BEER BITTERNESS

Reports of the Subcommittee on Determination of Isohumulones in Beer for 1967 and 1968 (Ref. 1) indicate that bitterness units (BU), as determined in Method A below, express the bitter flavor of beer satisfactorily, regardless of whether the beer was made with fresh or old hops. The European Brewery Convention has adopted the "E.B.C. Bitterness Units," determined in a similar way, as a uniform method that best expresses the true bitter flavor value of beer.

Method B, which has been archived, and Method C, below, determine iso-α-acids (IAAs). Method B employs solvent extraction, while Method C uses solid-phase extraction for isolating the IAAs from beer. Method D is an automated version of Method A for measuring BU.

While the results of the IAA methods are practically identical with those obtained by the BU method for beer brewed with fresh hops, the IAAs of beer brewed with old or poorly stored hops, and with certain special hop extracts, can be significantly lower than the BU figure.

A. BITTERNESS UNITS (BU)

(International Method)

Reagents

(a) *2,2,4-Trimethylpentane* (*isooctane*), spectrophotometric grade or equivalent. ASTM certified reference fuel grade isooctane may be used after one distillation, provided the absorbance at 275 nm meets the requirements for freedom from ultraviolet-absorbing substances specified below. A practical grade of isooctane may be used after purification by passage through a column of silica gel (12–28 mesh), such as that available from Fisher Scientific Company, Pittsburgh, PA 15219, under their designation S-156, grade 408. The isooctane reagent should have an absorbance at 275 nm in a 1-cm cell similar to that of freshly redistilled water from an all-glass still or an absorbance of not more than 0.005.

(b) *Hydrochloric acid,* 3*N*.

(c) *Octyl alcohol*, reagent grade or redistilled equivalent. One drop added to 20 mL 2,2,4-trimethylpentane (reagent a) must not increase absorbance reading at 275 nm by more than 0.005 in a 1-cm cell.

Apparatus

(a) *Mechanical shaker.* A platform type, or a "wrist-action" type shaker, with extending arm adjusted in a vertical plane so that tube will be held in a horizontal position.

(b) *Precision spectrophotometer*,[1] for use in the ultraviolet range.

(c) *Centrifuge tubes*, 50-mL, with glass stoppers or screw caps with Teflon lining.

(d) *Centrifuge* that will take 50-mL centrifuge tubes (apparatus c).

Method

Transfer 10.0 mL chilled carbonated beer (50°F) to a 50-mL centrifuge tube using a volumetric pipet that has had a minute amount of octyl alcohol (reagent c) introduced into the tip. Add 1 mL 3*N* HCl (reagent b) and 20 mL 2,2,4-trimethylpentane (reagent a). Stopper centrifuge tube tightly and place it on mechanical shaker for 15 min. The action must be vigorous. If required, centrifuge the tube long enough to separate the phases. As soon as possible, transfer sufficient clear, upper (isooctane) layer to cuvet of spectrophotometer. Set instrument to read zero absorbance at 275 nm for the 2,2,4-trimethylpentane-octyl alcohol blank (20 mL isooctane plus one drop octyl alcohol). Record absorbance at 275 nm (see Note).

Calculations

Calculate bitterness units of beer by the formula,

$$BU = \text{absorbance}_{275} \times 50.$$

Report bitterness units to nearest one-half unit.

Example

Absorbance of isooctane layer at 275 nm = 0.322.

$$BU = 0.322 \times 50 = 16.1.$$

Report BU as 16.0.

Note

Notice should be taken of recent findings that certain preservatives, such as *n*-heptyl *p*-hydroxybenzoate, and sorbates, and possibly some brewing adjuncts or coloring agents, may contribute to absorbance at the wavelengths specified in methods A and B. The possibility of picking up ultraviolet-absorbing extraneous substances would be greater in the BU methods than in the IAA methods. The possible effects of such materials should be checked before reporting bitterness values.

References

1. American Society of Brewing Chemists. Report of Subcommittee on Determination of Isohumulones in Beer. *Proc. 1967*, p. 269; *Proc. 1968*, p. 260.
2. Estimation of the Bitterness of Beer. *J. Inst. Brew*. 74:249, 1968.
3. Rigby, F. L., and Bethune, J. L. *J. Inst. Brew*. 61:325, 1955.
4. The E.B.C. Scale of Bitterness. *J. Inst. Brew* 73:525, 1967.
5. U.S. Pharmacopeia XVII, p. 1005.

[1] See **APPENDIX-1,A** for calibration of spectrophotometer.

1968, rev. 1975.

B. ISO-α-ACIDS (IAAs)

This section has been archived.

C. ISO-α-ACIDS BY SOLID-PHASE EXTRACTION AND HPLC

The iso-α-acids in degassed beer are adsorbed onto a solid-phase extraction (SPE) column and selectively desorbed (Ref. 3). The desorbed iso-α-acids are analyzed by high-performance liquid chromatography (HPLC) (Ref. 4).

Reagents

(a) *Calibration standard*, a sample of known total iso-α-acid content, such as a magnesium salt of iso-α-acids.
(b) *Tetraethylammonium hydroxide*, 10% in water.
(c) *Water,* HPLC grade.
(d) *Methanol,* HPLC grade.
(e) *Phosphoric acid,* 85% w/w.
(f) *Octanol.*
(g) *Desorbing solvent A*, 0.2 mL phosphoric acid (reagent e) in 100 mL water.
(h) *Desorbing solvent B,* 0.2 mL phosphoric acid (reagent e) in a mixture of 50 mL water and 50 mL methanol (reagent d).
(i) *Desorbing solvent C,* 0.1 mL phosphoric acid (reagent e) in 100 mL methanol (reagent d).

Apparatus

(a) *Volumetric flasks*, 2- and 100-mL.
(b) *Volumetric pipets*, 20-mL.
(c) *Graduated pipets*, 1-, 5-, and 10-mL.
(d) *C8 SPE octyl column*, 500 mg, 3-mL.
(e) *Plastic syringe*, 30-mL.
(f) *High-performance liquid chromatograph*, with a UV detector and 20-µL injection loop.
(g) *Integrator*.
(h) *Chromatographic column*, Shimadzu, Shim-pack CLC-ODS, 25 cm × 4.6 mm, or equivalent.
(i) *Analytical balance*, 0.1 mg capability.

Operating Conditions for Chromatography

The HPLC method described in **Hops-9,D** is used for chromatographic separation and quantitation of the three iso-α-acids. Variations from the **Hops-9,D** procedure are the elimination of the internal standard and the use of a 20-µL sample injection.

Method

Transfer approximately 200 mL beer to a beaker and degas by beaker transfer in excess of 20 times. Add one drop octanol (reagent f) and make additional transfers to assure foam collapse. Adjust pH to approximately 2.5 by adding 200 µL phosphoric acid (reagent e) per 100 mL beer.

Assemble the C8 SPE column equipment and apply the following adsorption/desorption sequence:

2 mL methanol (reagent d); discard eluate
2 mL water (reagent c); discard eluate
20 mL degassed beer using volumetric pipet; discard eluate
6 mL desorbing solvent A (reagent g); discard eluate
2 mL desorbing solvent B (reagent h); discard eluate
three successive 0.6-mL aliquots of desorbing solvent C (reagent i).

Collect eluates in a 2.0-mL volumetric flask. Make to volume with desorbing solvent C (reagent i), and mix thoroughly. Inject 20 µL for HPLC analysis. Analyze in duplicate.

Calibration

Weigh 20 mg of the calibration standard (reagent a), and record the mass to the nearest 0.1 mg. Dissolve the standard in methanol (reagent d) and make to volume in a 100-mL volumetric flask.

Inject 20 µL of the calibration solution four times (twice before and twice after the sample injections). Average the four response factors.

Calculations

$$RF = \frac{TA_{std}}{M_{std}(mg) \times \left(\frac{\%\ IAA_{std}}{100} \right)}$$

where

RF = response factor (average of four injections)

TA_{std} = total area of the three iso-α-acids peaks in the calibration assay

M_{std} = mass of calibration standard used

% IAA_{std} = percent purity of iso-α-acids in the calibration standard.

Since the calibration solution is a 100-mL volume and the method gives a 10-fold enrichment, calculation of iso-α-acids (mg/L) in beer is straightforward.

$$\text{Iso-α-acids (mg/ L)} = \frac{TA}{RF}$$

where

TA = total area of the three iso-α-acids peaks in the beer extract (average of duplicate assays).

Report results to the nearest 0.1 mg/ L.

Example

Mass of calibration standard = 20.7 mg

Reported purity of iso-α-acids in calibration standard = 92.3%

Average total area of calibration standard peaks = 12,770,000

Average total area of sample peaks = 7,527,520

$$\text{Response factor} = \frac{12{,}770{,}000}{\left(20.7 \times \frac{92.3}{100}\right)} = 668{,}373$$

$$\text{Iso-}\alpha\text{-acids (mg/ L)} = \frac{7{,}527{,}520}{668{,}373} = 11.3$$

Precision

Based on a collaborative study (Ref. 2), repeatability coefficients of variation of 2.0–3.9% and reproducibility coefficients of variation of 10.3–12.6% can be expected when iso-α-acid concentrations in beer are in the range of 10–30 mg/ L.

Note

Iso-α-acid calibration standards will deteriorate over time. Follow the supplier's recommendations for storage of calibration extracts.

References

1. American Society of Brewing Chemists. Report of Subcommittee on Iso-α-acids in Beer by Solid-Phase Extraction and High-Performance Liquid Chromatography. *Journal* 50:157, 1992.
2. American Society of Brewing Chemists. Report of Subcommittee on Iso-α-acids in Beer by Solid-Phase Extraction and High-Performance Liquid Chromatography. *Journal* 51:173, 1993.
3. Donley, J. R. *J. Am. Soc. Brew. Chem.* 50:89, 1992.
4. Ono, M., Kakudo, Y., Yamamoto, Y., Nagami, K., and Kumada, J. *J. Am. Soc. Brew. Chem.* 42:167, 1984.

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D. BITTERNESS BY AUTOMATED FLOW ANALYSIS

An automated flow analyzer is used in determining beer bitterness by simulating the manual isooctane extraction technique (Method A). A degassed beer sample is injected into a carrier stream containing a surfactant, combined with a 0.6*N* hydrochloric acid stream and an isooctane stream. After mixing in a coiled tube, the organic phase is separated from the flow stream, and the absorbance at 275 nm is determined.

Reagents

(a) *Deionized water (DI H_2O),* greater than 1 MΩ resistivity.
(b) *2,2,4-Trimethylpentane (isooctane),* 99%+ spectrophotometric grade.
(c) *Brij-35 stock, 1% (w/v).* Dissolve 1.0 g of Brij-35 (Aldrich No. 85,836-6 or equivalent) into 100 mL DI H_2O (reagent a).
(d) *Brij-35 carrier solution, 0.005% (v/v).* Pipet 5 mL of the Brij Stock (reagent c) into a 1,000-mL volumetric flask and dilute to volume with DI H_2O (reagent a).
(e) *Hydrochloric acid, 0.6N, with Brij-35, 0.005% (v/v).* Add 200 mL of DI H_2O (reagent a) to a 1,000 mL volumetric flask. Add 49.6 mL of concentrated reagent-grade hydrochloric acid and mix. Add 5 mL of Brij Stock (reagent c) to the flask and mix by swirling. Bring to volume with DI H_2O (reagent a).
(f) *Antifoam*, Antifoam-B (silicone emulsion) or equivalent.
(g) *Calibration beers*, beers containing a known beer bitterness units (BU) level as determined to the nearest 0.1 BU by Method A. At least two different BU levels should be used to define the linear calibration.

Apparatus

(a) *Automated flow analyzer*, configured and optimized for bitterness determination.
(b) *UV detector with flow cell*, capable of 275-nm detection with a bandpass of about 6 nm.
(c) *Ultrasonic bath*.
(d) *Eye dropper*.
(e) *Erlenmeyer flasks*, 250-mL or 500-mL.
(f) *Volumetric flasks*, 1,000-mL.
(g) *Volumetric pipets*, 5-mL.
(h) *Balance*, capable of 0.1 g.

Operating Conditions for Analyzer

(a) *Isooctane flow rate,* 2.0 mL/min or greater.
(b) *Carrier flow rate,* 0.4 mL/min or less.
(c) *0.6*N *HCl flow rate,* 0.4 mL/min.
(d) *Sample loop volume,* 200 µL.
(e) *Mixing coil length,* 6 m.

Calibration

Use the Brij carrier solution (reagent d) as calibration standard No. 1 for a zero BU calibration point. Place two drops of antifoam (reagent f) into an Erlenmeyer flask and slowly pour about 150 mL of a calibration beer (reagent g) into this flask. Swirl the flask several times to mix and slowly remove some carbonation. Touch the edge of the flask briefly (one or two seconds) into the ultrasonic bath to remove large volumes of carbonation. Repeat above step to remove enough carbonation to prevent foaming up the sides of the flask. Swirl the contents to remove any foam from the sides of the flask if necessary. Place the flask in the ultrasonic bath for 5 min. Label this flask calibration standard No. 2 or No. 3 based on its position within the calibration plot. Repeat the degassing procedure for the other calibration beer and label accordingly.

Inject the calibration standards in triplicate. Calibrate the instrument using the "known" BU values determined by Method A for each of the standards and their respective average peak heights obtained from the analyzer.

Method

Degas beer samples (see Notes) as described under the calibration section. Inject each sample in triplicate and record the average peak height for calculation of BU, or record the BU value calculated by the analyzer.

Calculations

The bitterness results are predicted from the linear relationship of beer BU and the absorbance intensity at 275 nm (based on peak height) of the bittering components extracted into the isooctane phase. A least squares fit of this relationship generates the slope and intercept used to calculate the sample bitterness from the sample peak height. Report the values obtained to the nearest 0.1 BU:

$$BU = (\text{slope} \times \text{peak height}) - \text{intercept}.$$

Example

Slope = 1.088×10^{-4}
Intercept = -8.397×10^{-2}
Peak height of beer injection = 118,186 units

$$BU = [(1.088 \times 10^{-4}) \times 118{,}186] - (-8.397 \times 10^{-2}) = 12.8$$

Precision

Based on a collaborative study (Ref. 2), repeatability coefficients of variation within a single laboratory of 0.7–1.5% and reproducibility coefficients of variation comparing two or more laboratories of 4.4–6.2% can be expected.

Notes

1. A collaborative study (Ref. 2) indicated that significantly lower BU results were obtained with this method compared with Method A when beers containing tetrahydro-iso-α-acids were analyzed. This problem was not encountered when analyzers with optimized extraction systems (Ref. 3) and grating monochrometers were used.

2. The collaborative studies (Refs. 1,2) did not investigate performance of the method on hexahydro-reduced hop constituents or on mixtures of the various bittering constituents.

References

1. American Society of Brewing Chemists. Report of Subcommittee on Beer Bitterness by Automated Flow Analysis. *Journal* 52:182, 1994.
2. American Society of Brewing Chemists. Report of Subcommittee on Bitterness in Beer by Automated Flow Analysis. *Journal* 53:216, 1995.
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4. Switala, K. J., and Schick, K. G. *J. Am. Soc. Brew. Chem.* 48:18, 1990.

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