Ultraviolet – Visible Spectroscopy for Determination of α- and β-acids in Beer Hops

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ABSTRACT:

In this experiment, the content of α- and β-acids in beer hops is found through UV-Vis spectroscopic analysis. Three samples will be prepared by extracting finely grained hops through methanol and diluting with methanolic NaOH. The spectrums obtained give a constant overall shape. The experiment was done to find out the concentration of the third component from degraded α- and β-acids that is also existing in the hops samples with the help of the calculated concentrations of α- and β-acids. From the calculated results, the average concentration of the third component in all three samples was 0.061 g/L.

INTRODUCTION:

UV-Vis spectroscopy is a useful absorption or reflectance spectroscopy that helps determine the quantity of analytes by detecting the absorptivity or reflectance of a sample under ultra-violet to visible light wavelength range (1). In this experiment, the absorptivity of the samples were measured and the content of different components were determined from the spectrum. In this lab, UV-Vis spectroscopy was used in to obtain absorbance spectrums of α- and β-acids found in difference hops samples. The structures of α- and β-acids are shown as the Fig. 1 below.

![Figure 1. Structures of major α- and β-acids found in hops](image-url)
By understanding the content of $\alpha$-acid in the hops, the bitterness flavor of beer can be controlled since the bitterness is formed by the iso-form of $\alpha$-acid through isomization of $\alpha$-acid. From the absorbance obtained from the spectroscopy, the concentration of the third component that is degraded from $\alpha$- and $\beta$-acids was also calculated.

**EXPERIMENTAL:**

**Theory.**

UV-vis spectroscopy measures the transition of $\pi$-electrons in molecules from their ground state to excited states (1). The $\pi$-electrons can absorb the ultra-violet or lights, and the wavelength of absorbance is depending on the energy level of transitions. The more easier excited the electrons, the longer the wavelength of light can be absorbed (2).

According to the Beer-Lambert Law:

$$A = \epsilon \cdot b \cdot C$$

where $\epsilon$ is the molar absorptivity ($M^{-1} \text{ cm}^{-1}$) or “strength of the absorber”, $b$ is the pathlength (cm) or “thickness of the absorber” (which is 1 cm in this case), and $C$ is the concentration ($M$) of the absorber. The relationship between absorbance and the concentration is proportional. Therefore, the concentration of the components can be found from the absorbance spectrum of the sample.

**Materials.**

A pack of commercial dried hops was used to prepare the three samples being analyzed in the experiment. Concentrated (6M) NaOH and spectrophotometric grade methanol solutions were also needed for preparing samples and blank solutions.

**Instrumental.**

The spectrophotometer used in the lab was the Shimadzu UV2450 double beam instrument.
Procedures.

Methanolic NaOH was first made by taking 0.5 mL of 6M NaOH and diluting with methanol in a 250-mL volumetric flask. Three portions (about 2.5 g) of a commercial dried hops were taken and finely grained, then were mixed with 50.0 mL of methanol separately and stirred for 30 minutes under room temperature. The solutions were filtered and 50 microliters of each solution were diluted with methanolic NaOH in a 25-mL volumetric flask. These diluted solutions were the samples and were going to be running with a blank solution that was prepared by diluting 50 microliters of methanol with methanolic NaOH in a 25-mL volumetric flask. All samples and blank solution were filled in four 1-cm quartz cuvettes to ¾-full. Each sample was run in the spectroscopy with the blank solution. The absorbance spectrum was set to show the wavelength range from 210 nm to 510 nm since it was believed that the absorbances of α- and β-acids, and the third component will be shown within this range.

Data Analysis.

Inside the hops, the α-acid, β-acid and unknown degraded component are compounds, and concentrations are unknown until calculated. Since they exist as a mixture in the extracted sample, in order to find out the concentration of the component, it is more practical to have the exact absorptivity than molar absorptivity. According to literature (2), the absorptivity at specific wavelength for pure α- and β-acids and the third degradation component are as shown in Table 2. It is shown that the unknown component 3 has the strongest absorbance at 275 nm. Due to the total absorbance at any wavelength is equal to the sum of absorbances by each component, therefore, the following three equations can be concluded:

\[ A_{355} = 31.8C_\alpha + 46.0C_\beta + 1.0C_{\text{Comp}3} \]  \hspace{1cm} (Eq. 1)
\[ A_{325} = 38.1C_\alpha + 33.1C_\beta + 1.5C_{\text{Comp}3} \]  \hspace{1cm} (Eq. 2)
\[ A_{275} = 9.0 C_\alpha + 3.7 C_\beta + 3.1 C_{\text{Comp3}} \]  
(Eq. 3)

which \( A \) is the absorptivity at that wavelength and \( C \) is the concentration of the component it is representing in the sample. After obtaining the all the data points of the spectrums, by using the equations above, the relative concentrations of the each component in each of the samples can be calculated.

According to Table 3, in hops sample 1, \( A_{355} = 0.41052 \), \( A_{325} = 0.39037 \), and \( A_{275} = 0.20172 \). So the following equations could be set up:

\[ \begin{align*}
0.41052 &= 31.8 C_\alpha + 46.0 C_\beta + 1.0 C_{\text{Comp3}} \\
0.39037 &= 38.1 C_\alpha + 33.1 C_\beta + 1.5 C_{\text{Comp3}} \\
0.20172 &= 9.0 C_\alpha + 3.7 C_\beta + 3.1 C_{\text{Comp3}}
\end{align*} \]  
(Eq. 4)

(Eq. 5)

(Eq. 6)

If the Eq. 4 was kept unchanged, and \( C_\beta \) was selected from Eq. 5 as an variable, by multiplying the Eq. 5 with the ratio of (\( C_\alpha \) in Eq. 4/\( C_\alpha \) in Eq. 5):

\[ 0.3258 = 31.8 C_\alpha + 27.627 C_\beta + 1.2520 C_{\text{Comp3}} \]

and then subtracting this equation from Eq. 4:

\[ \begin{align*}
0.41052 &= 31.8 C_\alpha + 46.0 C_\beta + 1.0 C_{\text{Comp3}} \\
-(0.3258 &= 31.8 C_\alpha + 27.627 C_\beta + 1.2520 C_{\text{Comp3}}) \\
0.08472 &= 0 + 18.373 C_\beta - 0.2520 C_{\text{Comp3}}
\end{align*} \]  
(Eq. 5')

In this result, there are two variables, \( C_\beta \) & \( C_{\text{Comp3}} \). They were then eliminated from Eq. 6 in the following two steps. First, \( C_\alpha \) was eliminated the same way as above, but by multiplying Eq. 6 by the ratio of (\( C_\alpha \) in Eq. 4/\( C_\alpha \) in Eq. 6):

\[ 0.71274 = 31.8 C_\alpha + 13.073 C_\beta + 10.953 C_{\text{Comp3}} \]

and then subtracting this equation from Eq. 4:

\[ \begin{align*}
0.41052 &= 31.8 C_\alpha + 46.0 C_\beta + 1.0 C_{\text{Comp3}} \\
-(0.71274 &= 31.8 C_\alpha + 13.073 C_\beta + 10.953 C_{\text{Comp3}}) \\
-0.30222 &= 0 + 32.927 C_\beta - 9.953 C_{\text{Comp3}}
\end{align*} \]  
(Eq. 7)
Second, $C_\beta$ was eliminated from Eq. 7 by multiplying Eq. 7 with the ratio of ($C_\beta$ in Eq. 5'/$C_\beta$ in Eq. 7):

$$-0.1686 = 0 + 18.373 C_\beta - 9.953 C_{\text{Comp3}}$$

, and then subtracting this equation from Eq. 5':

$$0.08472 = 0 + 18.373 C_\beta - 0.2520 C_{\text{Comp3}}$$
$$-(-0.1686 = 0 + 18.373 C_\beta - 9.953 C_{\text{Comp3}})$$

$$0.2533 = 0 + 0 + 5.302 C_{\text{Comp3}} \quad \text{(Eq. 6')}$$

At this point, there are three equations obtained:

$$0.41052 = 31.8 C_\alpha + 46.0 C_\beta + 1.0 C_{\text{Comp3}} \quad \text{(Eq. 4)}$$
$$0.08472 = 0 + 18.373 C_\beta - 0.2520 C_{\text{Comp3}} \quad \text{(Eq. 5')}$$
$$0.2533 = 0 + 0 + 5.302 C_{\text{Comp3}} \quad \text{(Eq. 6')}$$

By Eq. 6', $C_{\text{Comp3}}$ could be calculated:

$$C_{\text{Comp3}} = \left( \frac{0.2533}{5.302} \right) = 0.04777 \text{ g/L}$$

By substituting this $C_{\text{Comp3}}$ value into Eq. 5', $C_\beta$ could also be calculated:

$$0.08472 = 0 + 18.373 C_\beta - 0.2520 \times 0.04777$$
$$C_\beta = 0.005266 \text{ g/L}$$

By substituting the calculated $C_{\text{Comp3}}$ and $C_\beta$ values into Eq. 4, the value of $C_\alpha$ could be calculated:

$$0.41052 = 31.8 C_\alpha + 46.0 \times 0.005266 + 1.0 \times 0.04777$$
$$C_\alpha = 0.003790 \text{ g/L}$$

With the corrected significant digits, the concentration values should be as the following:

$$C_\alpha = 0.0038 \text{ g/L}$$
$$C_\beta = 0.0053 \text{ g/L}$$
$$C_{\text{Comp3}} = 0.048 \text{ g/L}$$

The same steps were taken to calculate concentration of the three components in rest of the samples.
RESULTS:

With the help of Mathematica and Excel, the concentrations, weights and %wt of α-acid, β-acid and the third component (indicated as Comp 3) in each sample were found as the following:

a)

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Concentration (g/L)</th>
<th>Grams in 25-mL solution</th>
<th>%wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acid</td>
<td>0.0038</td>
<td>0.00095</td>
<td>0.15</td>
</tr>
<tr>
<td>β-acid</td>
<td>0.0053</td>
<td>0.00013</td>
<td>0.21</td>
</tr>
<tr>
<td>Comp 3</td>
<td>0.048</td>
<td>0.0012</td>
<td>1.9</td>
</tr>
</tbody>
</table>

b)

<table>
<thead>
<tr>
<th>Sample 2</th>
<th>Concentration (g/L)</th>
<th>Grams in 25-mL solution</th>
<th>%wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acid</td>
<td>0.0022</td>
<td>0.000055</td>
<td>0.091</td>
</tr>
<tr>
<td>β-acid</td>
<td>0.0052</td>
<td>0.00013</td>
<td>0.22</td>
</tr>
<tr>
<td>Comp 3</td>
<td>0.067</td>
<td>0.0017</td>
<td>2.8</td>
</tr>
</tbody>
</table>

c)

<table>
<thead>
<tr>
<th>Sample 3</th>
<th>Concentration (g/L)</th>
<th>Grams in 25-mL solution</th>
<th>%wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acid</td>
<td>0.0024</td>
<td>0.000060</td>
<td>0.098</td>
</tr>
<tr>
<td>β-acid</td>
<td>0.0054</td>
<td>0.00014</td>
<td>0.22</td>
</tr>
<tr>
<td>Comp 3</td>
<td>0.069</td>
<td>0.0017</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Table 4. Calculated values from the spectrum of different samples

<table>
<thead>
<tr>
<th></th>
<th>Average concentration (g/L)</th>
<th>Average mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acid</td>
<td>0.0028</td>
<td>0.00070</td>
</tr>
<tr>
<td>β-acid</td>
<td>0.0053</td>
<td>0.00013</td>
</tr>
<tr>
<td>Comp 3</td>
<td>0.061</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

Table 5. Average concentrations and masses of the three components among three samples

From these table, it can be concluded that the third degraded component has the highest concentration among the three components in the sample. The β-acid has the second highest concentration and the α-acid has the lowest concentration.
**Figure 2.** Absorbance versus wavelength spectrum of all samples of beer hobs

The spectrum above shows the three samples being run during the experiment. The shape of the spectrums match to each other with small variations.

**DISCUSSION:**

When comparing the spectrums in Fig. 2 with the weights of the hops samples, the increase and decrease of the values match to the lift and drag of the spectrums. When the sample is heavier, the absorbance becomes stronger. This is because the concentration of analyte affects the absorbance which can be explained by the Beer-Lambert Law, the concentration is proportional to the absorbance. Since the degraded α-acid component can argue the absorption of α- and β-acids and can interfere with a standard two-component analysis (2), so by clarifying how many degraded component in the hops can know how much the spectrum has been affected. The component absorbs strongly at 275 nm but also has significant absorptions at 325 and 355 nm, where α- and β-acids have strong absorbance at (2).
LITERATURE CITED:


APPENDIX:

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Hops Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>2.479</td>
</tr>
<tr>
<td>Sample 2</td>
<td>2.415</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.445</td>
</tr>
</tbody>
</table>

Table 1. Weight of three samples used for analysis

<table>
<thead>
<tr>
<th>Component</th>
<th>355 nm</th>
<th>325 nm</th>
<th>275 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acid</td>
<td>31.8</td>
<td>38.1</td>
<td>9.0</td>
</tr>
<tr>
<td>β-acid</td>
<td>46.0</td>
<td>33.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Component 3</td>
<td>1.0</td>
<td>1.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 2. Absorptivies (L mg⁻¹) of pure α-acid, β-acid and the third component in hops in specific wavelengths (2)

<table>
<thead>
<tr>
<th>Sample</th>
<th>355 nm</th>
<th>325 nm</th>
<th>275 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.41052</td>
<td>0.39037</td>
<td>0.20172</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.37576</td>
<td>0.35648</td>
<td>0.18666</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.39534</td>
<td>0.37474</td>
<td>0.19286</td>
</tr>
</tbody>
</table>

Table 3. Absorbance of three samples in the specific wavelengths