

UV-Visible analysis of bitterness and total carbohydrates in beer

Key Words

Evolution 200, GENESYS, beer, bitterness, brewing, polyphenols, quality control, UV-Visible spectroscopy

Introduction

Beer is one of the oldest and most popular beverages known, with archeological evidence indicating it to be at least 7000 years old. In the intervening centuries, beer production has become a multi-billion dollar industry dominated by large multinational corporations. More recently, attention has turned to smaller craft breweries that cultivate a more artisanal approach. Proper preparation of both craft beers and high volume macro-brews requires a deep understanding of the available ingredients and processes in order to develop new brands and ensure batch-to-batch quality.

To control flavor and quality, brewers rely on a battery of tests to prove and maintain consistency. Brewers associations and governmental agencies have worked together to set guidelines for testing particular parameters important for determining batch quality. While alcohol content is the criterion most commonly thought of, a great many more specifications are measured and recorded, such as, initial and final gravity, carbohydrate content, protein content, color, polyphenols, and bitterness. The equipment required for testing may include wet chemistry, HPLC and other chromatographic techniques that can be expensive and require a highly trained technician. Fortunately, many of these tests can also be performed using relatively simple and inexpensive UV-Visible spectroscopy methods.



Thermo Scientific™ Evolution™ 200 Series
UV-Visible Spectrophotometer

Background

UV-Visible spectroscopy is one of the most basic techniques for testing a variety of materials. It is routinely used for qualitative and quantitative determination of different analytes including conjugated organic compounds, transition metal complexes, and biological macromolecules. Because of its flexibility, ease of use and ubiquitous presence of instrumentation in laboratories, UV-Visible spectroscopy is relied upon to test a large number of parameters important for Quality Control (QC) in beer production. The American Society of Brewing Chemists (ASBC), European Brewery Convention (EBC), Association of Official Analytical Chemists (AOAC) and other groups have developed and refer to a number of set guidelines for UV-Visible testing of specific parameters useful in determining and controlling beer quality.



Thermo Scientific™ GENESYS™ 50 UV-Visible Spectrophotometer

Some characteristics tested by UV-Visible methods include:

- Absorbance Integral
- α - and β -Acids in Hops
- Anthocyanogene
- Bitterness/Bitter Substances
- Color
- Diacetyl Content/Vicinal Diketone
- Ethanol (Ethyl Alcohol)
- Free Amino Nitrogen (FAN)
- Iodine
- Protein
- Sulfite
- Total Carbohydrates
- Total Polyphenols

Since consistent QC in beer production is important to ensure high quality products, collecting high quality data and reporting it accurately every time is extremely critical. To meet specific instrument requirements, modern UV-Visible spectrophotometers are designed to offer high performance, ease-of-use and improved productivity while allowing users to customize them for their specific analytical needs. The Thermo Scientific Evolution 200 Series UV-Visible Spectrophotometer is the instrument of choice with its exceptional strength in delivering high quality data consistently while requiring little operator training. Furthermore, the powerful Thermo Scientific™ INSIGHT™ 2 and CUE™ Software (Figure 1) allows users to customize their own workflows from measuring samples to analyzing data, thus minimizing errors and greatly improving productivity.

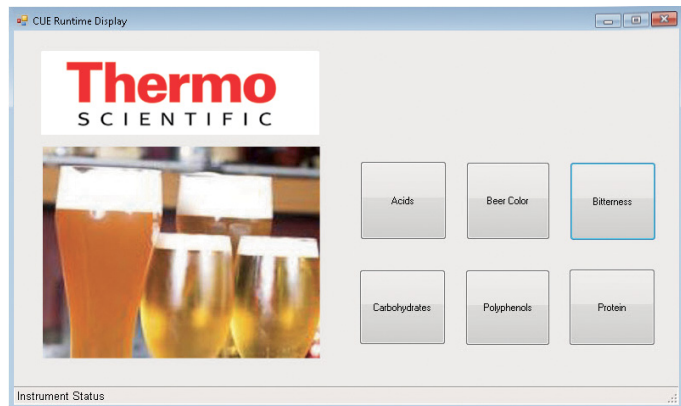


Figure 1: Customized beer analysis with CUE software

While UV-Vis is ideally suited to analyze multiple parameters, this application note describes the methods and results for the analysis of two of these: bitterness and total carbohydrates. Samples used for the analyses were obtained from beer kits purchased from Northern Brewer™ (Roseville, MN) and prepared according to the vendor's instructions. The samples included: a dark roasted Black IPA, dark Lakefront IBA, American Red Ale (Lakefront Fixed Gear) and a southern English style Nut Brown Ale. Sample preparation, analysis and results of the two methods are detailed below.

Experiment

Bitterness

Bitterness in beer is imparted from the use of hops and is one of the chief components in flavor quality. Isohumulones extracted out of the hop cones during brewing provide this bitter flavor and are usually measured in International Bitterness Units (IBU). The final IBU of the product is a result of both the amount and type of hops used during the brewing process as well as when the hops are added. Additionally, the type and amount of malt used in beers affects the flavor and can alter its apparent bitterness. In order to maintain consistency in quality, bitterness needs to be tightly monitored and controlled. While different types of beer tend to have different IBU values, these values typically range from 0 to 100. The general method for analysis is to extract the beer samples into isooctane and measure the peak height at 275 nm.

In this experiment, analysis of IBU was performed according to the ASBC method for Determination of Isohumulones in beer. Samples of the four different beers were prepared by placing 10 mL aliquots in 50 mL centrifuge tubes. One mL of 3N HCl and 20 mL of isooctane with 50 μ L octyl alcohol were added and the samples were shaken for 15 minutes. Emulsions were formed and broken with centrifugation to separate the aqueous layer from the organic layer. A portion of the upper organic layer was withdrawn from the centrifuge tube and measured at 275 nm using isooctane/octyl alcohol as the blank. Bitterness in IBU units was calculated using the following equation:

$$\text{Bitterness (IBU)} = \text{Abs}_{275} * 50$$

Figure 2 shows the full spectra of the four test beer samples. Note that there is considerable variability between the different samples indicating a large detectable difference in isohumulone content. The measurements at 275 nm for the samples along with the calculated IBU contents are included in Table 1.

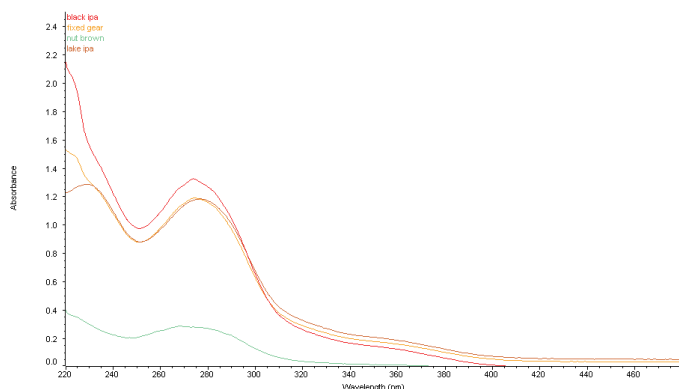


Figure 2: Bitterness of beer samples

Beer ID	Bitterness (IBU)
Black IPA	66
Lakefront IBA	59
Lakefront Fixed Gear	60
Nut Brown Ale	14

Table 1: IBU of beer samples

Total Carbohydrates

Total Carbohydrates include simple and complex sugars as well as larger starch molecules. In beer, the primary carbohydrates are dextrans produced by the partial hydrolysis of starches. The malting process of barley and other grains activates enzymes that break down starches into maltose and other simple sugars. Typically these simple sugars are consumed during fermentation, resulting in a final beer that should have very little or no remaining simple sugars. The remaining unfermented sugars and starches are a key component of the type and flavor of the beer. For example; higher carbohydrate contents are associated with high gravity stouts and porters. Carbohydrate content typically ranges from lows below 0.5 g/100 mL to highs over 6 g/100 mL. UV-Visible analysis of carbohydrates involves breaking them down with sulfuric acid and, subsequently, reacting them with phenol.

Total Carbohydrates analysis was performed using the ASBC BEER-41(A) method. Samples of beer were diluted 1:1000, and 2 mL aliquots of the diluted samples were placed in 50 mL centrifuge tubes. A standard 2% dextrose solution was similarly diluted. One mL of 5% phenol solution was added to the samples and standard and mixed. Five mL of concentrated sulfuric acid was then added into the tubes, which were sealed and mixed again. The tubes were vortex mixed for 10 minutes to allow for color development. A blank was similarly prepared using 2 mL of water.

The samples, standard and blank were measured at 490 nm. The Total Carbohydrates content (Figure 3) was calculated using the following equation:

$$\begin{aligned} \text{Total Carbohydrates} &= 0.9 \times \frac{(\text{sample abs.} - \text{blank abs.}) \times 2 \times 1000}{(\text{std abs} - \text{blank abs}) \times 1000} \\ &= \frac{\text{sample abs.} \times 1.8^*}{\text{std abs}} \end{aligned}$$

* Note: The blank absorbance is subtracted from all standards and samples during data collection.

Figure 4 shows the spectra of the samples and clearly indicates the measured peak at 490 nm. The calculated total carbohydrates amounts for the samples in g/100 mL are enumerated in Table 2.

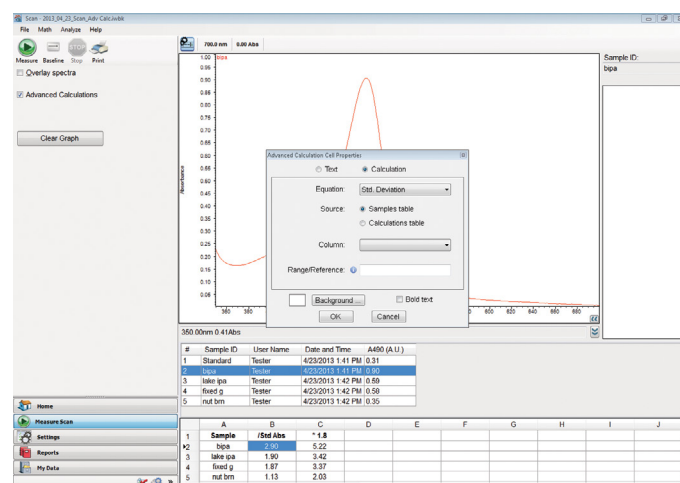


Figure 3: Total Carbohydrates Analysis with INSIGHT 2 software. This figure applies to the calculations shown for Total Carbohydrates.

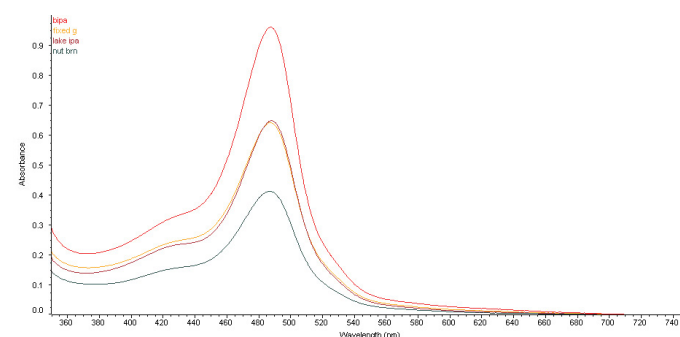


Figure 4: Total Carbohydrates in beer

Beer ID	Total Carbohydrates (g/100 mL)
Black IPA	5.22
Lakefront IBA	3.42
Lakefront Fixed Gear	3.37
Nut Brown Ale	2.03

Table 2: Total Carbohydrates data of beer samples

Conclusion

Many QC methods for beer analysis can be performed using UV-Visible spectrophotometer methods. As demonstrated above, the Evolution 200 Series Spectrophotometers with INSIGHT and CUE Software and optional accessories provide an optimal solution for performing these methods, delivering exceptional reliability and ease of use in a complete analytical package. For breweries with more limited analytical requirements, such as smaller batch sizes, batch quantities or number of required quality tests, the GENESYS 50 and GENESYS 150 UV-Visible Spectrophotometers are also available.



Thermo Scientific™ GENESYS™ 150 UV-Visible Spectrophotometers

Find out more at thermofisher.com/UVVis

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