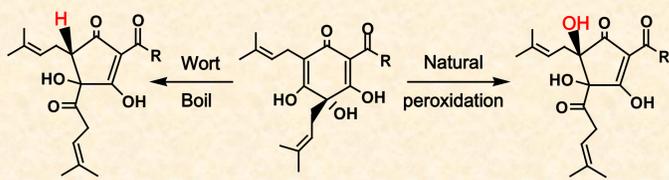


Introduction

In 2004 we reported that Humulinones, a hop bitter acids, are found in dried leaf hops and hop pellets and are closely related to iso- α -acids (1) **Fig. 1**. Humulinones can be produced in the laboratory via peroxidation of α -acids (2).

Fig 1. Formation of Iso- α -acids and Humulinones From Alpha Acids



R= isopropyl, isobutyl, sec-butyl

Humulinones are more polar and much more soluble in beer than iso- α -acids. When hops are added late in the kettle or dry-hopped, Humulinones readily dissolve and some dry-hopped beers contain levels high enough to influence bitterness (1). Recently it was reported that Humulinones are 65% as bitter as iso- α -acids (3).

Methods

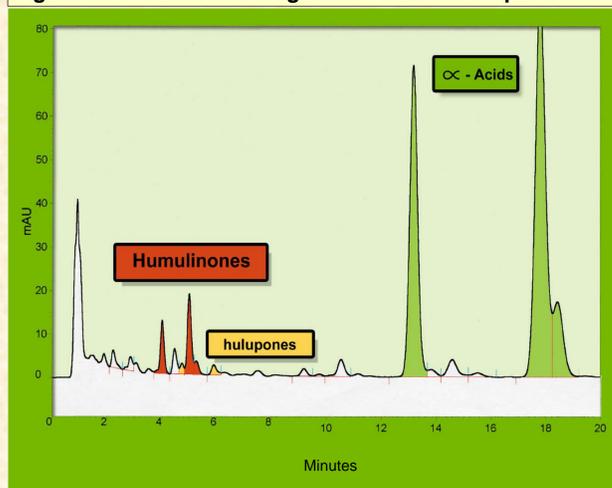
Extraction of Hops and Hop Pellets; HPLC Analysis

To 2.50 g of hop pellets or 5.00 g of ground hop cones were added 50 mL (or 100 mL) of acidic methanol (0.5 mL of 85% α -phosphoric acid in 1 L of methanol). This mixture was then extracted for 5 min. using a water bath sonicator. The subsequently filtered and diluted samples were eluted through a 3 μ m, C18 HPLC column using the mobile phase of Analytica-EBC method 7.9 (4); PDA integration was at 270 nm.

The rapid method of extraction resulted in a concentration of α -acids nearly identical to that of the current ASBC method of HOPS-14 (5).

Lab prepared Humulinone (2) was treated with one equivalent of dicyclohexylamine (DCHA) in methanol to make a Humulinone-DCHA salt. This salt was recrystallized twice in methanol, filtered, dried and determined to be 66.5% Humulinone. The Humulinone-DCHA salt was used as an HPLC standard. The α -acids were measured by HPLC using the ICE-2 standard. The relative elution of Humulinones and α -acids is shown in **Fig. 2**.

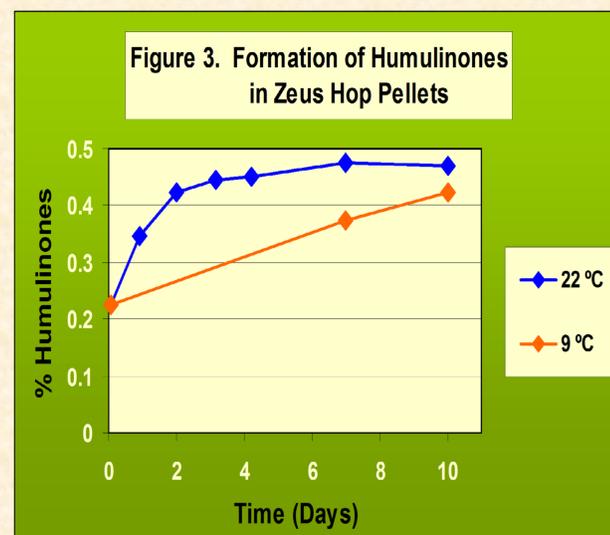
Figure 2. HPLC Chromatogram of Cascade Hop Pellets



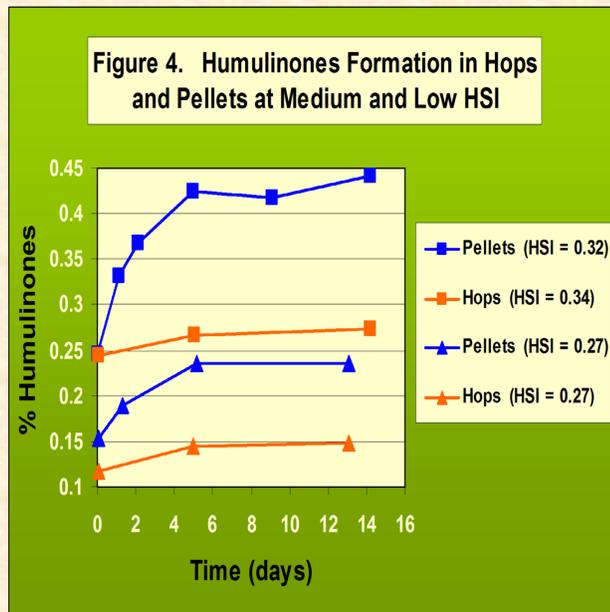
Results and Discussion

Humulinone Formation in Hops and Hop Pellets

Several varieties of freshly baled hops and their corresponding pellets were vacuum packed yet, in the near absence of air, showed an increase in Humulinone formation within days in the foil. This formation was faster at room temperature and slower at colder temperatures but formation appears to be unavoidable. (**Fig. 3**). What causes this post-pelleting formation of Humulinones is not yet known, however, we know air oxidation of alpha acids is one way Humulinones form.

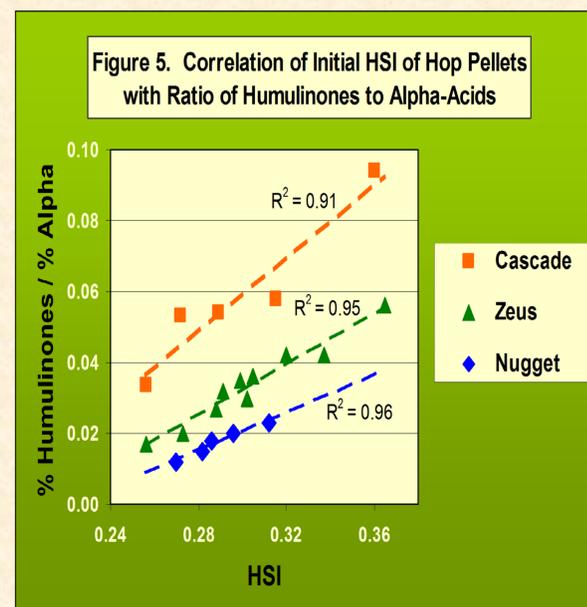


Humulinones form in baled hops at a slower rate and lower concentration than in hop pellets. The formation of Humulinones implies an equivalent loss of α -acids; however, this is not the case. α -acids are better preserved when pelletized and packaged under vacuum vs left in the bale. Furthermore, within any single variety of hops we see a correlation, the higher the HSI the higher the Humulinones concentration within the hops. **Fig. 4**



HSI and Humulinones Formation in Hop Pellets

An interesting correlation was found between the HSI of hop pellets, soon after pelletization, and the ratio of Humulinones to α -acids ("Humulinones Ratio") **Fig. 5**. We speculate that more highly oxidized hops contain more reactive oxygen species capable of oxidizing α -acids to their corresponding Humulinones.



Humulone Utilization in Dry-Hopped Beer

Centennial hop pellets measuring 0.35%w/w Humulinone by HPLC were used in a dry-hopping experiments to measure its utilization. Two beers were dry-hopped, a low IBU beer, measuring 8.6 ppm iso- α -acid and a high IBU beer, measuring 48 ppm iso- α -acids, both measured by HPLC. The beers were dry-hopped for three days at three levels plus a control, 0.5 lbs/barrel, 1.0 lbs/barrel, and 2.0 lbs/barrel. Regardless of the starting IBU between 87- 98% of the Humulinones in the hops dissolved into the beer. Interestingly, dry-hopping caused a decrease in iso- α -acids concentration in the high IBU beers however it had little effect with the low IBU beers, **Table 1**. Given that Humulinones are 65% as bitter as iso- α -acids, the loss in iso- α -acids in the high IBU beer is mostly offset by an increase in Humulinone concentration whereas the increase in Humulinones in the low IBU beers greatly impacted the bitterness of that beer.

Table 1. Humulinone & Iso- α -acid Concentration In Dry-Hopped Beer

Sample	lbs hop pellets Barrel of beer	ppm of Humulinone in beer	% Utilization Humulinone	ppm of Iso- α -acid in beer	*Calculated Bitterness Intensity
Low IBU Beer	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	0	1	-	48	49
	0.5	8	98	39	44
	1.0	14	91	35	44
	2.0	27	87	30	47

*ppm of Iso- α -acids + (ppm Humulinone x 0.65)

Implications for Dry-hopping

Hop pellets from less stable aroma varieties such as Cascade hops had higher Humulinones ratio than a more stable Nugget hops (**Fig. 5**). Heavily hopped dry-hopped beers using large dosages of Cascade or Centennial hops, are likely to contain relatively high levels of Humulinones. These levels of Humulinone can impact the bitterness of low IBU beers where as they may actually compensate for the loss of iso- α -acids in high IBU beers. We assayed 29 commercial india pale ales and found an average of 12 ppm Humulinones (range of 3 to 24 ppm). Given Humulinones relative bitterness of 0.65 IBU relative to iso- α -acids, 12 ppm of Humulinone would contribute about 8 IBU's of bitterness to beer.

Absence of Humulinones in CO₂ Extract

Supercritical CO₂ extraction of freshly prepared Cascade or Chinook hop pellets having concentrations of 0.1% and 0.3% Humulinones respectively resulted in CO₂ extracts containing no detectable Humulinones; via HPLC analysis and PDA spectrophotometer; detection limit of about 0.04%. It appears Humulinones are too polar to be solubilized by supercritical CO₂ extraction.

Conclusions

Hops and hop pellets (but not CO₂ extracts) contain mildly bitter Humulinones. Their formation post-kilning or post-pelleting is time and temperature dependent. Varietal factors are evident, and the HSI of the hops correlates positively, suggesting that oxidative damage during kilning or storage may encourage their formation. Beers dry-hopped with hops that contain high levels of Humulinone will see very high levels of incorporation into the beer and this can alter a beers bitterness.

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