Dry Hopping and Its Effect on Beer Bitterness, the IBU Test, and pH

IMPACT ANALYSIS | Dry hopping is very popular among craft brewers, however, few realize that it can significantly alter the hop acid composition of beer which can effect a beer's bitterness and interfere with the IBU Test. It can also increase a beer's pH. Dry hopping experiments were conducted and a number of analytical tests were performed on beers before and after dry hopping to better understand the impact it can have on bitterness, the IBU, and pH. In a separate follow-up paper dry hopping experiments were performed to evaluate its effect on beer foam stability.

HOPS CONTAIN two major organic acids, alpha acids and beta acids, and two minor organic acids, humulinones and hulupones. Humulinones are naturally formed by the oxidation of alpha acids within hops [1] and hulupones are formed by the oxidation of beta acids within hops and during the kettle boil [13]. Humulinones' molecular structure [2] is nearly identical to isoalpha acids, except humulinones contain an additional hydroxyl group (Fig. 1). This additional hydroxyl group makes humulinones more polar and more beer soluble than isoalpha acids and less bitter [3]. In fact, humulinones are reported to be 66 percent as bitter as isoalpha acids [4]. Baled hops of good quality generally contain less than 0.2 percent w/w humulinone, however, that concentration can increase up to 0.3 percent w/w or more after hop pelleting. If the hops are of questionable quality and have a high hop storage index, HSI, the humulinone concentration can be well over 0.4 percent [9]. These very low concentrations of humulinone contribute little to beer bitterness when hops are solely added to the kettle. However, if one dry hops at one pound per barrel of beer or more, most of the humulinones and some of the alpha acids will dissolve into the beer and this can impact a beer's bitterness. Additionally, during dry hopping the vegetative matter of the hop can absorb and remove a substantial amount of isoalpha acids if one dry hops a beer containing more than 40 mg/L isoalpha acids. This reduction in isoalpha acids and incorporation of humulinones and alpha acids can affect the overall bitterness of beer [9] and interferes with the International Bitterness Units, IBU, test [15]. Hulupones are reported to be about 84 percent as bitter as isoalpha acids [4], and their concentration in hops is generally less than 0.1 percent. As a result, the concentration of hulupones in dry hopped beers are very low, usually under two ppm, hence they contribute little bitterness [4]. Dry hopping increases the pH of beer and the unknown compound(s) responsible are found in the leaf material of the hop and not the lupulin glands [9].

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Test Methods Used and Sample Preparation

Hops and hop pellets were extracted using ASBC Method HOPS-14. High Performance Liquid Chromatography (HPLC) analysis was conducted using HPLC Method EBC 7.7 and the corresponding HPLC calibration standards, ICE-3, for alpha acids and beta acid analysis. HPLC Method EBC 7.9 and HPLC calibration standard ICS-I3, where used for isoalpha acid analysis. A humulinone-dicyclohexylamine HPLC calibration standard [5] was produced in-house and used to calibrate the HPLC for humulinone analysis.

The IBU test was conducted on beers us-

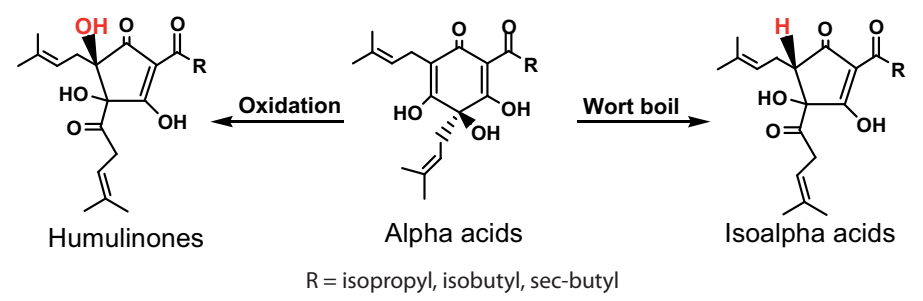

Fig. 1 The molecular structures of humulinones, alpha acids and isoalpha acids

ing ASBC Methods of Analysis, Beer Method 23A [11].

Pilot scale beers were brewed in a 15 gallon Sabco pilot brewery for the dry hopping experiments. Hop pellets were simply dumped on top of the beer at 16 °C, and stored under these conditions as stated.

Small-scale dry hopping of commercial beers for HPLC analysis and IBU test were performed in triplicate and accomplished as follows. 300 g of beer was added to a twelve 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 and CO_2 added and then capped. Samples were stored at 16 °C for three days before HPLC analysis. Beer was filtered through a Whatman GF/F filter (~0.7 µ) and five mL diluted to with acidic methanol and after cooling the volume was brought to 10 mL; 10 µL was injected onto HPLC column.

Dry Hopping and Humulinone Utilization

High Performance Liquid Chromatography (HPLC) analysis of 29 commercial beers labeled India Pale Ale (Fig. 2) showed humulinone concentrations ranging from three ppm to 24 ppm. To better understand the solubility characteristics of humulinone, a low IBU beer and a high IBU beer were dry hopped with Centennial hop pellets containing 0.35 percent humulinone as measured by HPLC. These beers were dry hopped at a dose rate of 0, 0.5, 1.0, and 2.0 pounds/barrel for five days at 16 °C. HPLC analysis of the beers after dry hopping showed the starting isoalpha acid concentration of the beer had no effect on humulinone solubility and utilization (Table 1). At the 0.5 lb dosage 98 percent of the humulinones dissolved into the beer, at the 1 lb dose rate 91 percent dissolved, and at the two lb dose rate 88 percent dissolved into the beer. HPLC analysis of the high IBU beer showed a considerable amount of isoalpha acids were removed from the beer due to dry hopping, however, very little was lost in the low IBU beer. At high concentrations, isoalpha acids are not very soluble in beer and HPLC analysis of the leaf material following dry hopping showed it contained isoalpha acids, thus the leaf material can absorb and remove some of the isoalpha acids during dry hopping.

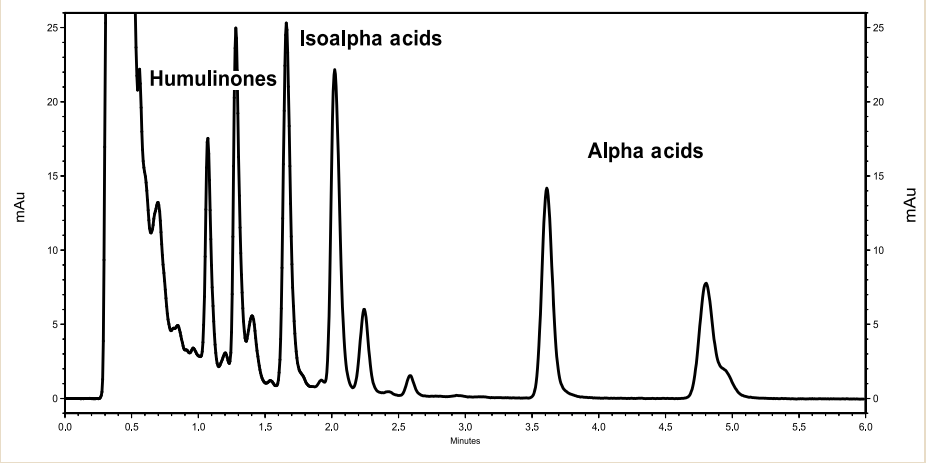

Fig. 2 HPLC trace of a commercial IPA

HUMULINONE UTILIZATION OF CENTENNIAL HOP PELLETS DRY HOPPED FOR FIVE DAYS

Beer	lbs of pellets added per barrel of beer	ppm of humulinone in beer	% Utilization of humulinone in beer	ppm of iso-alpha acids in beer
Low IBU beer 8.6 ppm isoalpha acids	0	0.8	-	8.6
	0.5	8	98	8.1
	1	14	91	7.9
	2	28	88	7.5
High IBU beer 48 ppm isoalpha acids	0	1	-	48
	0.5	8	98	39
	1	14	91	35
	2	27	87	30

Table 1

Dry Hopping and Bitterness

For some craft brewers dry hopping at one to two lbs/barrel of beer is not enough with some using as much as five pounds per barrel or more. To better understand what can happen to a beer that undergoes this kind of extreme dry hopping, a 42 ppm isoalpha acid beer was dry hopped with Cascade hop pellets assaying 5.7 percent alpha acids, 0.23 percent humulinone at a dose rate of 0, 1, 2, 3, 4, and 6 pounds per barrel for three days at 16 °C (Fig. 3). The results from this set of dry hopping experiments confirm the results in Table 1. That is, large losses of isoalpha acids occur with the first one to two lbs/bbl of hops added because one is dry hopping a high IBU beer. However, the additional pounds of hops added, three lb/bbl and more, remove very little isoalpha acids. It appears the "low IBU" designation of a beer is one containing about 25 ppm of isoalpha acids or less. This means each additional pound of hops removes less and less isoalpha acids because isoalpha acids are soluble at these lower concentrations in beer. With the addition of three lbs of hops or more per barrel of beer very little isoalpha acids are being removed while larger quantities of humulinones are still being added to the beer due to their high solubility. Also, a large quantity of alpha acids, which are $^1/_{10}$ as bitter as isoalpha acids, dissolves into the beer with concentrations reaching 37 ppm at the six pound per barrel dose rate. This change in hop acid composition can affect a beer's bitterness as the isoalpha acids concentration decreases and humulinones and alpha acids increase. One possible method for estimating the bitterness of a dry hopped beer analytically is with HPLC. HPLC analysis allows one to accurately measure the concentration of the three individual hop acids found in dry hopped beers and by using the below formula one can "calculate

the bitterness" by adding their relative bitterness to that of isoalpha acids.

Calculated Bitterness (HPLC) = ppm isoalpha acids + (0.66 x ppm humulinone) + (0.1 x ppm alpha acids

It should be noted that bitterness is not linear and after ~ 40 ppm of bitterness the intensity becomes less and less with each additional ppm of hop acids. In fact, many craft brewers say their dry hopped beers don't taste anywhere as bitter as the IBU test result suggests. By comparing the calculated bitterness of these six beers to the IBU test results (Fig. 4); one gets a more realistic estimate of a beer's bitterness by calculating it than what the IBU test result would suggest. Interestingly, dry hopping at a dose rate of three lb/bbl and more can make a beer more bitter. This is due to the fact that more bitterness is getting into the beer in the form of humulinones and alpha acids than is leaving the beer in the form of isoalpha acids.

Dry Hopping and The IBU Test

The IBU test was designed to measure only isoalpha acids in beer, not beer that contains humulinones and alpha acids. To better understand the impact various hop acids have on the IBU test, non-dry hopped beers were treated with purified isoalpha acids, alpha acids, and humulinones and assayed by HPLC and the IBU test (Table 2). One way to learn the effect individual hop acids have on the IBU test result is to measure their response factor, that is, divide the increase in IBU by the increase in hop acid concentration by HPLC. For example, the data in Table 2 shows that increasing the isoalpha acids concentration in beer from 36 to 49 ppm, an increase of 13 ppm, results in an increase of 9 IBU units. Therefore 9/13 = 0.7 or 1 ppm of isoalpha acids will measure ~ 0.70 IBU's. By adding 17 ppm of purified alpha acids to beer one gets an increase of 10.3 IBU; thus the response factor for alpha acids is 10.3/17 = 0.6. The addition of 14.8 ppm of purified humulinones causes the IBU to increase by 8.9, thus the response factor for humulinones is 8.9/14.8 = 0.60. The reason for the different response factors is due to the fact that at the wavelength used for the IBU test (275 nm) each and every hop acid can absorb light differently. Thus alpha acids and humulinones absorb 86 percent as much as isoalpha acids in the IBU test method. To see if these three hop acids are responsible for a dry hopped beer's IBU result, a control beer containing 51 ppm

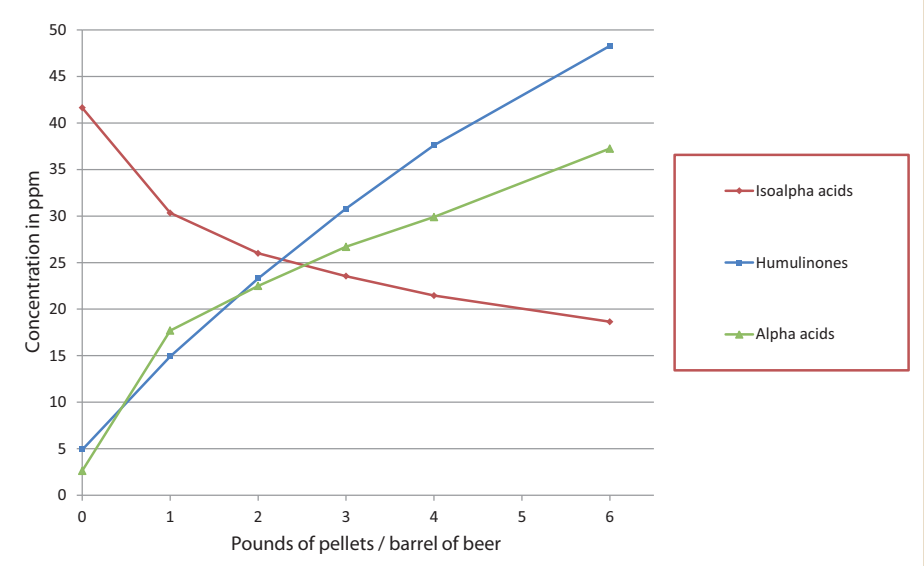

Fig. 3 Hop acid concentration vs. lbs/bbl Cascade pellets dry hopped

DETERMINING THE IBU RESPONSE FACTOR FOR ISOALPHA ACIDS, ALPHA ACIDS, AND HUMULINONES IN BEER

Sample	ppm isoalpha acid	ppm alpha acids	ppm humulinone	IBU	Δ IBU Δ Hop Acid
36 ppm isoalpha acid beer	35.8	4.3	1.3	34.8	
36 ppm IAA beer + 12.9 ppm IAA	48.7	4.3	1.3	43.9	0.70
36 ppm IAA beer + 17.1 ppm AA	35.8	21.4	1.3	45.1	0.60
36 ppm IAA beer + 14.8 ppm humulinone	35.8	4.3	16.1	43.7	0.60

Table 2 *IAA refers to isoalpha acids and AA refers to alpha acids*

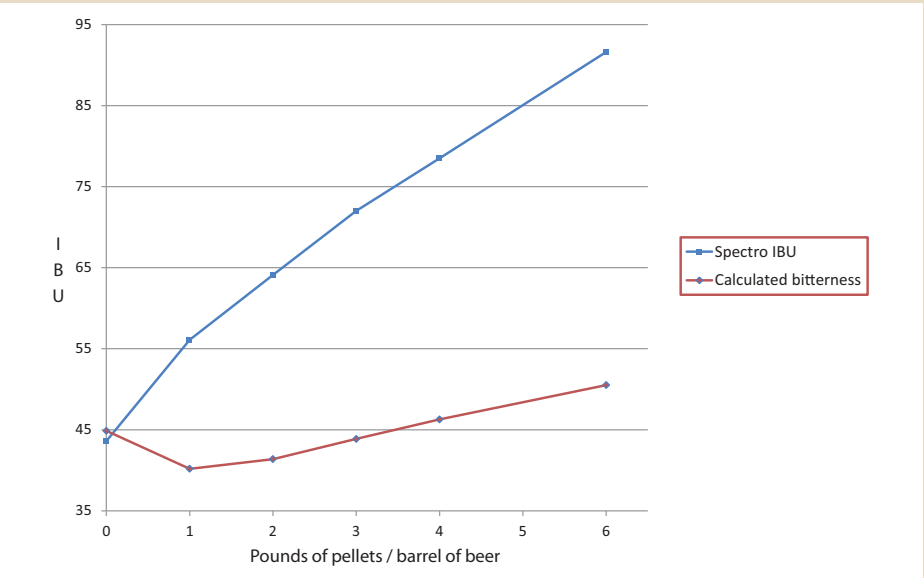

Fig. 4 Calculated bitterness & IBU vs. lbs/bbl Cascade pellets dry hopped

ABOUT 12 % OF THE IBU TEST RESULTS COME FROM OTHER HOP COMPOUNDS

Sample	ppm isoalpha acid	ppm alpha acids	ppm humulinone	IBU	Δ IBU
Control beer	51	-	-	40	
Control + 1 lb/bbl Cascade hop pellets	32	13	13	49	+9
32 ppm IAA beer + 13 ppm AA + 13 ppm humulinone	32	13	13	43	+3

Table 3 *IAA refers to isoalpha acids and AA refers to alpha acids*

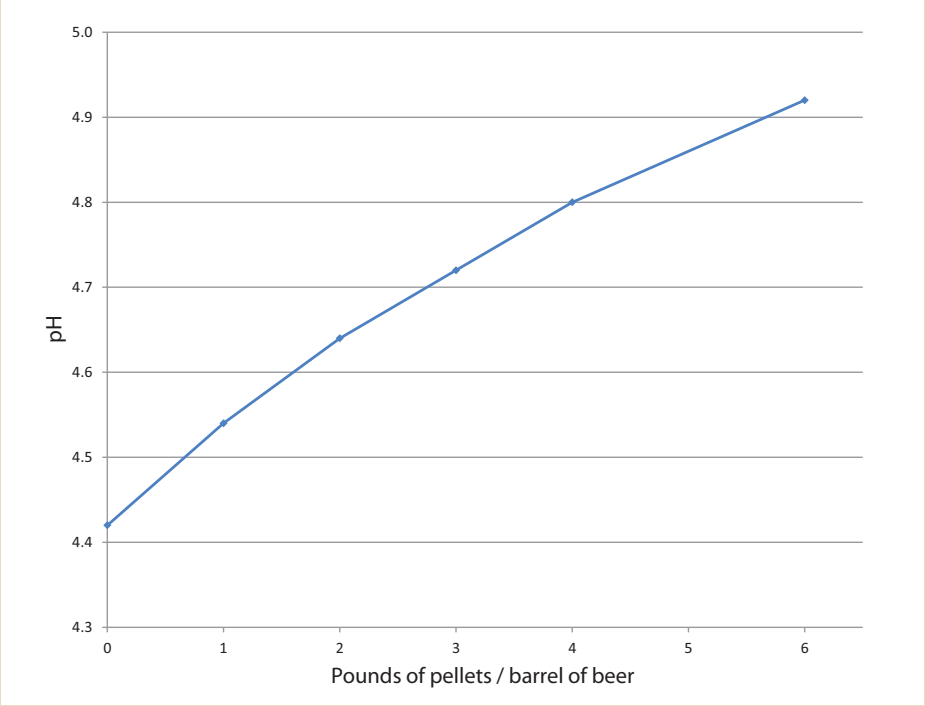

Fig. 5 pH of beer vs. lbs/bbl Cascade pellets dry hopped

of isoalpha acids was dry hopped with one pound of Cascade hop pellets for three days at 16 °C. Following HPLC analysis of the dry hopped beer, a non-dry hopped beer was spiked with alpha acids and humulinones and isoalpha acids to mimic the hop acid composition of the dry hopped beer. All three beers were assayed by the IBU test method (Table 3), and the results show that the beer with a hop acid composition similar to the dry hopped beer had an IBU measurement of 43, whereas the dry hopped beer measured 49. That means dry hopping adds additional hop compounds that increase a beer's IBU, in this example by twelve percent. Given that dry hopping causes a change in a beer's hop acid composition and that the IBU response factors of each hop acid is different means the IBU test cannot be used to estimate a dry hopped beer's bitterness.

Dry Hopping and pH

There are some unknown compounds in the leaf material of hops that's responsible for the increase in beer pH when one dry hops [9]. To study this in more detail the beers that were dry hopped with 0, 1, 2, 3, 4, and 6 lbs/bbl Cascade hop pellets for three days at 16 °C had their pHs measured. The results showed that dry hopping increased the pH by about 0.1 pH units per pound of hop pellets dosed and the effect is nearly linear (Fig. 5). It should be noted that this increase can vary somewhat based on a particular beers buffering capacity. Brenner [6] and Meilgaard [7] reported that beers with the same isoalpha acid concentration tasted more bitter at higher pHs vs. lower pHs. It was also reported by Rigby [8] that hop acids in their dissociated form have a stronger or more intense bitterness than hop acids in their undissociated form; the higher the pH the more dissociated the hop acids. To test these observations, a dry hop beer having a pH of 4.91 was treated with ten percent sulfuric acid to reduce its pH to 4.5. The beer with the higher pH tasted slightly more bitter than the beer with the lower pH, confirming what Brenner, Meilgaard and Rigby reported. Brewers concerned about the microbiological stability of a high pH dry hopped beer shouldn't worry given the large quantities of alpha acids in those beers. Alpha acids are extremely antibacterial and are reported to inhibit gram positive bacteria growth at concentrations as low as two ppm [16].

Conclusion/Summary

Brewers who dry hop a high IBU beer, 40 ppm of isoalpha acids or more, with one to two pounds of hops per barrel of beer can significantly alter the hop acid composition of beer and reduce its bitterness. However, dry hopping with three pounds per barrel or more can actually make the beer more bitter. A substantial amount of isoalpha acids can be removed from a high IBU beer when one dry hops; however little isoalpha acids are removed when the isoalpha acid concentration in the beer is 25 ppm or less. Thus dry hopping a beer containing 25 ppm isoalpha acids or less can make a beer more bitter due to the incorporation of humulinones and alpha acids. Low bitter humulinones are very soluble in beer and very low bitter alpha acids can also dissolve into the beer but not as efficiently. Because isoalpha acids, humulinones, and alpha acids absorb light at 275 nm differently, the wavelength used for the IBU test method, the IBU test cannot be used to estimate a dry hopped beer's bitterness or even measure the total hop acid concentration. Only HPLC can accurately measure the concentrations of these three individual hop acids and by taking into account their relative bitterness, to that of isoalpha acids, one can calculate a beers bitterness to better estimate its perceived bitterness. Dry hopping also increases the

pH of beer by about 0.1 pH units per pound of hops added per barrel of beer and this increase can lead to a slight increase in the sensory bitterness but it's small, about 2 - 3 IBU.

The follow-up article will be published in BRAUWELT International No. 2, 2018 and discuss the effect of dry hopping on beer foam stability. ■

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