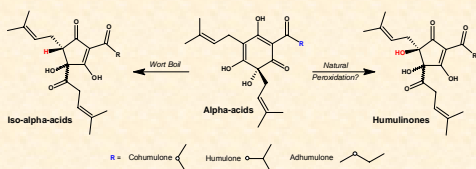


Introduction

Humulinones are hop bitter acids that have been identified in hops and hop pellets (1,2) and have been produced in the laboratory via peroxidation of α -acids (3). They are closely related to the iso- α -acids, having an extra hydroxyl group in the cyclopentenone ring (Fig. 1):

Figure 1. Formation of Iso- α -acids and Humulinones from Alpha-acids



Humulinones are slightly more polar than the iso- α -acids and, having a pK_a of 2.8 (3), they will be mostly in the ionized form in beer. When hops or hop pellets are added late in the kettle boil, or used for dry-hopping, these compounds should be readily dissolved, and in fact humulinones have been identified in some dry-hopped beers at levels sufficiently high to influence the beer bitterness (1).

Humulinones added to beer are reported to be about 0.4 times as bitter as iso- α -acids (4).

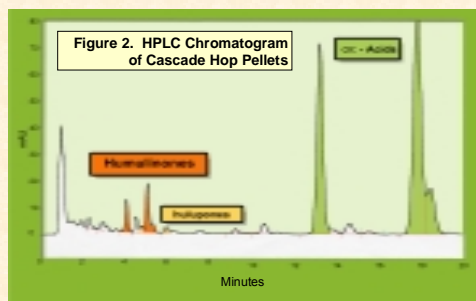
Methods

Extraction of Hops and Hop Pellets; HPLC Analysis

To 2.50 g of hop pellets or 5.00 g of ground hops were added 50 mL (or 100 mL) of acidic methanol (0.5 mL of 85% o-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 (5); 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 (6).

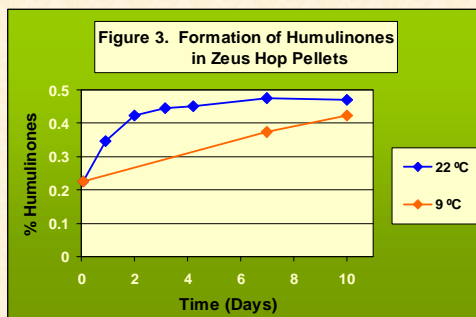
The concentrations of humulinones (calculated as for iso- α -acids) and α -acids were determined by using ICS-11 and ICE-2 standards, respectively. The relative elution of humulinones and α -acids is shown in Fig. 2.



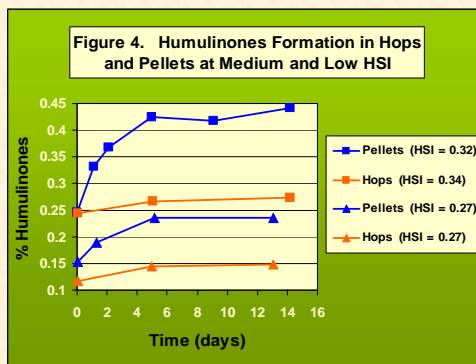
Results and Discussion

Factors affecting Humulinones Formation in Hops and Hop Pellets

When hops - of several different varieties - were pelleted (and then vacuum packed) a relatively rapid increase in the concentration of humulinones was found to take place, typically reaching a half-maximal concentration in less than 2 days, and an apparent maximum in about 6-10 days (Fig. 3). However, for some samples (not shown) a continuing, very slow increase in concentration was observed. The initial rate of formation was considerably slower in refrigerated samples (Fig. 3).



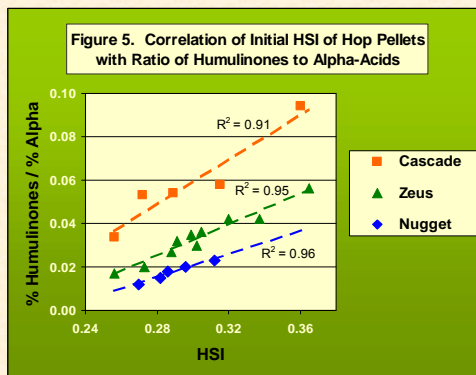
Humulinones were found to form in kiln-dried hops (sampled at time of pelleting and stored in plastic bags) at a slower rate than in hop pellets, as shown for ambient temperature-stored Galena hops and pellets in Fig. 4. Furthermore, pellets made from hops having a relatively high hop storage index (HSI) typically developed a higher concentration of humulinones.



Formation of humulinones implies an equivalent loss of α -acids; however, long-term decline of α -acids content in baled hops is nevertheless almost always much greater than in corresponding vacuum-packed pellets.

HSI and Humulinones Formation in Hop Pellets

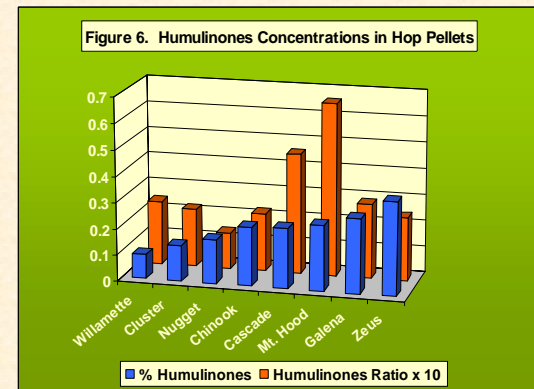
A good correlation was found between the HSI of hop pellets and the ratio of humulinones to α -acids ("Humulinones Ratio"), as shown in Fig. 5. We speculate that more highly oxidized hops may contain more reactive peroxides (possibly lipid peroxides and/or endogenous H₂O₂) capable of oxidizing α -acids to their corresponding humulinones.



Humulinones Concentration and the Humulinones Ratio in Commercial Hop Pellets, as related to Hop Variety

The humulinones content and the humulinones ratios ("HR") for pellets of eight US hop varieties are presented in Fig. 6, these values being shown for each variety at an HSI value of 0.27 (two values having been obtained by minor extrapolation).

Nugget hop pellets had the lowest HR value. This variety has good storage stability. On the other hand, Cascade and Mt. Hood hop pellets had the highest HR, and it is possibly not coincidental that these two varieties of hops have relatively poor storage stability. However, if so, it is not clear why this did not also apply to the relatively unstable variety, Zeus. The pellets of this super high-alpha variety did, though, have the highest actual concentration of humulinones.



Absence of Humulinones in CO₂ Extract

Mild supercritical CO₂ extraction of freshly prepared Cascade or Chinook hop pellets (having concentrations of humulinones of 0.1 and 0.3% respectively) resulted in CO₂ extracts containing no detectable humulinones (via HPLC analysis and PDA spectrophotometer; detection limit of about 0.04%).

Implications for Dry-hopping (or Late Kettle Hop Addition)

Heavily hopped beers, perhaps especially if dry-hopped with pellets of an unstable variety such as Cascade, are likely to contain relatively high levels of humulinones. (Indeed, in a limited survey, we found that commercial dry-hopped ales had humulinones concentrations ranging from 4 to 18 ppm). Consequently, because of the lower bittering impact of the humulinones - relative to the iso- α -acids - their significant presence (and, typically, also that of non-bitter α -acids) in such beers will result in over-estimation of the perceived bitterness when tested by a spectrophotometric BU method (1).

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, but occurs most rapidly, and to greater extent, after pelleting. Varietal factors are evident, and the HSI of the hops correlates positively, suggesting that oxidative damage during kilning or storage may encourage later formation of humulinones.

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