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Application News

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Spectrophotometric Analysis

Measurement of Color, Bitterness, and Diacetyl Content of Beer Using UV-Vis Spectrophotometer

Among alcoholic beverages, beer is extremely popular and enjoys a strong market, and diverse beers with distinctive individual characters, exemplified by craft beers, have sold well in recent years. Although sensory evaluation is an essential technique, evaluations using various types of analytical instruments are also effective for ensuring the uniform quality of these beers.

The UV-Vis spectrophotometer is one useful instrument for beer analysis. The standard international methods for sensory evaluation of beer are the methods of the American Society of Brewing Chemists (ASBC) and the European Brewery Convention (EBC), and in Japan, the Brewery Convention of Japan (BCOJ), centering on major beer producers, has established analytical methods for beer.

Among the 12 inspection items in the ASBC Methods of Analysis, color (Beer-10) and bitterness (Beer-23) are inspected by UV-Vis spectrophotometry. UV-Vis spectrophotometry also makes it possible to analyze the diacetyl, iron, polyphenol, and other components contained in beer.

This article introduces measurements of the color, bitterness, and diacetyl content of commercially-available beers using a Shimadzu UV-1900i UV-Vis spectrophotometer.

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Measurement Samples (Color and Bitterness)

Four types of commercially-available beer were prepared, and their color and bitterness were measured. Fig. 1 shows the appearance of the measurement samples, and Table 1 shows the details of each sample.

Fig. 1 Appearance of Measurement Samples

Table 1 Types and Features of Measurement Samples

	Type	Features
A	Belgian white	Color is extremely light, and has a spicy, acidic taste. Has a clean, refreshing taste.
B	Pilsner	World's most widely-consumed type. The main beers of major beer producers are this type. Yellow-gold color.
C	Indian pale ale (IPA)	Has a strong aroma and bitter taste due to the large amount of hops used. Alcohol content is also high.
D	Pale ale	Gold to copper color. Rich aroma is a distinctive feature.

Color

Beer has a wide range of colors, from the light-colored beers called white ale to black beers such as stout. The units used to express the color intensity of beer are the SRM (Standard Reference Method) system specified by the ASBC and the EBC (European Brewery Convention) system of the EBC. In these standards, the values are calculated using the absorbance of a single wavelength. Fig. 2 shows a reference diagram of the colors in these two systems.

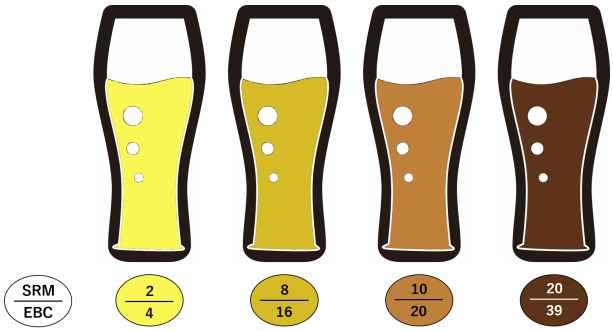

Fig. 2 Reference Diagram of Colors in SRM and EBC Systems

The colors of the four beers shown in Fig. 1 were measured using a Shimadzu UV-1900i. The measurement method referred to ASBC Methods of Analysis, Beer Methods, Beer-10A [(1)] and the BCOJ Beer Analysis Methods, 8.15 Bitterness Unit [(2)]. Fig. 3 shows the flowchart of the measurement, and Fig. 4 shows the measurement instrument. Although absorbance value at 430 nm is used in color calculations, absorbance value at 700 nm is also measured to check turbidity.

◆ Flowchart of Color Measurement

1) Introduce distilled water into a cell and conduct a baseline measurement.
↓
2) Introduce deaerated beer into a cell, and measure absorbance values at 430 nm and 700 nm.
↓
3) Convert the absorbance values to the absorbance values for a 1/2 inch optical path length ($A^{1/2}$).
Conversion formula $A^{1/2} = 1.27 \times$ (Absorbance value of 10 mm optical path length)
↓
4) Confirm that the absorbance value at 700 nm is not more than the value obtained by multiplying the absorbance value at 430 nm by 0.039. (If the result exceeds this value, filter and clarify.)
↓
5) SRM unit = 10 × dilution ratio × $A^{1/2}$, 430 nm (Eq. 1)
EBC unit = SRM unit × 1.97 (Eq. 2)

Note: In actual measurements, be sure to refer to ASBC and BCOJ.

Fig. 3 Flowchart of Color Measurement

Fig. 4 UV-1900i UV-Vis Spectrophotometer

Table 2 shows the absorbance values of the four samples at 430 nm and 700 nm and the results of the measurements of SRM and EBC calculated by Eq. 1 and Eq. 2, respectively. Due to the observable turbidity (cloudiness) of beer D, this beer was measured after filtration. From the appearance in Fig. 1, the color becomes darker in the order of A, B, C, D. The SRM and EBC values also increased in the same order.

Table 2 Results of Color Measurements of Samples

Sample	Absorbance value (430 nm)	Absorbance value (700 nm)	SRM value	EBC value
A	0.310	0.008	3.9	7.7
B	0.482	0.008	6.1	12.1
C	0.725	0.013	9.2	18.1
D	1.078 (after filtration)	0.051 (after filtration)	13.7	27.0

In addition to the single-point (single wavelength) measurement methods introduced here, the tristimulus method is also used to measure the color of beer. In the tristimulus measurement method, after measuring the transmission spectrum in the visible light region, L*, a*, and b* are obtained by the CIELAB method. This method makes it possible to acquire color-related information that cannot be obtained by the single-point method.

Bitterness

Bitterness is one of the important components which determine the flavor of beer, and originates from the hops used to give beer its aroma. Alpha (α) acids are eluted from the hop flowers when the hops are boiled, and are then converted to iso-α acids, which are the bitterness components of beer.

In measurements of bitterness, the International Bitterness Units (IBU) of the sample is obtained by solvent elution of the bitterness components in the beer, followed by measurement of the absorbance value at 275 nm.

Here, the bitterness of the four types of beer shown in Fig. 1 was measured using the UV-1900i. The measurement method referred to the ASBC Methods of Analysis, Beer Methods, Beer-23A [3]. Fig. 5 shows the flowchart of the measurement.

◆ Flowchart of Bitterness Measurement

1) Introduce 10.0 mL of deaerated beer cooled to 10 °C into a 50 mL centrifuge tube.
↓
2) Add a small amount of octyl alcohol.
↓
3) Add 1 mL of 3 mol/L hydrochloric acid and 20 mL of isooctane.
↓
4) Using a shaker, shake the centrifuge tube for 15 min.
↓
5) Conduct centrifugal separation for 5 min at 3,000 rpm, and remove the isooctane layer.
↓
6) Take the isooctane layer in a 10 mm cell, and measure its absorbance value at 275 nm.
(In the baseline measurement, use pure isooctane.)
↓
7) Calculate bitterness (IBU) by using the following formula.
IBU = 50 × (Absorbance value at 275 nm)

Note: In actual measurements, be sure to refer to ASBC.

Fig. 5 Flowchart of Bitterness Measurement

Table 3 shows the results of the bitterness measurements of the samples. Beer C had the highest value, and beer A had the lowest value. Beer C is an Indian pale ale (IPA), which has a strong aroma and bitterness due to the large amount of hops used. The measurement results also showed that this beer had the highest bitterness value in comparison with the other samples.

Table 3 Results of Bitterness Measurements of Samples

Sample	Absorbance value (275 nm)	Bitterness unit (BU)
A	0.253	12.5
B	0.661	33.0
C	1.146	57.5
D	0.838	42.0

Diacetyl Level

The unpleasant flavor that spoils the inherent good taste of beer is called "off-flavor" [4]. This unpleasant off-flavor is known to occur when beer has high contents of diacetyl and 2,3-pentanedione (both compounds are included under the general name vicinal diketone: VDK). If the VDK content exceeds a certain level, the beer will have a buttery flavor called "butterscotch." However, VDKs can be reduced and deodorized by dehydrogenase in the presence of yeast. Therefore, in the beer production process, a maturation process in which VDKs are consumed by yeast is necessary after the fermentation process is completed. Thus, checking the VDK concentration after the fermentation process is completed is extremely important for the flavor of beer [5].

VDK evaluations are mainly conducted by sensory evaluation, but quantitative analysis of diacetyl, which is one VDK, is also possible with a UV-Vis spectrophotometer if pretreatment is conducted.

In this experiment, five types of beer which had completed the fermentation process were prepared, and changes in the concentration of diacetyl with time were measured using the UV-1900i. The measurement method referred to the ASBC Methods of Analysis, Beer Methods, Beer-25C [6]. Fig. 6 shows the flowchart of the measurement.

◆ Flowchart of Diacetyl Measurement

[Preparation of Reagents]

A Dipotassium phosphate (aq)
Dissolve 33 g of K_2HPO_4 in 100 mL of water.

B Hydroxylammonium chloride (aq)
Dissolve 11 g of $NH_2OH \cdot HCl$ in 250 mL of water.

C Sodium acetate (aq)
Dissolve 35 g of CH_3COONa in 100 mL of water.

D Hydroxylamine acetate buffer solution
Mix the above 3 reagents at a ratio of A : B : C = 2 : 4 : 1.

E Acetone-Dipotassium phosphate
Dissolve 29 g of anhydrous K_2HPO_4 in water, add 40 mL of acetone, and add water to make up a total volume of 200 mL.

F Ammonium tartrate hydroxide (aq)
Mix 12 mL of a saturated solution of potassium sodium tartrate and 3 mL of concentrated ammonia water.

G Iron sulfate (aq)
Dissolve 5 g of $FeSO_4 \cdot 7H_2O$ in 100 mL of 1 % H_2SO_4.

H Diacetyl standard solution
Prepare an aqueous solution with a concentration of 500 mg/L, and store in a dark place.

[Measurement Procedure]

1) Introduce 30 mL of deaerated beer in a sample test tube.
↓
2) Introduce 1 mL of reagent D in a trap test tube, and connect to the sample test tube with a tube.
↓
3) Set the N_2 gas flow rate at 100 to 150 mL/min, and flow the gas in the sample test tube.
↓
4) Place only the sample test tube in a water bath at 65 °C, and aerate for approximately 1.5 h.
↓
5) After aeration, remove the trap test tube, heat for 10 min in a water bath at 70 to 80 °C, and add 0.5 mL of reagent E while warm.
↓
6) After cooling, add and mix 1.5 mL of reagent F, further add and mix 0.1 mL of reagent G, and add reagent A to make up a total volume of 5 mL.
↓
7) After mixing, wait 2 to 3 min, then measure absorbance value at 530 nm.

[Standard samples]

Using reagent H, prepare standard samples with concentrations of 0.5, 1.0, 1.5, 3.0, and 4.0 mg/L. Add 1 mL of the standard sample to 1 mL of reagent D, then measure by the same procedure as in steps 5) to 7) of the above flowchart.

Note: In actual measurements, be sure to refer to ASBC.

Fig. 6 Flowchart of Diacetyl Measurement

Table 4 shows the measurement results of the standard samples, and Fig. 7 shows the calibration curve. Table 5 shows the measurement results for diacetyl in each sample. It was found that the concentration of diacetyl decreases with time after the fermentation process is completed, and the lowest concentration is reached before bottling. From this, it can be said that providing an adequate maturation period after completion of the fermentation process is necessary in order to reduce the diacetyl level in beer. (Beer is generally matured for 7 to 10 days at approximately the same temperature as the fermentation temperature [5].)

Table 4 Measurement Results of Standard Samples

Sample concentration (mg/L)	Absorbance value (530 nm)
0.5	0.004
1.0	0.007
1.5	0.013
3.0	0.026
4.0	0.032

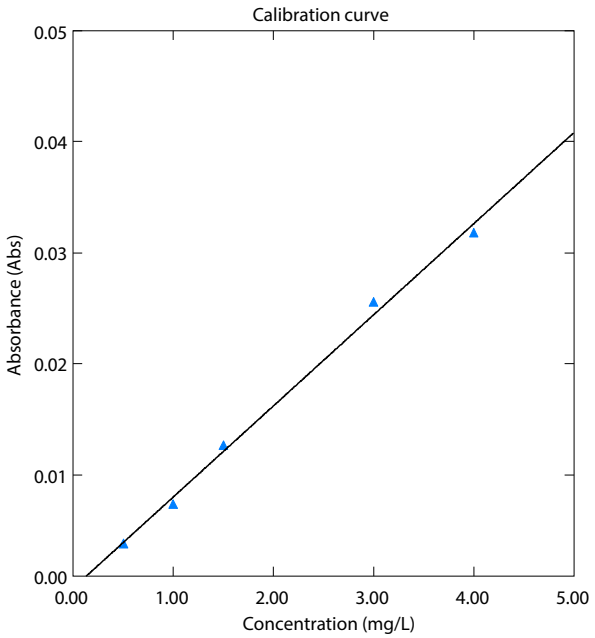

Fig. 7 Calibration Curve of Standard Samples

Table 5 Measurement Results of Diacetyl in Samples

Time after completion of main fermentation	Absorbance value (530 nm)	Diacetyl concentration (mg/L)
4th day	0.023	2.81
5th day	0.019	2.30
7th day	0.015	1.90
Before bottling	0.007	0.74

Conclusion

The color, bitterness, and diacetyl concentration of beer samples were obtained by using a UV-1900i UV-Vis spectrophotometer. These values are extremely important indexes for maintaining the quality of beer. Use of a UV-Vis spectrophotometer enables quantitative evaluation of various beer indexes.

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