Abstract
The bitterness of beer is expressed by the International Bitterness Units (IBU) scale which measures the concentration of isomerized hop alpha acids including isocohumulone, isohumulone and isoadhumlone (collectively called isohumulones). Two common factors accelerating beer deterioration include light or heat exposure, which leads to oxidation of isohumulones and develops off-flavors. To keep beer tasting fresh, preventing oxidation of isohumulones is necessary. The objective of this study was to determine the effect of antioxidants on the oxidation of isohumulones in beer exposed to light or heat. Three antioxidants (ascorbic acid, caffeic acid and crocin) and mixture were added at 0.1% level in fresh beer from a local microbrewery. Samples were exposed to blue light (460 nm) for 10 hours or placed in an oven at 70°C for 8 hours. Isohumulone levels were analyzed by HPLC in an isocratic mobile solution (75% methanol, 24% water, 1% phosphoric acid) using a C$_{18}$ column. Column temperature, flow rate, injection volume, and detector wavelength were set at 35°C, 0.65 mL/min, 5 µL, and 280 nm, respectively. Total isohumulone content (mg/L, IBUs) was calculated using a standard curve and data was analyzed using SPSS at a 3% confidence level. Results showed when beer samples were exposed to light, a mixture of ascorbic acid and crocin (AACR), ascorbic acid (AA) or crocin (CR) had a slower rate of decline (0.5, 0.9, and 1.1 IBUs per hour) than either control and caffeic acid (CA) (0.9 and 1.2 IBUs per hour). In samples exposed to heat, AACR, AA and CR also reduced the concentration of isohumulones (0.2, 0.3 and 0.6 IBUs per hour) than either control and caffeic acid (CA) (0.9 and 1.2 IBUs per hour). These results indicated that ascorbic acid and crocin were recommended for use in beer to mitigate the oxidation of isohumulones.

Introduction
• Hops impart a bitter flavor in beer which is necessary to balance the sweetness of the malt.
• The bitter compounds come from hop acids, α-acids (humulones) and β-acids (lupulones), found in the female flowers of the vinous plant humulus lupulus.
• Because hop resins are insoluble in water, they must be boiled during the brewing process until isomerized, and are then called isohumulones.
• In the presence of light and riboflavin (a photosensitizer), isohumulones irreversibly convert to mercaptans, which yield a “skunky” off-flavor in beer.
• In the brewing industry, brown glass packaging is commonly used to filter harmful light and modified hop extracts may be also be used (particularly in the case of beer sold in clear and green glass bottles).
• Because heat also increases the rate of oxidation, beer is often shipped and stored at refrigerated temperatures.

Objective
• To determine the effect of antioxidants (ascorbic acid, caffeic acid, and crocin) on the oxidation of isohumulones in beer by light or heat.

Methodology
1. Beer (a hoppy India Pale Ale) was sourced from a local microbrewery. Beer antioxidant treatments were prepared by mixing the antioxidants to a final concentration of 0.1% (w/w). The control samples contained no antioxidants. The antioxidants used include ascorbic acid, caffeic acid, crocin and ascorbic acid/crocin mixture (1:1 ratio).
2. Prepared beer samples were filtered into clear HPLC vials using a 0.45 µm membrane filter.
3. Samples were set on an orbital shaker under light (emitting wavelengths in the blue spectrum, 460 nm) for up to 10 hours, or were placed in an oven at 70°C for up to 8 hours.
4. After each time interval, three vials from the control and each antioxidant treatment were removed and analyzed by HPLC.
5. HPLC (Agilent 1260 system) analysis was performed with an isocratic mobile solution (75% methanol, 24% water, 1% phosphoric acid) and Agilent Zorbax C$_{18}$ column. Column temperature was 35°C, the flow rate was 0.65 mL/min, the injection volume was 5 µL and the detector was set to 280 nm. The chromatograms from the HPLC produced four peaks and the four areas under the curve were summed to compute the total isohumulones. The total area was compared with a standard curve to determine isohumulone content in mg/L (IBUs) and data was analyzed using SPSS at a 3% confidence level.

Results
• When exposed to light, a mixture of ascorbic acid and crocin, ascorbic acid or crocin had a slower rate of decline (0.2, 0.3, and 0.6 IBUs per hour) than either no treatment or caffeic acid (0.9 and 1.2 IBUs per hour).
• When exposed to heat, a mixture of ascorbic acid and crocin, ascorbic acid or crocin had a slower rate of decline (0.5, 0.9, and 1.1 IBUs per hour) than either no treatment or caffeic acid (1.7 and 1.9 IBUs per hour).
• These results indicate that ascorbic acid and crocin are recommended for use in beer to mitigate the oxidation of isohumulones.

Conclusion & Recommendations
• Ascorbic acid and crocin should be studied further to determine feasibility of use as an antioxidant in beer and the ideal concentration.
  o Ascorbic acid can impart a tart flavor to beer.
  o Crocin will add a yellow-orange hue.
• Caffeic acid could be studied further to determine whether the concentration level was a factor, but overall it is not recommended for use in beer.

References

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