Dry Hopping and Its Effects on the International Bitterness Unit Test and Beer Bitterness

John Paul Maye and Robert Smith
S.S. Steiner, Inc., New York, NY 10065, U.S.A.

ABSTRACT

The international bitterness unit (IBU) test was developed to measure the iso-α-acid concentration in finished beer and give the brewer a general sense of his beer's sensory bitterness. This test method was not designed for nor did it take into account beers that are heavily dry hopped. Craft brewers performing the IBU test on dry-hopped beers are finding that the IBU test results do not correlate with the bitterness they taste in their beers. Many brewers are unaware that dry hopping can significantly alter the hop acid composition within their beer. High-performance liquid chromatography was used to accurately measure the hop acid composition of dry-hopped beers, and purified hop compounds were added to non-dry-hopped beers to simulate the hop acid composition of dry-hopped beers. The IBUs of the spiked beers were measured to see what effect non-iso-α-acid hop acids have on the IBU test. The results of this work will explain why and how dry hopping affects the IBU test and beer bitterness.

Keywords: Dry hopping, International bitterness unit (IBU), Bitterness

In 1955 Rigby and Bethune published their research showing α-acids within hops isomerize into iso-α-acids when hops are boiled and that iso-α-acids are primarily responsible for beer bitterness (10). It was not long before analytical test methods were developed to measure the concentration of iso-α-acids in beer (3,4,7). Ultimately, in 1968 the world-leading brewing organizations agreed on one method for measuring beer bitterness, the international bitterness unit (IBU) test (2). This spectrophotometric test focused on measuring the iso-α-acids in beer, and it did not take into account beers that are heavily dry hopped (i.e., beers dry hopped with a dose rate of 1 lb of hops, or more, per barrel of beer). It was generally accepted over the years that other hop compounds contribute bitterness, and the IBU test was regarded more as a close estimate of the iso-α-acid concentration in beer, and for the most part it worked quite well. Recently, it was reported that beers that are heavily dry hopped can lose a large percentage of iso-α-acids owing to absorption onto the leafy material and can contain large amounts of non-bitter α-acids and large amounts of low-bitter humulinones (9). Humulinones are reported to be 66% as bitter as iso-α-acids (1,12,13), and their molecular structure (5,11) is nearly identical to iso-α-acids, except they contain an additional hydroxyl group (Fig. 1). α-Acids and humulinones absorb at 275 nm, the wavelength used to measure iso-α-acids via the IBU test, but their extinction coefficients are different from iso-α-acids; thus, their concentrations are not measured accurately. The IBU test was not designed to measure α-acids or humulinones, only iso-α-acids. In dry-hopped beer, the IBU test measures all the iso-α-acids, most of the α-acids, and most of the humulinones, but it cannot differentiate what hop acid it is measuring. The following experiments and tests will explain why the IBU test results increase with dry hopping and why the dry-hopped beers taste less bitter. To better understand the significance dry hopping has on the IBU test, dry-hopped beers were compared with several non-dry-hopped beers spiked with purified iso-α-acids, humulinones, α-acids, and combinations thereof and analyzed by HPLC and the IBU test.

Experimental

HPLC analysis was conducted following EBC 7.7 and the corresponding HPLC calibration standard, ICE-3, for α-acids. HPLC method EBC 7.9 and HPLC calibration standard ICS-I3 were used for iso-α-acid analysis. A humulinone–dicyclohexylamine HPLC calibration standard (8) was produced in-house and used to calibrate the HPLC for humulinone analysis.

The IBU test was performed as described in the ASBC Methods of Analysis, Method Beer 23A (2).

Beers containing 32 and 51 ppm of iso-α-acids were prepared by adding a dilute aqueous solution of iso-α-acids (Hopsteiner Iso-Extract 30%) to a commercial beer containing 11 ppm of iso-α-acids, 0 ppm of α-acids, and 0 ppm of humulinones. Beer (300 g) assaying 51 ppm of iso-α-acids by HPLC 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. Cascade hop pellets con-

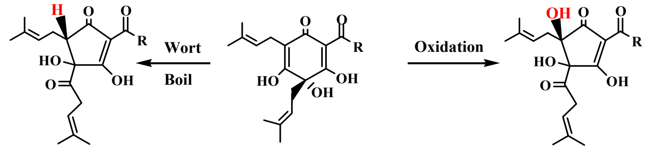

Figure 1. The molecular structures of iso-α-acids, α-acids, and humulinones.

E-mail: jmaye@hopsteiner.com

http://dx.doi.org/10.1094/TQ-53-3-0808-01

taining 6.1% α-acids and 0.37% humulinone were added to each bottle at a dose rate equivalent to 1 lb/bbl, and then the air was removed in a vacuum desiccator, CO_2 added, and the bottle capped. "Bottle dry hopping" gives nearly identical results to pilot-brewery-scale dry hopping. Samples were stored at 16°C for three days prior to HPLC analysis. Beer was filtered through a Whatman GF/F filter (≈0.7 µm), and 5 mL was diluted to 10 mL with acidic methanol; 10 µL was injected onto the HPLC column.

Small-scale simulated dry-hopping was conducted by spiking beer with the appropriate amount of purified and diluted potassium salt solutions of α-acids (Hopsteiner AlphaExtract 20%), iso-α-acids (Hopsteiner Iso-Extract 30%), and 10% humulinone (prepared in lab) to correspond to the levels found in a dry-hopped beer, separately and combined. Five samples of each beer were prepared, and their average HPLC results were compared with average IBU test results.

To 300 g of degassed commercial IPA beer assaying 43.2 ppm of iso-α-acids, 5.5 ppm of humulinones, and 1.1 ppm of α-acids was added 1.5, 3.0, and 4.5 mg of Cascade steam-distilled hop oil dissolved in isopropyl alcohol. Air was removed from the neck of the bottle under vacuum, and nitrogen was added back and the bottle capped. Bottles were stored two days at 19°C and then as analyzed via the IBU test.

Results and Discussion

Beers containing 51 ppm of iso-α-acids as measured by HPLC were dry hopped at a dose rate of 1 lb/bbl using Cascade hop pellets containing 6.1% α-acids and 0.37% humulinone. Following three days of dry hopping at 16°C, the beers were assayed by HPLC (Fig. 2) and shown to contain on average 32 ppm of iso-α-acids, 13 ppm of α-acids, and 13 ppm of humulinone (Table 1). Dry hopping under these conditions caused a 19 ppm loss of iso-α-acid bitterness, and because humulinones are 66% as bitter as iso-α-acids it only added 8.5 ppm of bitterness (13 ppm × 0.66 = 8.5 ppm). By using HPLC one can calculate the bitterness of a beer by adding the bitterness intensities of the various hop acids relative to iso-α-acids. Thus, the dry-hopped beer had a calculated bitterness of 32 + (13 × 0.66) = 40.5, whereas the pre-dry-hopped beer had a calculated bitterness of 51. This difference in calculated bitterness explains why many craft brewers experience less bitterness after they dry hop. α-Acids have been reported to contribute no bitterness to beer at concentrations below 14 ppm (6), so their contribution to the calculated bitterness is zero. Bitterness is calculated as follows: **calculated bitterness (HPLC) = ppm iso-α-acids + (0.66 × ppm humulinone).**

When the dry-hopped beers were assayed via the IBU test method the IBUs increased to 49, compared with the pre-dry-hopped beers (IBU = 40), even though the HPLC calculated bitterness of the dry-hopped beer went down to 40.5 from 51 (Table 1). To better understand the effect various hop acids have on the IBU test, non-dry-hopped beers were treated with purified iso-α-acids, α-acids, and humulinones and assayed by HPLC and the IBU test (Table 2). One way to compare the effect hop acids have on IBUs is to look at the response factor, that is, divide the increase in IBU by the increase in the concentration for that hop acid by HPLC. For example, the data in Table 2 show that increasing the iso-α-acids concentration in beer from 32.5 to 51 ppm, an increase of 18.5 ppm, resulted in an increase in IBU of 13 IBU units. Therefore, 13 ÷ 18.5 = 0.7, or 1 ppm of iso-α-acids will measure ≈0.70 IBU. By adding 13 ppm of purified α-acids to beer, one gets an IBU increase of 8. Thus, the response factor for α-acids is 8 ÷ 13 = 0.62. The addition of 13 ppm of purified humulinones causes the IBU to increase by 7; thus, the response factor for humulinones is 7 ÷ 13 = 0.54. The reason for the different response factors is because each and every hop acid absorbs light differently and thus has a different extinction coefficient. Thus, α-acids and humulinones contribute about 88.5 and 77%, respectively, as much to the IBU measurement as iso-α-acids. The spiked beer that had a hop acid composition similar to the dry-hopped beer had an IBU of 43, which is about 12% less than the 49 IBU of the actual dry-hopped beer. That means dry hopping adds hop

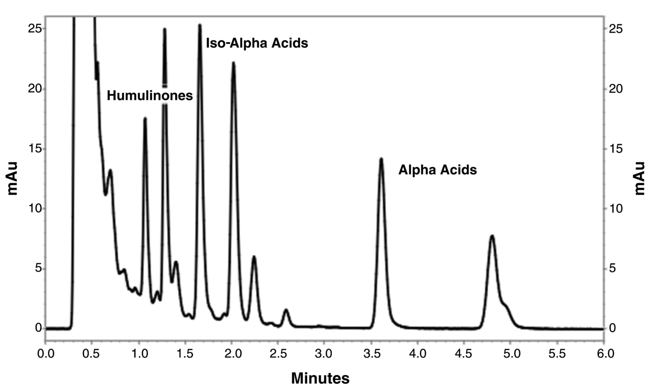

Figure 2. HPLC trace of dry-hopped beer.

Table 1. HPLC and IBU analysis of control beer and dry-hopped beer after three days of dry hopping[a]

Sample	IAA (ppm)	AA (ppm)	Humulinone (ppm)	IBU	Calculated bitterness
Control	51	0	0	40	51
Control + 1 lb/bbl Cascade hop pellets	32	13	13	49	40.5

[a] IAA = iso-α-acids, and AA = α-acids. Calculated bitterness (HPLC) = ppm IAA + (ppm humulinone × 0.66).

Table 2. Hop acid concentration in beer by HPLC versus IBU test[a]

Sample	IAA (ppm)	AA (ppm)	Humulinone (ppm)	IBU
Control	51	…	…	40
Control + 1 lb/bbl Cascade hop pellets	32	13	13	49
32 ppm IAA beer	32.5	…	…	27
32 ppm IAA beer + 13 ppm AA	32	13	…	35
32 ppm IAA beer + 13 ppm humulinone	32	…	13	34
32 ppm IAA beer + 13 ppm AA+13 ppm humulinone	32	13	13	43

[a] IAA = iso-α-acids, and AA = α-acids.

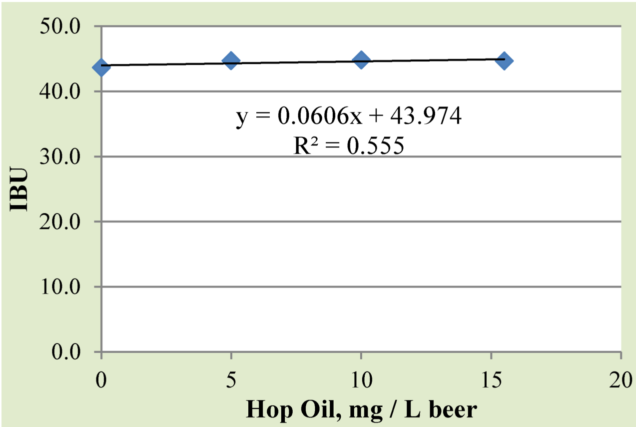

Figure 3. Hop oil and its effect on the IBU test result.

compounds other than iso-α-acids, humulinones, and α-acids that increase the IBUs by about 12%. Reverse-phase HPLC analyses of the IBU test isooctane layer from the dry-hopped beers revealed hop compounds that eluted before humulinones and thus are more polar and beer soluble. Hop compounds that elute this early tend to be highly oxidized hop acids of very low bitterness intensity, such as humulinic acid. Given that dry hopping causes a change in a beer's hop acid composition and that the IBU response factors of each hop acid are different, the IBU test cannot be used to estimate a heavily dry hopped beer's bitterness. By performing HPLC analyses on dry-hopped beers one can more accurately compare sensorial bitterness to calculated bitterness.

Brewers who dry hop are generally interested in incorporating hop oil into their beer. To see if hop oil affects the IBU test result, beer measuring 43 IBU was treated with 5, 10, and 15 ppm of Cascade steam-distilled hop oil (Fig. 3). The results indicate, at the dose levels tested, hop oil does not absorb at 275 nm, the wavelength used to measure IBU, and thus does not affect the IBU test result.

Summary and Conclusion

HPLC analysis of beers that are heavily dry hopped show dry hopping removes a considerable amount of iso-α-acids and adds nonbitter α-acids and less bitter humulinones to beer. Both α-acids and humulinones are measured via the IBU test but not accurately, because the IBU test cannot differentiate between what hop acids it is measuring and because the different hop acids have different response factors. This means the IBU test cannot even be used to measure the total hop acid concentration in beer. In addition, the IBU test of dry-hopped beers also measures hop compounds other than iso-α-acids, humulinones, and α-acids; such hop compounds are expected to further exacerbate the difference between the IBU result and a beer's sensory bitterness. HPLC analysis allows one to separate and accurately measure the various hop acids in dry-hopped beers. Once the hop acid composition and concentrations are determined one can use that data to calculate a beer's bitterness. Brewers concerned about achieving consistent bitterness of their dry-hopped beers may want to consider comparing sensory bitterness to HPLC calculated bitterness. Finally, hop oil, one of the main reasons brewers dry hop, does not affect the IBU test results.

REFERENCES

1. Algazzali, V. A., and Shellhammer, T. 2016. Bitterness intensity of oxidized hop acids: Humulinones and hulupones. J. Am. Soc. Brew. Chem. 74:36-43.
2. ASBC Methods of Analysis. Beer Method 23A. Bitterness units (International Method). Approved 1968, rev. 1975. American Society of Brewing Chemists: St. Paul, MN. doi: 10.1094/ASBCMethod-Beer-23.
3. Bishop, R. L. 1967. European Brewery Convention: The E.B.C. scale of bitterness. J. Inst. Brew. 73:525-527.
4. Brenner, M. W., Vigilante, S., and Owades, J. L. 1956. A study of hop bitters (isohumulones) in beer. Proc. Am. Soc. Brew. Chem., 48-61.
5. Cook, A. H., and Harris, G. 1950. The chemistry of hop constituents. Part I. Humulinone, a new constituent of hops. J. Chem. Soc., 1873-1876.
6. Fritsch, A., and Shellhammer, T. H. 2007. Alpha acids do not contribute bitterness to lager beer. J. Am. Soc. Brew. Chem. 65:26-28.
7. Howard, G. A. 1968. Institute of Brewing analysis committee estimation of the bitterness of beer. J. Inst. Brew. 74:249-251.
8. Maye, J. P., Smith, R., and Leker, J. 2016. Preparation of dicyclohexylamine humulinones and dicyclohexylamine hulupones. J. Am. Soc. Brew. Chem. 74:57-60.
9. Maye, J. P., Smith, R., and Leker, J. 2016. Humulinone formation in hops and hop pellets and its implications for dry hopped beers. Tech. Q. Master Brew. Assoc. Am. 53:23-27.
10. Rigby, F. L., and Bethune, J. L. 1955. Rapid methods for the determination of total hop bitter substances (iso-compounds) in beer. J. Inst. Brew. 61:325-332.
11. Shoolery, J. N., Verzele, M., and Alderweireldt, F. 1960. On the structure of humulinone. Tetrahedron 9(3-4):271-274.
12. Verzele, M., and De Keukeleire, D. 1991. *Chemistry and Analysis of Hop and Beer Bitter Acids*. Elsevier Science: Amsterdam, the Netherlands, p. 52.
13. Whitear, A. L., and Hudson, J. R. 1964. Hop resins and beer flavor III: Hop resins in beer. J. Inst. Brew. 70:24-30.