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High Speed Analysis of α-Acids and β-Acids in Hops by using UHPLC

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1. Introduction

The hops used as a raw material for beer contain α-acids (humulones) and β-acids (lupulones). The α-acids are converted to iso-α-acids (isohumulones), which are bitterness components of beer, by isomerization in the brewing process. The β-acids are not strongly related to the strength of beer bitterness but are thought to influence the balance of bitterness.
International Bitterness Units (IBU) are generally used in measurements of the bitterness value. The IBU value is calculated based on the results of solvent extraction of the bittering components in beer and spectrophotometric measurement. Although this is a simple method, overestimation is possible if the sample includes substances.
High performance liquid chromatography (HPLC) is generally used in analyses of these compounds. This article describes examples of a high speed analysis of the α-acids and β-acids in hops with a Shimadzu Nexera XR HPLC, referring to EBC (European Brewery Convention) and ASBC (American Society of Brewing Chemists) and IBU was also measured with a UV-1900i UV-Vis spectrophotometer and compared with the results of the HPLC

UV-1900i UV-Vis spectrophotometer

Nexera XR UHPLC System

2. Methods and Materials

2.1. Analysis of Iso-α-Acids,α-Acids, and Humulinones in beer using HPLC

The reagents used in formulation of the standard solution were “DCHA-Iso, ICS-I4,” “International Calibration Extract 4,” and “DCHA-Humulinones, ICS-Hum1.”
Samples of five commercially-available beers were prepared referring to EBC 9.47. Table 1 shows the details of each sample. Fig. 1 shows the calculation method for quantitative values in accordance with EBC 9.47.

2.2. Measurement of IBU

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, which is near the maximum wavelength of iso-α-acids. The measurement method referred to the ASBC Methods of Analysis, Beer Methods, Beer-23A. Fig. 2 shows the flowchart of the measurement.

Table 1 Types of Measurement Samples

Sample	Beer Type	Country of manufacture
Beer I	Lager	Japan
Beer II	Lager	USA
Beer III	Lager	Italy
Beer IV	Ale	Japan
Beer V	IPA	Japan

$$F = A_{std} \times 50 \times 100 / (V_{std} \times V_{inj} \times M_{std} \times C_{std} / 100)$$

where:
F = response factor (average of 4 injections) (area per mg)
A_{std} = total area of the peaks representing a particular type of iso-α-acids in the standard
M_{std} = weight of international calibration standard in mg
C_{std} = concentration of a particular type of iso-α-acids in the international calibration standard in % (m/m)
V_{std} = volume of stock standard solution in mL diluted at 50 mL (working standard solution)
V_{inj} = volume injected in mL

$$Cs = As \times D \times 1000 / (V_{inj} \times F)$$

where:
Cs = concentration of a particular type of iso-α-acids in the beer expressed as mg/L
As = total area of the peaks representing a particular type of iso-α-acids in the sample (average of 2 injections)
F = response factor in area per mg
D = dilution factor = 2 x 0.967 = 1.934 where 0.967 is due to the volume change when mixing methanol and beer 1:1 by volume.

Fig. 1 Calculation method in accordance with EBC 9.47

◆ 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. 2 Flowchart of IBU Measurement

2. Methods and Materials

2.3. Analysis of α-Acids,β-Acids in hop using HPLC

Fig. 3 shows the structural formulas of the *α*-acids and *β*-acids contained in hops. Both the *α*-acids and the *β*-acids consist of three homologues. Here, “International Calibration Extract 4” (purchased from ASBC or Labor Veritas) was used as a reagent when preparing the standard solution. Solvent extraction and analysis were conducted using two types of commercial hop pellets. Fig. 4 shows the sample preparation protocol and the quantitative values were calculated by the formula in Fig.5.

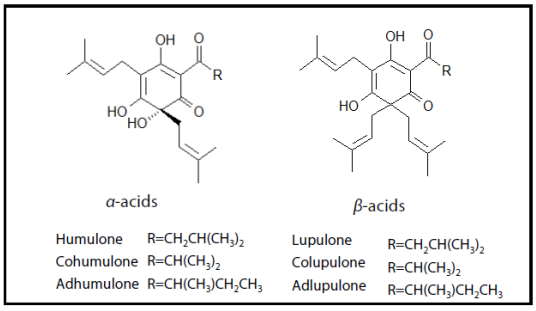

Fig.3 Structural Formulas of *α*-Acids and *β*-Acids

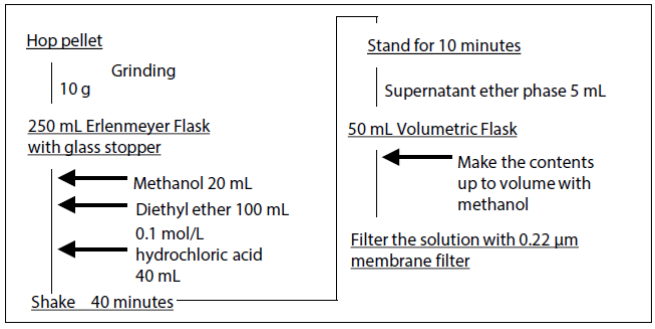

Fig.4 Sample Preparation for Hop Analysis

$$C_i=(DF \times M_{CS} \times C_{iC} \times A_i)/(M_s \times A_{ic})$$

where:
C_i = concentration of component i in the sample expressed as percent by weight
DF = dilution factor, DF = 2 for hops and hop powder products
M_{cs} = weight of the calibration standard in g
C_{ic} = concentration of component i in the calibration standard expressed as percent by weight
A_i = peak area of component i from the sample run (average)
M_s = weight of the sample in g
A_{ic} = peak area of component i from the calibration run (average)

Fig.5 Calculation method for *α*-Acids and *β*-Acids in Hop pellet

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3. Result

3.1. Comparison the results of IBU method and HPLC method

Fig. 6 shows the chromatogram of Beer V obtained by an analysis conforming to EBC 9.47, and an analysis under the high speed analysis condition, respectively (regarding to conditions, see in Table2 and 3). Because the reagents used to prepare the standard solution contain multiple homologues, grouping of iso-α-acids, α-acids, and humulinones were carried out here. Under the EBC-compliant conditions, a pH meter is necessary in preparation of the mobile phase and one analysis requires 45 min, but under the high speed analysis conditions, a pH meter is not necessary and the analysis can be completed in only 5min, reducing the analysis time by about 90%. Under the high speed analysis conditions, EDTA is added to the mobile phase to improve the peak shape of the iso-α-acids.

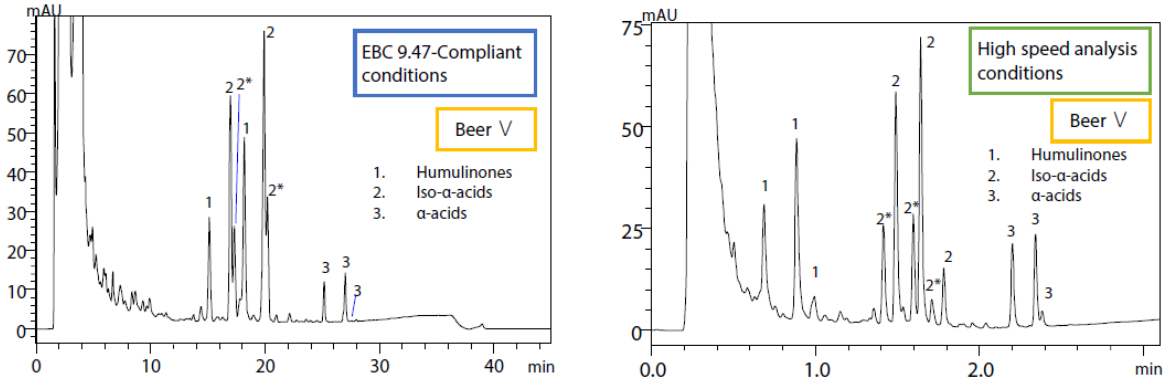

Fig.6 Chromatograms of Beer Sample Solutions

Table 2 HPLC Analytical Conditions (EBC 9.47-Compliant)

System	: Nexera XR
Column	: Shim-pack™ GIST C8 (250 mm × 4.6 mm I.D., 5 µm)*1
Mobile Phase A	: Acetonitrile/1% citric acid buffer(pH7.0)=30:70
Mobile Phase B	: Methanol
Flow Rate	: 1.0 mL/min
Time program	: B Conc. 15%(0-5min)-80%(30-33 min)-15%(35-45 min)
Column Temp.	: 35 °C
Injection Vol.	: 50 µL
Detection	: UV 270 nm
Vial	: Shimadzu Vials, LC, 1.5 mL Clear Glass *2

* 1 P/N: 227-30173-09 , *2 P/N: 227-34001-01

Table 3 UHPLC Analytical Conditions

System	: Nexera XR
Column	: Shim-pack Velox™ C18 (50 mm × 3.0 mm I.D., 1.8 µm)*4
Mobile Phase A	: 10 mmol/L (Sodium) phosphate buffer (pH2.6)+0.2 mmol/L ETDA・2Na aq.
Mobile Phase B	: Acetonitrile
Flow Rate	: 0.8 mL/min
Time program	: B Conc. 40%(0 min)-90%(2.1-3.5 min)-40%(3.51-5 min)
Column Temp.	: 40 °C
Injection Vol.	: 5 µL
Detection	: UV 270 nm
Vial	: Shimadzu Vials, LC, 1.5 mL Clear Glass *2

*4 P/N: 227-32008-01

3. Result

3.1. Comparison the results of IBU method and HPLC method

Table 4 shows the results of HPLC methods and Table 5 shows the results of measurements of the IBU values with a UV-1900i UV-Vis spectrophotometer. A larger IBU value was obtained for Beer V, which is an IPA(Indian Pale Ale). A distinctive feature of IPA beers is a unique bitter taste obtained by using a large amount of hops. Because hops are also added to the boiled and cooled wort (mixture of malt extract and water before fermentation), a higher level of α-acids and humulinones in comparison with other beers is thought to be a factor in the distinctive flavor of IPAs.

Table 4 Results of HPLC analysis

Unit: mg/L

Sample	Humulinones	Iso-α-acids	α-acids
Beer Ⅰ	1.2	21.0	0.7
Beer Ⅱ	0.6	7.0	0.1
Beer Ⅲ	1.3	18.2	0.9
Beer Ⅳ	6.4	19.7	3.6
Beer Ⅴ	14.9	36.8	18.7

Table 5 Results of IBU Measurement using UV-1900i

Sample	IBU
Beer Ⅰ	16.3
Beer Ⅱ	5.1
Beer Ⅲ	14.1
Beer Ⅳ	23.6
Beer Ⅴ	50.5

3. Result

3.2. Simultaneous analysis for α-acids, β-acids and iso-α-acids in hop

As explained before, IPA beers require additional hops during other stages of the beermaking process. Since these hops are added at low temperature, no iso-α-acids are produced. Instead, other hop acids, such as β-acids, are extracted from the hops into the beer. These hop acids are significantly less bitter than iso-α-acids. However, these hop acids also have strong UV absorbance around 275 nm and thus influence the IBU reading. Fig.7 shows the chromatogram of high speed analysis of the *α*-acids and *β*-acids in hop, referring to EBC (European Brewery Convention) 7.7 and ASBC(American Society of Brewing Chemists) Hops-14 (regarding to analytical condition, see in Table6).

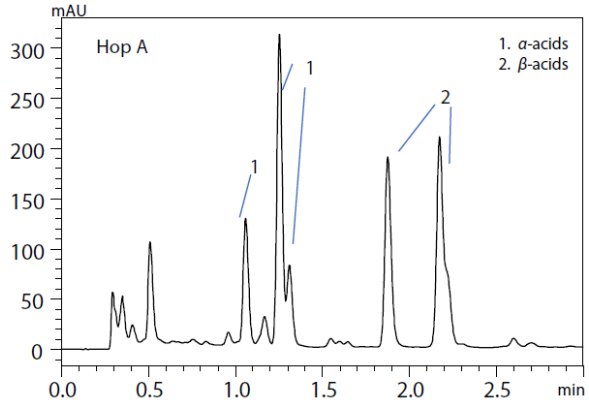

Fig.7 Chromatograms of Extracts of Hop

Table 6 UHPLC Analytical Conditions for Hop Analysis

System	:	Nexera XR
Column	:	Shim-pack Velox C18 (50 mm × 3.0 mm I.D., 1.8 µm)*1
Mobile phase A	:	10 mmol/L (Sodium) phosphate buffer (pH2.6)+ 0.2 mmol/L ETDA・2Na aq.
Mobile phase B	:	Methanol
Flow rate	:	0.7 mL/min
Time program	:	B Conc. 80% (0 min)-90% (3 min)-80% (3.01-5 min)
Column temp.	:	40 °C
Injection vol.	:	2 µL
Detection	:	UV 314 nm (SPD-M40), Standard cell
Vial	:	Shimadzu Vials, LC, 1.5 mL Clear Glass*2

*1 P/N: 227-32008-01 *2 P/N: 227-34001-01

Standard addition was carried out by spiking the solvent before shaking with a 1/10 concentration of the standard solution, and a spike-and-recovery test was conducted. Table 7 shows the results. The quantitative values and recovery rates indicate the average values when the analysis was repeated 6 times.

Table 7 Quantitation Results and Recovery Rates (N=6)

	Concentration (%)		Relative standard deviation of concentration (%RSD)		Addition recovery rate (%)		Relative standard deviation of recovery rate (%RSD)	
Sample	*α*-acids	*β*-acids	*α*-acids	*β*-acids	*α*-acids	*β*-acids	*α*-acids	*β*-acids
Hop A	5.1	14.3	1.4	1.2	98	90	1.1	1.0
Hop B	9.5	24.0	1.4	1.3	102	97	2.4	2.4

4. Conclusion

- The IBU values were measured with a UV spectrophotometer.
- HPLC was employed to achieve a more accurate quantitative analysis.
- In the analysis for some types of beer, the result of IBU may be affected by β-acids derived from hop.
- UHPLC analysis allows simultaneous analysis of the *α*- and *β*-acids extracted from hop pellets referring to EBC and ASBC.

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