.1)	75.9 (4.7)
Tetra								
a	2.5	54 (4.4)	103 (3.7)	150 (3.1)	21.2 (1.9)	1005	49 (9.2)	73.2 (5.8)
b	4.3	65 (0.8)	132 (1.4)	207 (0.6)	19.8 (0.7)	1001	39 (1.4)	63.4 (9.5)
c	6.3	85 (4.1)	168 (4.0)	247 (4.2)	21.0 (3.5)	1014	46 (1.2)	70.9 (5.3)
d	8.1	100 (2.9)	188 (4.7)	269 (4.0)	19.9 (0.2)	1007	42 (1.3)	64.3 (8.3)
e	10.6	119 (8.1)	210 (7.0)	295 (5.6)	20.1 (1.0)	1002	43 (5.8)	57.6 (2.3)

[a] The percentages in parentheses are coefficients of variation (% cv) of five measurements. [b] When % cv is less than 0.1%, it is indicated as −. [c] Atmospheric pressure differences for consecutive measurements were negligible (maximum % cv = 0.1%). [d] No cling was observed for the control beer.
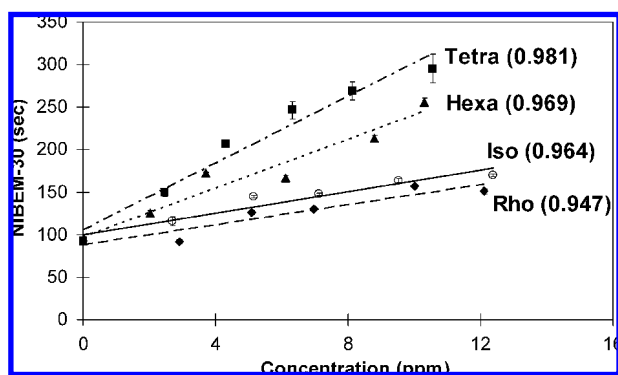

Figure 2. Linear correlations (Pearson *r* in parentheses) between Iso concentrations and NIBEM-30 for Iso, Rho, Hexa, and Tetra. Control base beer is included as 0 ppm for all linear models. Error bars indicate ± one standard deviation (*n* = 5). Variation in all observations was less than 5.6% cv with an average of 2.3% cv. All linear correlations were significant at *p* < 0.004.

Corp., Seattle, WA). Confidence intervals (CIs) (95%) were produced for all models to determine at what concentration one compound became significantly different from another.

RESULTS AND DISCUSSION

Foam Stability. The NIBEM-TPH method was highly reproducible and reliable. The average % cv of the five foam replicate measurements showed low variability, averaging 2.3% across all compound−concentration combinations (**Table 2**).

Statistically significant linear correlations were obtained between NIBEM-30 values and the five concentrations of Iso, Rho, Tetra, and Hexa (**Figure 2**). Inclusion of the control foam data improved the degree of linear fit (increased r^2) in all cases. Tetra had the strongest effect on foam stability among the four compounds. By adding 5 ppm of Tetra to unhopped beer, its stability, in terms of NIBEM value, was increased approximately

2-fold as compared to the unhopped beer. Hexa had the second strongest effect among the four. These results confirmed the strong foam-stabilizing ability of Tetra and Hexa that are noted in the literature (*11, 15, 22*). Both Iso and Rho had a relatively smaller effect on foam stability as compared to Tetra and Hexa; however, the positive influence on foam stability of these two compounds is recognized. Similar relationships between NIBEM-10 and concentration and NIBEM-20 and concentration were also clearly observed (figures not included).

Linear regression models with high r^2 values (>0.89 in all cases) were created for each of the four compounds using six data points including the unhopped control. CI bands (95% CI) were used to determine at what dosage level these compounds became significantly different from each other. For instance, in **Figure 3**, at low concentrations, the difference between Iso and Tetra is insignificant, but as the concentration of each rises, there comes a point at which Tetra is significantly different from Iso in terms increased foam stability. We identified this concentration as that point where the upper bound of the CI of Iso crossed the lower bound of the CI of Tetra, 2.4 ppm in this case. At concentrations greater than 2.4 ppm Tetra, its foam-stabilizing effects became significantly higher than that of Iso at the same concentration. For Hexa, this differentiating concentration was 4.2 ppm (**Figure 4**). No statistically significant difference in the foam-stabilizing capability existed between Iso and Rho. Tetra was significantly stronger than Hexa between concentrations of 5.5 and 9.6 ppm in stabilizing foam, but above 9.6 ppm, these two compounds could not be statistically differentiated due to the nature of 95% CI (data not shown). To confirm differences between Tetra and Hexa over a broader range of concentrations, more data points are required.

The surface activity of the foam-stabilizing materials is directly linked to their ability to produce and stabilize foam in which the higher the hydrophobicity of the compound is, the stronger the foam-stabilizing effect is (*9, 23*). Considerable work

8632 *J. Agric. Food Chem.,* Vol. 56, No. 18, 2008
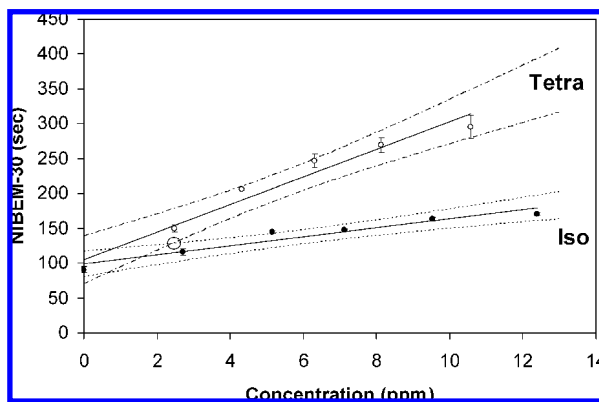
Kunimune and Shellhammer

Figure 3. CIs (95%) for Iso and Tetra (dashed lines). The circle indicates the point (2.4 ppm) where the lower bound of Tetra and upper bound of Iso 95% CIs cross each other, that is, where Tetra becomes significantly different from Iso at a given concentration in terms of NIBEM-30 values. Linear regressions of NIBEM-30 against hop acid concentration are indicated by solid lines. Error bars indicate ± one standard deviation ($n = 5$).
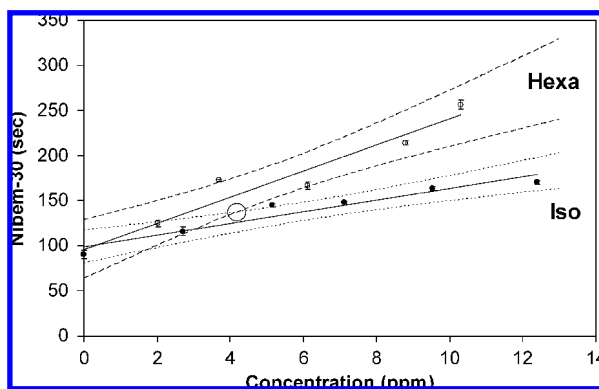

Figure 4. CIs (95%) of Iso and Hexa (dashed lines). The circle indicates the point (4.2 ppm) where the lower bound of Hexa and upper bound of Iso 95% CIs cross each other, that is, where Hexa becomes significantly different from Iso at a given concentration in terms of NIBEM-30 values. Linear regressions of NIBEM-30 against hop acid concentration are indicated by solid lines. Error bars indicate ± one standard deviation ($n = 5$).

has been performed examining the influence of protein hydrophobicity and beer foam (*9, 14, 24*). Additionally, the hydrophobicity of Isos is another critical characteristic of foam stabilization (*9, 12, 23, 24*). From this perspective, it is quite reasonable that Tetra and Hexa had much stronger foam-stabilizing abilities as compared to the other two hop acids given that the reduction of the carbon–carbon double bonds with hydrogen in Isos will significantly enhance the hydrophobicity of the compound (*24, 25*). In the present study, analysis of the HPLC retention time data from the Iso and reduced Iso standards confirmed the relative hydrophobicities being ordered as Tetra, Hexa, Iso, and Rho from the most to least hydrophobic. These hydrophobicity data positively correlated with the obtained NIBEM data. These results support work published by Simpson and Hughes (*25*) and to a large degree the work by Smith, Davidson, and Wilson (*11*), although they reported that dihydroisohumulone (the humulone analogue of Rho) had a greater foam-stabilizing action relative to isohumulone and isocohumulone at 5 and 10 ppm.

Two hypotheses have been postulated describing hop acid–beer foam interactions. Simpson and Hughes (*25*)
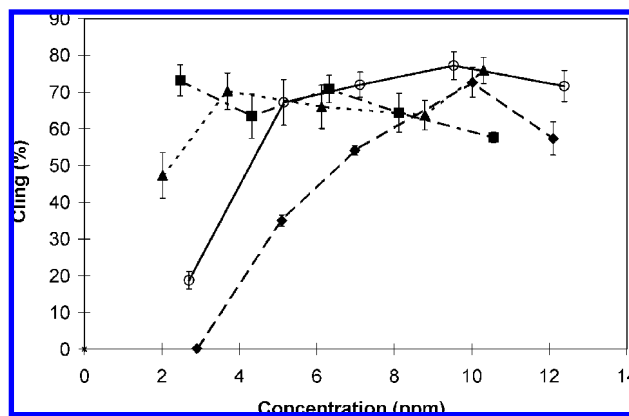

Figure 5. Effect of Iso, Rho, Hex, and Tetra on the cling area measured by NIBEM-CLM. Error bars indicate ± one standard deviation ($n = 5$).

postulated that the primary interaction between protein amino groups and Iso carbonyl and enolate groups is strengthened by hydrophobic interactions of the Iso side chains and hydrophobic regions of polypeptides. Furthermore, this interaction is strengthened by divalent and trivalent cations. Lusk, Ting, Goldstein, Ryder, and Navarro (*24*) suggest that the Isos induce a surface activity of the bittering acid–protein complex; therefore, greater hydrophobicity will lead to greater surface activity of the complex.

Cling Area. The cling area percentages measured by NIBEM-CLM did not show the same linear relationship over the entire concentration range as did the NIBEM-30 data (**Figure 5**). Both Iso and Rho displayed concentration-dependent effects on cling area at low concentrations with Iso reaching its plateau at 5 ppm and Rho at 10 ppm. Hexa displayed a similar trend but only over the first two data points (up to 4 ppm). Interestingly, these three hop acids did not produce a continual increase in cling area with increasing concentration. That is, the highest levels produced cling area less than a maximum at lower concentrations. Tetra showed a greater ability to produce cling beginning with the lowest concentration (2–3 ppm) and remaining relatively unchanged across all higher concentrations. Very small amounts of Tetra or Hexa (approximately 2 and 4 ppm, respectively) are sufficient to create a substantial amount of cling. In contrast, the control (unhopped beer) and the lowest concentration of Rho (approximately 2 ppm) produced no cling formation.

The average variance, expressed as the average % coefficient of variation (% cv) of cling area percentages, was 6.7% excluding one outlier (the outlier had the cling area percentage ranging from 0.0 to 0.6%). Two possible reasons for this relatively higher % cv as compared to the NIBEM-30 values are the difficulty in preparing the standard glass to achieve exactly the same condition for each measurement, the technological limitation of NIBEM-CLM, and the inherent variation in foam collapse and cling phenomena.

Digital photos of cling were taken immediately after NIBEM-CLM analysis. The beer foam in this study displayed three major types of cling formation patterns: "ring", "mesh", and "powder" (**Figure 6**). **Figure 7** shows the cling patterns of Iso, Rho, Hexa, and Tetra within the range of concentrations. Generally, at the lower concentrations, one or two rings of cling formation were observed. For the higher concentration of Iso and Rho and also the lower concentration of Tetra and Hexa, the meshlike pattern was observed. The size and fineness of this meshlike pattern varied from a sample to sample, but in general, the mesh pattern was observed at the
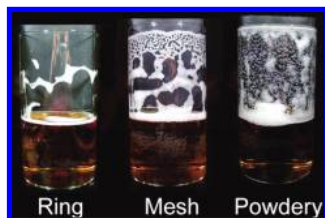
Foam Stability and Cling Formation of Reduced Hop Acids

J. Agric. Food Chem., Vol. 56, No. 18, 2008 **8633**

Figure 6. Three major types of cling that can be created by hop acids in lager beer.

Figure 7. Cling patterns of all compound−concentration combinations and cling area % (in parentheses).

top part of the glass. At the higher concentration of Tetra and Hexa, powdery, snowlike cling was observed. The distribution of the cling for this pattern was not uniform, and also, the size of bubbles varied. The control showed no cling.

There are several things to be noted about the cling measurements. First, NIBEM-CLM can give the overall cling area percentage but cannot indicate what cling pattern was obtained. Each compound produced very unique, characteristic cling patterns of its own depending on the concentration. For example, Iso-d, Rho-d, Tetra-a, and Hexa-e had relatively similar cling percentages (approximately 75%) according to NIBEM-CLM, although they clearly look different from each other to the human eye (**Figure 7**). Second, the area that NIBEM-CLM measures is a band between 10 and 40 mm below the rim of standard glass. The cling above and below the area is not measured by NIBEM-CLM, which exclude important foam lacing phenomena. The overall actual cling area percentage might be different from that of NIBEM-CLM as well as the consumers' perception. Further research should be conducted to determine the foam-stabilizing effect and

cling capability of multiple Isos combinations in addition to the consumer appeal of cling patterns.

ABBREVIATIONS USED

Iso, iso-α-acid; Rho, rho-iso-α-acid; Hexa, hexahydro-iso-α-acid; Tetra, tetrahydro-iso-α-acid; % cv, coefficient of variation.

LITERATURE CITED

(1) Bamforth, C. W. The foaming properties of beer. *J. Inst. Brew.* **1985**, *91* (6), 370–383.

(2) Bamforth, C. W. Perceptions of beer foam. *J. Inst. Brew.* **2000**, *106* (4), 229–238.

(3) Smythe, J. E.; O'Mahony, M.A.; Bamforth, C. W. The impact of the appearance of beer on its perception. *J. Inst. Brew.* **2002**, *108* (1), 37–42.

(4) Smythe, J. E.; Bamforth, C. W. A study of perceived beer history on reported preferences by sensory panels with different levels of training. *J. Inst. Brew.* **2002**, *108* (1), 34–36.

(5) Roza, J. R.; Wallin, C. E.; Bamforth, C. W. A comparison between the instrumental measurement of head retention/lacing and perceived foam quality. *MBAA TQ* **2006**, *43* (3), 173–176.

(6) Ronteltap, A. D.; Hollemans, M.; Bisperink, C. G. J.; Prins, A. Beer foam physics. *Tech. Q. Master Brew. Assoc. Am.* **1991**, *28* (1), 25–32.

(7) Bamforth, C. W. The relative significance of physics and chemistry for beer foam excellence: Theory and practice. *J. Inst. Brew.* **2004**, *110* (4), 259–266.

(8) Dale, C. J.; Walker, S. G.; Lyddiatt, A. Dynamic changes in the composition and physical behaviour of dispensed beer foam. *J. Inst. Brew.* **1993**, *99* (6), 461–466.

(9) Hughes, P. S. Hydrophobic interactions and their significance for beer foam quality. In E.B.C. Monograph 27, E.B.C. Symposium on Beer Foam Quality; Verlag Hans Carl Getranke-Fachverlag Amsterdam: The Netherlands, 1998; pp 158−167.

(10) Todd, P. H.; Held, R. W.; Guzinski, J. G. The development and use of modified hop extracts in the art of brewing. *Tech. Q. Master Brew. Assoc. Am.* **1996**, *33* (2), 91–95.

(11) Smith, R. J.; Davidson, D.; Wilson, R. J. H. Natural foam stabilizing and bittering compounds derived from hops. *J. Am. Soc. Brew. Chem.* **1998**, *56* (2), 52–57.

(12) Hughes, P. The significance of iso-a-acids for beer quality. *J. Inst. Brew.* **2000**, *106* (5), 271–276.

(13) Hollemansn, M.; Tonies, T. R. J.M.; Bisperink, C. G. J.; Ronteltap, A. D. The role of malt lipids in beer foam. *Tech. Q. Master Brew. Assoc. Am.* **1991**, *28* (4), 168–173.

(14) Evans, D. E.; Surrel, A.; Sheehy, M.; Stewart, D. C.; Robison, L. M. Comparison of foam quality and the influence of hop a-acids and proteins using five foam analysis methods. *J. Am. Soc. Brew. Chem.* **2008**, *66* (1), 1–10.

(15) Baker, C. W. Impact of post-filtration addition of selected hop extracts on beer foam and clarity. *Tech. Q. Master Brew. Assoc. Am.* **1990**, *27* (2), 33–38.

(16) Bamforth, C. W.; Jackson, G. Aspects of foam lacing. *Proc. Eur. Brew. Conv. Cong.* **1983**, 331–338.

(17) Huvaere, K.; Andersen, M. L.; Skibsted, L. H.; Heyerick, A.; DeKeukeleire, D. Photooxidative degradation of beer bittering principles: A key step on the route to lightstruck flavor formation in beer. *J. Agric. Food Chem.* **2005**, *53* (5), 1489–1494.

(18) Huvaere, K.; Sinnaeve, B.; Bocxlaer, J. V.; DeKeukeleire, D. Photooxidative degradation of beer bittering principles: Product analysis with respect to lightstruck flavour formation. *Photochem. Photobiol. Sci.* **2004**, *3* (9), 854–858.

(19) Fritsch, A.; Shellhammert, T. H. Relative bitterness of reduced and nonreduced iso-alpha-acids in lager beer. *J. Am. Soc. Brew. Chem.* **2008**, *66* (2), 88–93.

(20) American Society of Brewing Chemists, Beer 23-C. Iso-alpha-acids by solid phase extraction and HPLC. In *Methods of Analysis*, 9th ed.; American Society of Brewing Chemists: St. Paul, MN, 2004.

(21) European Brewery Convention, Method 9.42. Foam stability of beer using the NIBEM-T meter. Analytica-EBC; Zoeterwoude: The Netherlands, 2006.

(22) Weiss, A.; Schönberger, Ch.; Mitter, W.; Biendl, M.; Back, W.; Krottenthaler, M. Sensory and analytical characterization of reduced, isomerised hop extracts and their influence and use in beer. *J. Inst. Brew.* **2002**, *108* (2), 236–242.

(23) Moir, M.; Smith, R. J. Foam-enhancing and bitter, reduced iso-a-acids derived from hops. *Proc. Eur. Brew. Conv. Cong.* **1995**, 125–134.

(24) Lusk, L.; Ting, P.; Goldstein, H.; Ryder, D.; Navarro, A. Foam tower fractionation of beer proteins and bittering acids. E.B.C.

(25) Monograph 27, E.B.C. Symposium on Beer Foam Quality; Verlag Hans Carl Getranke-Fachverlag Amsterdam: The Netherlands, 1998; pp 166−187.

(25) Simpson, W. J.; Hughes, P. S. Stabilization of foam by hop derived bitter acids chemical interactions in beer foam. *Cerevisia Biotech.* **1994**, *19* (3), 39–44.

(26) Maye, J. P.; Mulqueen, S.; Xu, J.; Weis, S. Spectrophotometric analysis of isomerized alpha-acids. *J. Am. Soc. Brew. Chem.* **2002**, *60* (3), 98–100.

(27) Raumschuh, J. Determination of iso-alpha-, alpha-, and beta-acids in hop extracts and isomerized hop extracts by high-performance liquid chromatography. *J. Am. Soc. Brew. Chem.* **1999**, *57* (4), 162–165.