MBAA TQ vol. 53, no. 1 • 2016 • pp. 23–27

PEER-REVIEWED PAPER

Humulinone Formation in Hops and Hop Pellets and Its Implications for Dry Hopped Beers

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ABSTRACT

Humulinones are a natural hop bitter acid that can be found in leaf hops, and their concentration can increase over several days following hop pelleting. Hops and hop pellets with a high hop storage index (HSI) have higher concentrations of humulinones than hops and hop pellets with a lower HSI. High concentrations of humulinones have recently been found in beers that have been dry hopped, in some cases as high as 24 ppm. Dry hopping experiments show humulinones readily dissolve in beer and can impact the bitterness profile of dry hopped beers. Additionally, dry hopping can cause a significant loss in iso-α-acid concentration, especially in beers containing high concentrations of iso-α-acids.

Keywords: Humulinone, Dry hopping

Humulinones are naturally formed by the oxidation of α-acids within the hop (3). Their molecular structure (7) is nearly identical to iso-α-acids, except humulinones contain an additional hydroxyl group (Fig. 1). This hydroxyl group makes humulinones more polar and more soluble in beer than iso-α-acids. In addition, humulinones have been reported to be less bitter than iso-α-acids, about 66% as bitter (1,8). Baled hops generally contain less than 0.3%w/w humulinone; however, that concentration can slowly increase to 0.5%w/w or slightly more after hop pelleting. These low concentrations of humulinone contribute little to a beer's bitterness when hops are solely added to the kettle. However, if one dry hops at dose rates of 1–2 lbs per barrel of beer, nearly all the humulinone will dissolve into the beer, and that can affect the overall bitterness of that beer.

Experimental

Hops and hop pellets were extracted by the ASBC method Hops-14. HPLC analysis was conducted using HPLC method EBC 7.7 and the corresponding HPLC calibration standards, ICE-3, for α-acid and β-acid analysis. HPLC method EBC 7.9 and HPLC calibration standard ICS-I3 were used for iso-α-acid analysis. A humulinone–dicyclohexylamine HPLC calibration standard (4) was produced in-house and used to calibrate the HPLC for humulinone analysis.

Beers were brewed in a 15 gal Sabco pilot brewery for the dry hopping experiments.

Small-scale dry hopping of commercial beers was accomplished as follows. Beer (300 g) was added to a 12 oz bottle with one drop of octanol and purged with CO_2 and then lightly degassed by careful bath sonication to prevent overfoaming. Hop pellets were added to each bottle and air removed in a vacuum desiccator, CO_2 was added, and then the bottle was capped. Samples were stored at 16°C before HPLC analysis. Beer was filtered through a Whatman GF/F filter (≈0.7 µm); 5 mL was diluted with acidic methanol, and after cooling the volume was brought to 10 mL. Then 10 µL was injected onto the HPLC column.

Concentration of Humulinones in Hops and Hop Pellets

A freshly prepared bale of the Zeus hop variety was analyzed to contain 0.23%w/w humulinone, by HPLC (Fig. 2). Following hop pelleting, the concentration of humulinones was monitored over the next 10 days. Even though the pellets were stored under vacuum and in one case cold, the concentration of humulinones slowly increased to about 0.47%w/w and then stopped (Fig. 3). In a separate experiment, two different lots of Galena hop bales and their corresponding pellets had their humulinone concentrations monitored over 14 days. It was found that the lot with the higher hop storage index (HSI) not only contained more humulinone but their

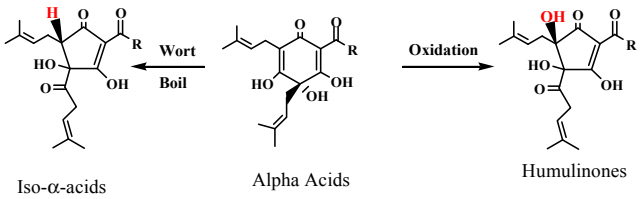

Figure 1. The molecular structures of iso-α-acids, α-acids, and humulinones.

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http://dx.doi.org/10.1094/TQ-53-1-0227-01

resulting pellets also produced more humulinone (Fig. 4). Interestingly, there appears to be a linear relationship between the ratio of humulinone concentration/α-acid concentration versus HSI in hop pellets, and this linear relationship is variety dependent (Fig. 5). Using these variety-specific equations of a line, one can estimate the humulinone concentration in hop pellets if one knows the HSI and α-acid concentration of their hop pellets.

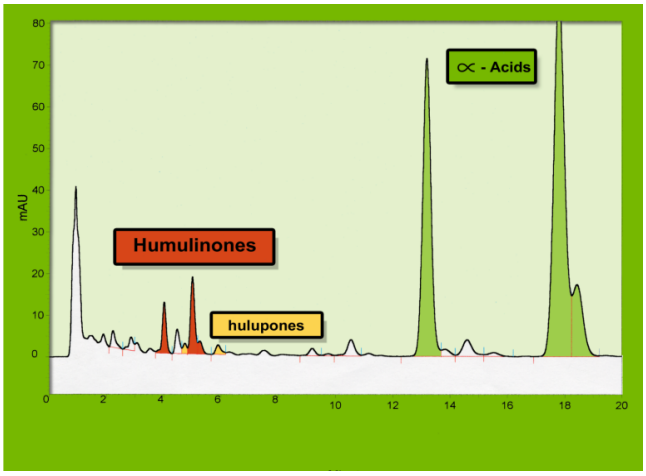

Figure 2. HPLC trace of humulinones and α-acids in hops.

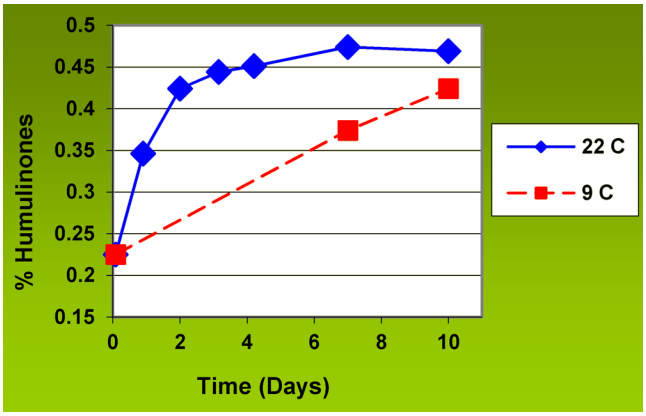

Figure 3. Formation of humulinones in Zeus hop pellets stored at 9°C and 22°C under vacuum.

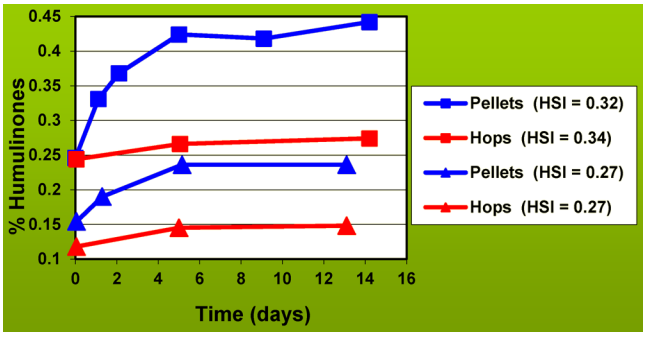

Figure 4. Humulinone formation in Galena hops and hop pellets having different hop storage indexes (HSI).

What Causes Humulinones to Form in Hops and Hop Pellets?

The exact mechanism of how humulinones form in hops and hop pellets is still unknown. When hops are baled, about 10–20% of the lupulin glands are broken, whereas when they are pelletized nearly 100% of the glands are broken. With this in mind, it appears there is something in the leaf material of the hop that facilitates the oxidation and isomerization of α-acids to form humulinone. Whatever it is, it appears to be limiting, and this is why the concentration of humulinones increases following hop pelleting and then stops. Exposing hop pellets or hop powder to air caused little to no increase in the overall humulinone concentration.

Humulinone Concentration in Dry Hopped Beers

HPLC analysis of 29 commercial beers labeled India pale ale (Fig. 6) showed humulinone concentrations ranging from 3 to 24 ppm. To better understand the solubility characteristics of humulinone, a low-IBU beer (8.6 ppm of iso-α-acids, by HPLC) and a high-IBU beer (48 ppm of iso-α-acids, by HPLC) were dry hopped with Centennial hop pellets assaying 0.35% w/w humulinone, by HPLC. The hop pellets were simply dumped on top of the beer, at 16°C, for 5 days. The dry hopping dose rates for both beers were 0, 0.5, 1.0, and 2.0 lbs/bbl. After 5 days HPLC analysis was conducted on all the beers (Table 1). The

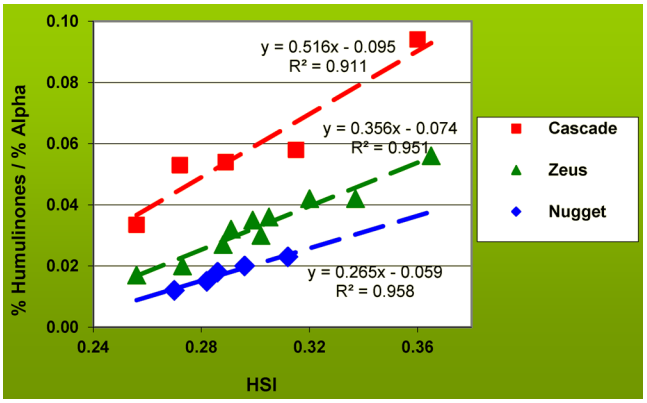

Figure 5. The linear relationship of (%humulinones ÷ %α-acids) versus hop storage index (HSI).

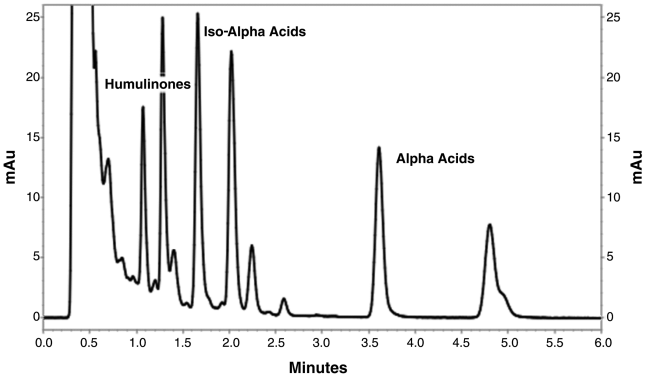

Figure 6. HPLC trace of dry hopped beer.

results showed that at a 0.5 lb/bbl dose rate nearly 98% of the humulinones dissolved into the beer, regardless of the starting IBU. At 1.0 lb of hop pellets per barrel 91% of humulinones dissolved, and at 2 lbs/bbl about 87–88% of the humulinones dissolved into the beer. One unexpected result was with regard to the significant loss in iso-α-acid concentration in the high-IBU beer versus nearly no loss in iso-α-acids in the low-IBU beer. By increasing the dry hopping dose rates from 0 to 0.5, 1.0, and 2.0 lbs/bbl in the high-IBU beer, one sees the iso-α-acid concentration drop from 48 to 39, 35, and 30 ppm, respectively. This significant loss in bitterness was offset, however, by the large increase in humulinones that dissolved in the beer. If one calculates the bitterness intensity of dry hopped beers as (0.66 × ppm of humulinones) + ppm of iso-α-acids, then the overall bitterness after dry hopping is about 3–4 organoleptic IBUs lower than the control. It should be noted that humulinone has an IBU spectrophotometric response factor similar to iso-α-acids (Fig. 7). That means, for example, if one performs the spectrophotometric IBU test on the 2 lb/bbl dry hopped beer the instrument results would measure just under 57 IBU for 27 ppm of humulinone + 30 ppm of iso-α-acids (not taking into account other IBU absorbing compounds); however, the organoleptic bitterness will taste more like 47 IBU beer (27 × 0.66 + 30), or about 17% less bitter than the instrument results would suggest. This discrepancy between analytical IBU and organoleptic bitterness can only be discerned by HPLC analysis, which can accurately measure these two hop acids separately, and by taking into account that humulinones are 66% as bitter as iso-α-acids. Interestingly, the low-IBU beer (8.6 ppm of iso-α-acids) showed less than a 1 ppm loss in iso-α-acids after dry hopping, yet its calculated bitterness went up dramatically owing to the high incorporation of humulinones into that beer. So it is possible to make a more bitter beer by dry hopping as long as the starting IBUs are low, estimated to be ≈25 ppm of iso-α-acids or less.

Humulinones and Other Nonvolatile Hop Compounds That Dissolve in Dry Hopped Beers

To better understand the rate of how fast nonvolatile compounds such as humulinones and α-acids dissolve in beers and how fast iso-α-acids are lost during dry hopping a second dry hopping experiment was conducted. In this study a 51 ppm (HPLC) iso-α-acid beer was brewed and dosed with Cascade hop pellets (Table 2) at a dose rate of 0, 0.5, 1.0, and 2.0 lbs/bbl. The hop pellets were simply dumped on top of the beer at 16°C, and the contact time was 1, 2, and 5 days. Samples of each beer were taken after 1, 2, and 5 days and analyzed by HPLC for α-acids, β-acids, iso-α-acids, humulinones, hulupones (oxidized β-acids), and xanthohumol, a prenylated polyphenol (Tables 3, 4, and 5). HPLC analysis showed that it takes about 2 days for the humulinone and α-acids to achieve their maximum solubility in beer; however, it appears that some loss in iso-α-acids can still occur at 5 days. Xanthohumol appears to hit saturation when 1 lb of hops is added per barrel of beer, with no additional increase at 2 lbs/bbl. Little to no β-acids or hulupones were seen in any of the beers, mainly owing to the low solubility of β-acids in beer and the low concentration of hulupones in the hops. Because the humulinone concentration in the Cascade hop pellets used was only 0.26%w/w, the calculated bitterness of this beer was dramatically lower than the control when 1 or 2 lbs of hops were added per barrel of beer (Table 6). This significant reduction in bitterness shows what an important role humulinones can play in contributing bitterness to dry hopped beers.

Dry Hopping and pH

It was noticed in all of the dry hopping experiments that the pH increased as the dry hop dosage increased. To study this in more detail, five commercial beers with different IBUs and different pHs were degassed and dry hopped with Cascade hop

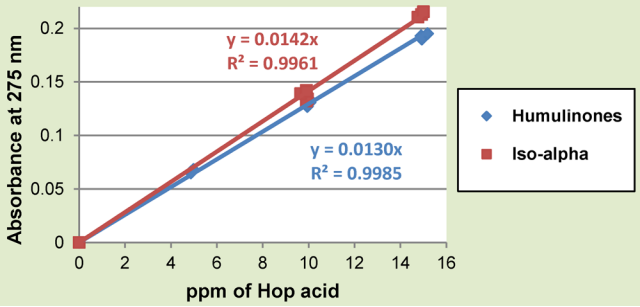

Figure 7. Absorbance intensity at 275 nm of humulinone and iso-α-acids in isooctane via IBU extraction of a low-IBU beer spiked with different concentrations of humulinone and iso-α-acids.

Table 2. Cascade hop pellet analysis on the day of dry hopping

Compound	Concentration (%)
α-Acids (ICE-3)	5.6
β-Acids (ICE-3)	5.8
Humulinones (dicyclohexylamine–humulinones, 65.9%)	0.26
Xanthohumol (99.7% pure, in-house standard)	0.26
Hulupones (dicyclohexylamine–hulupones, 67.0%)	0.05

Table 1. Dry hopping with Centennial hop pellets assaying 0.35% humulinone for 5 days[a]

Sample	Pellets (lbs) per barrel of beer	Humulinone (ppm) in beer	% Utilization of humulinone	Iso-α-acid (ppm) in beer	Calculated bitterness intensity
Low-IBU beer (8.6)	0	0.8	…	8.6	9.1
	0.5	8	98	8.1	13
	1.0	14	91	7.9	17
	2.0	28	88	7.5	26
High-IBU beer (48)	0	1	…	48	49
	0.5	8	98	39	44
	1.0	14	91	35	44
	2.0	27	87	30	47

[a] Calculated bitterness = (ppm of humulinone × 0.66) + ppm of iso-α-acids.

pellets for 3 days at 16°C. The results showed that regardless of the beers' starting IBU or pH, drying hopping had a linear impact on pH, with the pH rising by about 0.14 pH units per pound of hop pellets dosed (Fig. 8). This rise in pH could further counteract the loss in iso-α-acid bitterness that occurs with dry hopping. That is, Brenner et al. (2) and Meilgaard and Trolle (5) reported that beers with the same iso-α-acid concentration tasted more bitter at higher pHs versus lower pHs. It was also reported by Rigby (6) that hop acids in their dissociated form have a stronger or more intense bitterness than hop acids in their undissociated form; thus, the higher the pH the more dissociated hop acids. To test these observations, a dry hopped beer having a pH of 4.91 was treated with 10% sulfuric acid to adjust its pH to 4.5. The beer with the higher pH tasted slightly more bitter than the beer with the lower pH. Therefore, an increase in pH owing to dry hopping can affect the bitterness and perhaps other flavors in beer.

Summary and Conclusions

Humulinones are found in leaf hops at concentrations generally below 0.3%w/w as measured by HPLC; however, following hop pelleting this concentration can increase to 0.5%w/w. Hops or hop pellets with high HSIs will have higher concentrations of humulinone than hops or hop pellets with low HSIs; however, this is variety dependent. Humulinones are more polar than iso-α-acids, and over 87% will dissolve into beer within 2–3 days of dry hopping. Dry hopping under the conditions tested showed that a substantial loss in iso-α-acids can occur when high-IBU beers are dry hopped. However, that significant loss in bitterness can be offset by dry hopping with hops containing 0.35%w/w humulinone or more. Dry hopping also affects the pH of beer, regardless of its starting pH or IBU, and that effect is linear, about 0.14 pH units per pound of hop pellets dosed per barrel of beer. This increase in pH can also lead to a slight increase in sensory bitterness. Finally, sensory evaluation of a very low IBU beer spiked with 22 ppm

Table 6. Calculated bitterness for dry hopping with Cascade hops pellets after 5 days[a]

Hops (lbs) per barrel	Iso-α-acid (ppm)	Humulinone (ppm)	Calculated Bitterness
0	51.3	4.2	54.1
0.5	42.5	10	49.1
1.0	29.4	14	38.6
2.0	25.9	23	41.1

[a] Calculated bitterness = (ppm of humulinone × 0.66) + ppm of iso-α-acids.

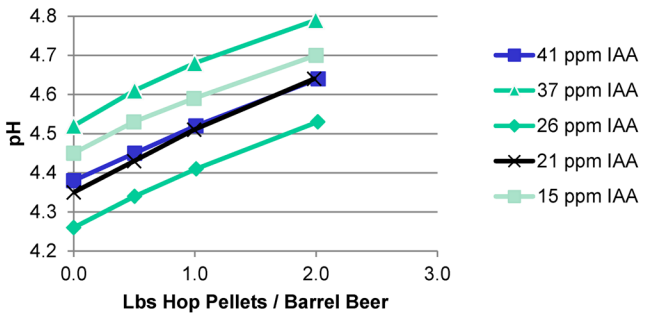

Figure 8. The change in beer pH after 3 days of dry hopping with Cascade hop pellets.

Table 3. HPLC analysis of beers dry hopped for 1 day with Cascade hop pellets[a]

Hops (lbs) per barrel	Iso (ppm)	α-Acids (ppm)	Hum (ppm)	XN (ppm)	% Utilization			
					Iso	α-Acids	Hum	XN
0	51.3	9	4.2	0.4	…	…	…	…
0.5	48	17	9	0.6	–7	8	88	12
1.0	38	21	11	0.6	–26	6	62	12
2.0	36	25	13	0.65	–39	4	39	13

[a] Iso = iso-α-acids; Hum = humulinone; and XN = xanthohumol.

Table 4. HPLC analysis of beers dry hopped for 2 days with Cascade hop pellets[a]

Hops (lbs) per barrel	Iso (ppm)	α-Acids (ppm)	Hum (ppm)	XN (ppm)	% Utilization			
					Iso	α-Acids	Hum	XN
0	51.3	9	4.2	0.4	…	…	…	…
0.5	45	24	9	0.93	–12	12	91	18
1.0	33	32	14	1.11	–36	10	90	21
2.0	29	31	20	0.8	–43	5	72	15

[a] Iso = iso-α-acids; Hum = humulinone; and XN = xanthohumol.

Table 5. HPLC analysis of beers dry hopped for 5 days with Cascade hop pellets[a]

Hops (lbs) per barrel	Iso (ppm)	α-Acids (ppm)	Hum (ppm)	XN (ppm)	% Utilization			
					Iso	α-Acids	Hum	XN
0	51.3	9	4.2	0.24	…	…	…	…
0.5	42	22	10	0.6	–17	11	100	12
1.0	29	27	14	0.8	–43	8	97	8
2.0	25	35	23	0.8	–49	6	90	4

[a] Iso = iso-α-acids; Hum = humulinone; and XN = xanthohumol.

of humulinone was compared with the same beer spiked with 14.5 ppm of iso-α-acids. The bitterness intensity of the two beers appeared to be similar, confirming that humulinones are about 66% as bitter as iso-α-acids. The bitterness profile of the humulinone beer, however, appeared smoother, and there was less lingering on the tongue than with the iso-α-acid beer. This smooth bitterness makes sense given humulinones are more polar than iso-α-acids and should therefore not stick or linger on the tongue as long as iso-α-acids.

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