HPLC determination of α- and β-acids in hops

Adam Sahlstrom and Stephen Rostad
Department of Chemistry, Concordia College, 901 8th St S, Moorhead, MN 56562

Abstract

The weight percents of six α- and β-acids found in common brewing hops were determined in three varieties of hops using HPLC analysis. The results confirmed data included by the hops supplier regarding the percent weight of hops present in each type. Data analysis indicated that multiple extractions should be used in order to increase extraction efficiency of α-acids.

Introduction

The many characteristics of a beer are caused by variations in the brewing process and the ingredients used. All beers consist of grain, hops, yeast, and water at a minimum. The grain is often barley but is also commonly rice or wheat. The grain provides the sugar maltose and sweetness of the beer. The yeast turns the maltose into ethanol and carbon dioxide. The bitterness and aroma of the beer is provided by the hops, the female flower of the hop plant.

The chemical source of hops’ bitterness are the α- and β-acids. The α-acids consist of humulone, cohumulone, and adhumulone. The β-acids consist of lupulone, colupulone, and adlupulone. α-acids undergo isomerization during the brewing process to produce the very bitter iso-α-acids, which are the primary chemical source of a beer’s bitterness. α-acids are significantly less bitter than the β-acids and are generally considered to impart undesirable characteristics to the beer.

Considering the significant impact α- and β-acids have on the flavor of beer, it is important for brewers to be able to accurately measure their concentration in order to maintain standards for a known brand or to create a new brew with desired characteristics.

The average α- and β-acid weight percent varies among the varieties of hops but is typically between 3 and 15% with β-acid concentration between 2 and 8%. The concentrations of the α- and β-acids can be determined by a HPLC separation followed by UV-Vis detection. In this experiment the α- and β-acid concentration in the Chinook, Challenger, and Bullion varieties of hops using HPLC and international calibration extract III (ICE-III) for calibration are determined.

Experimental

The method was followed according to Baker HPLC analysis of α- and β-acids in hops.
Extraction Solvent Preparation

An extraction solvent was prepared in a 1-L plastic bottle by mixing 509 mL of HPLC grade methanol with 91 mL of ultrapure, deionized, and degassed water making a total volume of 600 mL. The solution was then acidified with 0.17 mL of 88% formic acid.

Sample Preparation

Three different varieties of hops were obtained in pellet form in sealed 1-oz packages from RiteBrew.com. The varieties used were Bullion, Challenger, and Chinook. 0.5780 g of Bullion, 0.5866 g of Challenger, and 0.4970 g of Chinook hops were weighed separately on an analytical balance and then quantitatively transferred to three separate 100-mL beakers. Aliquots of extraction solvent of approximately 20 mL were then added to each beaker. A glass stir rod was used to crush the hops pellets into powder. Magnetic stir bars were then added to each beaker and each was allowed to stir for an hour on a magnetic stir plate. Following that hour, the contents of each beaker was vacuum filtered separately using medium porosity filter paper. The solid collected on the filter paper was rinsed with approximately 10 mL of extraction solvent in 2-mL aliquots and all of the filtrate was collected. Following filtration, the solid was disposed of and each filtrate was quantitatively transferred to a labeled 50-mL volumetric flask. Each was diluted to volume using extraction solvent. Small volumes of these solutions were then filtered directly into HPLC sample vials using 0.45-μm nylon syringe filters. One sample of each hop variety was prepared by this method.

ICE-3 Standard Preparation

International calibration extract 3 (ICE-3) for HPLC analysis of α-acids and β-acids was obtained from the American Society of Brewing Chemists in a 40-g jar. The extract contained 44.64% total α-acids (13.88% cohumulone, 30.76% n+adhumulone), 24.28% β-acids (13.44% colup8ulone, 10.34% n+adlupulone). 0.1482 g of this standard was weighed into a 100-mL beaker on an analytical balance. The standard was then dissolved in approximately 20 mL of extraction solvent by stirring. This solution was then quantitatively transferred to a 50-mL volumetric flask and diluted to volume with extraction solvent. Approximately 25 mL of this solution was then filtered through a 0.45-μm nylon syringe filter into a 100-mL beaker. Different volumes of this filtrate were then transferred to five autosampler vials labeled 1 through 5. The volumes were as follows: Std. 1=40 μL standard solution/160 μL extraction solvent; Std. 2=80/120; Std. 3=120/80; Std. 4=160/40; Std. 5=2 mL of standard solution. The standards were then diluted to 1/20 of their concentration in order to prevent overloading of the HPLC. These five solutions formed the standards for HPLC analysis.

HPLC Analysis

All five standards were run through a Kinetex 2.6u XB-C18 100A column 50 mm x 4.6 mm purchased from Phenomenex. The separation was isocratic with a mobile phase consisting of 80% methanol and 20% water acidified with formic acid to a pH ~2.5 at a
flow rate of 1mL/min and temperature of 35°C. The HPLC used was a Varian Prostar equipped with a Varian Prostar 325 LCD detector. The absorbances of the analytes were detected at a wavelength of 326 nm.

Results and discussion

Figures 1-4 show a standard chromatogram and the chromatograms for each of the samples. The peaks at ~2.8, 3.7, 4.9, and 6.3 matched the cohumulone, humulone/adhumulone, colupulone, and lupulone/adlupulone from Baker et. al. The samples show all four of these peaks as well as two peaks that elute in under 2 minutes.

![Figure 1](image1.png)

Figure 1. Standard 1 chromatogram showing the cohumulone peak at 2.8, the humulone/adhumulone peak at 3.7, the colupulone peak at 4.9, and the lupulone/adlupulone peak at 6.3.

![Figure 2](image2.png)

Fig. 2. Bullion chromatogram showing the same peaks as standard 1 with two unidentified peaks before 2 minutes.
Figure 3. Challenger chromatogram showing peaks at the same times as Bullion and Chinook.

Figure 4. Chinook chromatogram showing peaks at the same time as bullion and challenger.

Figure 5 shows the calibration plots of cohumulone, humulone/adhumulone, colupulone, and lupulone/adlupulone versus signal peak area. Each graph shows a positive linear relation between concentration and signal peak area as expected.
The peak areas for the hops samples was used with the equation for the calibration plots to give the concentration of each of the α- and β-acids. This information is recorded in Table 1.

Table 1. α- and β-acid concentrations and peak areas for hops samples.

<table>
<thead>
<tr>
<th>Hop Variety</th>
<th>Cohumulone Peak area</th>
<th>Cohumulone mg/mL</th>
<th>Hum/Adhumulone Peak area</th>
<th>Hum/Adhumulone mg/mL</th>
<th>Colupulone Peak area</th>
<th>Colupulone mg/mL</th>
<th>Lup/Adlupulone Peak area</th>
<th>Lup/Adlupulone mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullion</td>
<td>4.2</td>
<td>0.2703</td>
<td>6.8</td>
<td>0.4551</td>
<td>3.6</td>
<td>0.2528</td>
<td>2</td>
<td>0.1392</td>
</tr>
<tr>
<td>Challenger</td>
<td>1.4</td>
<td>0.0868</td>
<td>6.0</td>
<td>0.4011</td>
<td>2.4</td>
<td>0.1673</td>
<td>3.3</td>
<td>0.2328</td>
</tr>
<tr>
<td>Chinook</td>
<td>3.9</td>
<td>0.2508</td>
<td>8.7</td>
<td>0.5834</td>
<td>1.8</td>
<td>0.1245</td>
<td>1.5</td>
<td>0.1021</td>
</tr>
</tbody>
</table>

The concentrations of the α-acids in the hops samples were compared to the distributor's values to give the percent differences in Table 2. The α-acid percentages differed from the distributor's values by an average of 28%. This difference is likely the difference in extraction efficiency from the method we used and the method used by the hops distributor.
Table 2. α- and β-acid concentrations according to distributor.

<table>
<thead>
<tr>
<th>Hop Variety</th>
<th>Distributor % α-acids</th>
<th>Experimental % α-acids</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullion</td>
<td>7.9-9</td>
<td>6.3</td>
<td>25</td>
</tr>
<tr>
<td>Challenger</td>
<td>5.6</td>
<td>4.2</td>
<td>25</td>
</tr>
<tr>
<td>Chinook</td>
<td>12.5</td>
<td>8.4</td>
<td>33</td>
</tr>
</tbody>
</table>

Conclusions

The amounts of α- and β-acids in three different varieties of hops were effectively
determined by using liquid chromatography. The effectiveness of this method to give
reproducible results is evident in the fact that, allowing for extraction efficiency, the data
achieved is congruent with the literature values of α-acids in each variety of hops.

Acknowledgments

Special thanks to Dr. Jensen for his guidance throughout the project and to Kyle
Czech for his HPLC expertise.

References