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LZV936 Brewery Analysis Software

User Manual

07/2014, Edition 2

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1.1 Safety information

NOTICE

The manufacturer is not responsible for any damages due to misapplication or misuse of this product including, without limitation, direct, incidental and consequential damages, and disclaims such damages to the full extent permitted under applicable law. The user is solely responsible to identify critical application risks and install appropriate mechanisms to protect processes during a possible equipment malfunction.

Please read this entire manual before unpacking, setting up or operating this equipment. Pay attention to all danger and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment.

Make sure that the protection provided by this equipment is not impaired. Do not use or install this equipment in any manner other than that specified in this manual.

1.1.1 Use of hazard information

⚠DANGER

Indicates a potentially or imminently hazardous situation which, if not avoided, will result in death or serious injury.

⚠WARNING

Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

⚠CAUTION

Indicates a potentially hazardous situation that may result in minor or moderate injury.

NOTICE

Indicates a situation which, if not avoided, may cause damage to the instrument. Information that requires special emphasis.

1.1.2 Precautionary labels

Read all labels and tags attached to the instrument. Personal injury or damage to the instrument could occur if not observed. A symbol on the instrument is referenced in the manual with a precautionary statement.

	This symbol, if noted on the instrument, references the instruction manual for operation and/or safety information.
	Electrical equipment marked with this symbol may not be disposed of in European domestic or public disposal systems. Return old or end-of-life equipment to the manufacturer for disposal at no charge to the user.

1.1.3 Chemical and Biological Safety

DANGER	
	Chemical or biological hazards. If this instrument is used to monitor a treatment process and/or chemical feed system for which there are regulatory limits and monitoring requirements related to public health, public safety, food or beverage manufacture or processing, it is the responsibility of the user of this instrument to know and abide by any applicable regulation and to have sufficient and appropriate mechanisms in place for compliance with applicable regulations in the event of malfunction of the instrument.

Normal operation of this device may require the use of chemicals or samples that are biologically unsafe.

- Observe all cautionary information printed on the original solution containers and safety data sheets prior to their use.
- Dispose of all consumed solutions in accordance with the local and national regulations and laws.
- Select the type of protective equipment suitable to the concentration and quantity of the dangerous material being used.

1.2 Product overview

The Brewery Analysis software LZV936 is a set of spectrophotometric procedures that are relevant for brewery analysis. The procedures agree with the MEBAK user manual, the 1st edition published in 2012 or with the American Society of Brewing Chemists (ASBC), AACC International Approved Methods of Analysis, 11th Edition.

1.3 Install instrument updates

1. Select SYSTEM CHECKS>INSTRUMENT UPDATE.
2. Connect the USB flash drive to a USB connection (type A) of the instrument to install the LZV936 Brewery Analysis Software.
3. Push **OK**.
   Wait until the software is installed.
4. Set the instrument to off.
5. Wait at least 20 seconds, then set the instrument to on.

1.4 Select a test

1. Select **STORED PROGRAMS**.
   The programs are shown alphabetically. The procedures of the LZV936 Brewery Analysis Software are at the end of the list, with the test numbers 2001 to 2026.
2. Push **SELECT BY NUMBER** and enter the number.
3. Push **START**.

1.5 Use the SIP 10 sipper

Use the SIP 10 sipper module and a Pour-Thru cell for a fast and easy measurement. Refer to SIP 10 documentation for installation, configuration and information about sample insertion.

1.6 Sample collection and storage of beer

Correct sampling and storage are critical for accurate testing. Whether the analysis will be chemical, physical or microbiological, make sure to get a representative sample. It is necessary to prevent contamination during microbiological analyses.

In most procedures for chemical and physical analyses, it is necessary to degas the beer sample. Refer to Degassing methods and alternatives on page 6 or Degas with a rotary shaker on page 5 to degas the sample.

1.6.1 Degas with a rotary shaker

The rotary shaker method is a thorough, consistent method of beer decarbonation. This method standardizes decarbonation variables such as temperature, shaking velocity and time. The decarbonated beer is then measured by the standard method.

Based on a collaborative study[*], repeatability coefficients of variation for original gravity, alcohol and real extract in beer samples showed ranges of 0.1–0.4, 0.1–0.5 and 0.1–0.2%, which were judged acceptable. Reproducibility coefficients of variation for original gravity, alcohol and real extract showed ranges of 0.6–1.0, 0.8–1.6 and 0.4–1.0%, which were judged acceptable.

For nonalcoholic beer samples decarbonated by the rotary shaker method, repeatability coefficients of variation for original gravity, alcohol and real extract were 0.3, 1.7 and 0.5%. Reproducibility coefficients of variation for original gravity, alcohol and real extract were 1.0, 6.4 and 0.8%.

Note: *Beers degassed with the rotary shaker method had significantly lower original gravity and real extract determinations, as compared to beer degassed according to* Degassing methods and alternatives *on page 6*. *This difference was anticipated, as Constant and Collier[**] found that the rotary shaker method gives more complete decarbonation of beer samples, which ameliorates the influence of residual CO_2 on specific gravity determinations.*

1.6.1.1 Items to collect

Reagent

- Tributyl phosphate (TBP)

Accessories

- Water bath, 20°C (±1°C) (68°F)
- Rotary shaker, approved up to 300 rpm
- Platform for the rotary shaker
- Erlenmeyer flasks, 500-mL wide-mouth baffled flask, premark to 250-mL
- Aluminum foil
- Pipetter, 10–100 µl
- Pipet tips, 10–100 µl

1.6.1.2 Sample preparation

1. Modify the beer sample temperature to 20°C (68°F).
2. Use a pipet to add 10 µl of TBP into a 500-mL wide-mouth baffled Erlenmeyer flask.
3. Fill to the 250-mL mark with sample.
4. Add 10 µl of TBP again.
5. Make a 10-mm diameter hole in the center of the aluminium foil.

[*] American Society of Brewing Chemists, *Report of Subcommittee on Beer Decarbonation by a Rotary Shaker Method*, Journal 56:196, 1998

[**] *Constant, M. D., and Collier, J. E. , J. Am. Soc. Brew. Chem. 51:1, 1993, 1998*

6. Put the aluminum foil over the flask. Crimp the aluminum foil cover over the mouth of the flask.
7. Put the flask on the rotary shaker and shake for exactly 12 minutes at 190 rpm. The beer sample is now ready for analysis.

1.6.2 Degassing methods and alternatives

Method	Procedure	Time	Residual CO_2	Pros and Cons
Beer-1 A	Add the sample to a 500-mL Erlenmeyer flask. Shake by hand until there is no more gas in the sample.	5 to 20 minutes	Moderate to low (0.2 to 0.06)	The quantity of residual CO_2 depends on time and vigor of shaking.
Beer-1 D (Rotary shaker)	Add the sample to a 500-mL wide-mouth baffled Erlenmeyer flask. Put the sample in a rotary shaker for 12 minutes.	12 minutes	Low	Very specific in procedure with uniform results.
Pouring[1]	Pour the sample back and forth between two 500-mL beakers until there is no more gas in the sample.	5 to 20 minutes	Moderate to low (0.2 to 0.01)	The quantity of residual CO_2 depends on time and amount of pouring.
Ultrasonic bath[1]	Put the sample in the ultrasonic bath until there is no more gas in the sample.	15 minutes	Higher (0.56 vol)	The least effective of all methods, but the easiest to process a large number of samples.
Filtration	Filter the sample with fluted filter paper.	10 to 15 minutes	Higher (0.529 vol)	—
Ultrasonic bath and filtration[1]	Put the sample in the ultrasonic bath, then filter the sample with fluted filter paper.	10 minutes ultrasonic bath 10 to 15 minutes filtration	Moderate (0.41)	More effective than ultrasonic bath or filtration alone, still higher.
Gas purging	The sample has other inert gas bubbled through it to remove CO_2.	25 minutes	None	Not automated and is time consuming.
Membrane degassing[1]	The sample goes through an instrument that contains a semi-permeable membrane with negative pressure on one side to remove CO_2.	30 to 40 mL/min (5 to 10 minutes for a complete sample)	None	Not automated.
Water vacuum pump[1]	Put the sample under a water vacuum pump to remove CO_2 from negative pressure.	5 to 10 minutes	None	Not automated, removes other volatiles.
Microwave	Put the sample in a microwave to remove CO_2.	15 to 20 seconds	Low	Faster and more effective than filtration and ultrasonic bath.

[1] These degassing methods are usually used in the industry and found in literature. However these methods have not been validated through normal ASBC protocols.

1.7 Sample collection and storage of wort

NOTICE

Do not let wort samples stay at ambient temperature for any length of time. It is necessary to prevent contaminations. Put foil (or a similar covering) over the sample, then refrigerate immediately. Keep the samples cold until brought to the laboratory.

All worts are not fully stable and are subject to microbial, physical and chemical deterioration. The form and the color of the precipitates are time and temperature dependent. Make sure that the cooling rates, clarification procedures and handling of wort samples are standardized before the comparison of analytical results can start.

1. Get a sufficient sample of plant wort for analysis.
2. Keep the wort in a refrigerator or fill the wort in beer bottles and pasteurize it.
3. Mix the sample well before portions are removed for analysis.
4. Modify the wort temperature to 5–8°C (41–46°F).
5. Decant the wort from any sludge.
6. Filter the wort at 5–8°C (41–46°F) through filter paper.
7. If the wort filtrate is not brilliant, filter it again.

Note: *Do not use a filter aid, except for the portion of the filtrate that will processed again for color determination.*

1.8 List of abbreviations

Reagents, if not otherwise recorded, are of analytical purity. Solutions, if not otherwise recorded, are aqueous.

Abbreviation	Definition
s	Standard deviation
r	Repeatability (95 %)
R	Comparability (95 %)
V_K	Variation coefficient
m	Mean value

1.9 Literature

Literature	Description
ASBC literature	*AACC International Approved Methods of Analysis,* 11th Edition Published by: American Society of Brewing Chemists (ASBC) 3340 Pilot Knob RoadSt. Paul, MN 55121 USA
MEBAK brew-technical analysis methods	*Methods collection of the Mitteleuropäischen Brautechnischen Analysenkommision* (MEBAK, Central European brew-technical analysis commission), 1st edition, 2012 Published by the Chairman Dr. Heinrich Pfenninger, Self-publication of the MEBAK, D-85350 Freising-Weihenstephan

1.10 Procedure overview

Procedure	Range	Program	Wavelength	Zero	Vial
MEBAK Anthocyanogens	0–100 mg/L	2005	550	reagent blank	10 mm OS
MEBAK Beer color	0–60 EBC units	2006	430	distilled water	10 mm OS
ASBC Beer color (Beer-10A)	0–40°	2020	430	distilled water	10 mm OS
MEBAK Bitter units beer	10–40 BU	2001	275	iso-octane	10 mm QS
MEBAK Bitter units wort	20–60 BU	2003	275	iso-octane	10 mm QS
ASBC Bitter units beer (Beer-23)	10–100 IBU	2021	275	alcohol blank	10 mm QS
ASBC Bitter units wort (Wort-24)	20–200 IBU	2022	275	alcohol blank	10 mm QS
MEBAK FAN light wort	0–400 mg/L	2007	570	reagent blank	10 mm OS
MEBAK FAN light beer	0–400 mg/L	2008	570	reagent blank	10 mm OS
ASBC FAN beer (Beer-31)	0–400 mg/L	2024	570	reagent blank	10 mm OS
MEBAK FAN dark wort	0–400 mg/L	2015	570	reagent blank	10 mm OS
MEBAK FAN dark beer	0–400 mg/L	2016	570	reagent blank	10 mm OS
ASBC FAN wort (Wort-12)	0–400 mg/L	2025	570	reagent blank	10 mm OS
MEBAK Iron	0–1 mg/L	2017	560	sample blank	40 mm OS
MEBAK Iso-α and α acids	0–60 mg/L	2013	255 + 360	reagent blank	10 mm QS
MEBAK Photometric iodine sample	0–1 iodine value	2010	578	reagent blank	40 mm OS
MEBAK Thiobabituric acid number TAN c-wort	0–100	2011	448	distilled water	10 mm OS
MEBAK Thiobabituric acid number TAN beer/wort	0–100	2012	448	distilled water	10 mm OS
MEBAK Total polyphenols	0–800 mg/L	2002	600	sample blank	10 mm OS
ASBC Total polyphenols (Beer-35)	0–800 mg/L	2026	600	sample blank	10 mm OS
MEBAK Vicinal diketones	0–1 mg/kg	2014	335	reagent blank	20 mm QS

Procedure	Range	Program	Wavelength	Zero	Vial
ASBC Diacetly (Beer-25B)	0–1 mg/L	2023	530	reagent blank	10 mm OS
MEBAK Reducibility	0–100%	2004	520	sample blank	10 mm OS

Section 2 Accessories

Description	Item no.
Rectangular cuvettes set OS, (light pass = 10 mm, aligned pair)	2095100
Rectangular cuvette QS, light pass = 10 mm (3.5 mL)	2624410
Rectangular cuvette QS, light pass = 20 mm	LZV008
Rectangular cuvette OS, light pass = 20 mm	LZP331
Rectangular cuvette QS, light pass = 50 mm (17.5 mL)	2624450
Rectangular cuvette OS, light pass = 50 mm	LZP167
Pour-Thru cell QS, light pass = 50 mm	SM01X176501040
Pour-Thru cell QS, light pass = 10 mm	LZV510
SIP 10 sipper module set for the DR 6000 comes with a tray, tube set and a 1 cm quartz glass Pour-Thru cell	EU: LQV157.99.30001 US: LQV157.99.30002

Anthocyanogens

Harris and Ricketts method **Method 2005**

0 to 100 mg/L **MEBAK[1] method**

Scope and application: For beer and wort.

[1] *MEBAK Wort, beer, beer-based beverages*, 1st edition 2012, P. 226 et seq

Test preparation

Items to collect

Reagents

- MN-polyamide SC 6
- Solution 1: n-Butanol / 37 % hydrochloric acid 5+1 (V/V)
- Solution 2: Dissolve 120 mg iron(II)sulfate ($FeSO_4$ x 7 H_2O) in 100 mL of solution 1

Accessories

- Spectrophotometer, 550 nm
- 10-mm rectangular cuvette OS
- Shaking machine
- Centrifuge
- Vacuum pump
- Mixing flask, 50-mL, with ground-in stopper
- Reagent flask, 30-mL, graduation a maximum of 25-mL, with ground-in stopper
- Frit 1 G4
- Filter flask

Sample preparation

1. Centrifuge worts and young beers for 10 minutes at 3000 rpm.
2. Add 5 mL of beer (or wort) and 5 mL of distilled water to a 50-mL mixing flask.
3. **Prepare the blank:** Add 10 mL of distilled water to a 50-mL mixing flask.
4. Add 0.5 g of polyamide powder rinsed in 10 mL of distilled water in both mixing flasks (sample and blank mixing flasks).
5. Mechanically shake both mixing flasks for 40 minutes.
6. Filter each suspensions through a 1 G4 frit. Rinse twice with approximately 20 mL of distilled water.
7. Vacuum both frits (sample and blank frits) and polyamide powder dry. Then transfer the residue respectively with a spatula quantatively into two reagent flasks (sample and blank) and purge both frits with 15 mL of solution 1.
8. Add 0.5 mL of solution 2 to both reagent flasks.
9. Put both reagent flasks in a boiling water bath for 30 minutes.
10. During the first 5 minutes of the boiling water bath, stir each flask well with a glass rod.
11. Remove the glass rods and rinse with a little of solution 1.
12. Keep both reagent flasks at 20 °C (68 °F) and then fill both reagent flasks to the 25-mL mark with solution 1.
13. Fill the prepared sample and prepared blank in two 10-mm rectangular cuvettes.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2005. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in mg/L.

Principle

Anthocyanogens (leucoanthocyanidins) are phenolic compounds that change by hot hydrochloric acid into red-colored anthocyanidins. Quantity and condensation or polymerization degree of these compounds have an effect on the formation of colloidal turbidities in the beer. Stabilization measures with PVPP correlate to a reduction of the anthocyanogen content.

The anthocyanogens are adsorbed to polyamide. The adsorbate is dissolved in butanol-hydrochloric acid and heated. A red solution forms, whose intensity is measured photometrically.

More information

Option	Description
Results specifications	mg/L without decimals
Accuracy	r = 9
Standard values	50–70 mg/L (depending on raw materials and technological measures, a stabilization with PVPP correspondingly less)

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07/2014, Edition 2

Beer color

Spectrophotometric EBC method **Method 2006**

0 to 60 units **MEBAK[1] method**

Scope and application: For beer, wort and liquid malt.

[1] *MEBAK Wort, beer, beer-based beverages*, 1st edition 2012, P. 185 et seq

Test preparation

Items to collect

Reagents

- 0.1 % diatomaceous earth

Accessories

- Spectrophotometer, 430 nm (± 0.5 nm)
- 10-mm rectangular cuvette OS
- Membrane filter

Sample preparation

1. Dilute the sample so that the extinction is within the linearity of the spectrophotometer.
2. Filter the sample through a membrane filter. Filtration is not necessary if the turbidity of the diluted sample is below 1 EBC turbidity unit.
3. Clarify the beer as necessary by addition of 0.1 % diatomaceous earth and a filter upstream of the membrane filter.
4. Fill the prepared sample in a 10-mm rectangular cuvette.
5. **Prepare the blank:** Fill distilled water in a 10-mm rectangular cuvette.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2006. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in the display.

Principle

This method prevents the subjective influences of the human eye and differences in color impression with comparison of samples with a color standard. This technical method is considered the official reference method.

The extinction is measured in a 10-mm rectangular cuvette at a wavelength of 430 nm. The color in EBC units is derived by converting with a suitable factor.

Interferences

A spectrometric absorption curve does not reflect the color impression of the human eye, as light of equal intensity in various parts of the spectrum influences the eye differently. Also the extinction curves are at 430 nm very steep, so that slight measuring errors can occur. There are also differences during comparison of light beers with diluted dark beers.

Results specifications

EBC units with two indicative numerals.

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07/2014, Edition 2

Beer color

Spectrophotometric color method **Method 2020**

0 to 40° **ASBC[1] method Beer-10A**

Scope and application: For beer, wort and liquid malt.

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Test preparation

Items to collect

Accessories

- Spectrophotometer, 430 nm (± 0.5 nm), 700 nm
- 10-mm rectangular cuvette OS
- Membrane filter
- Centrifuge

Sample preparation

1. Decarbonate the sample.
2. Fill the prepared sample in a 10-mm rectangular cuvette and measure the absorbance at 430 nm and 700 nm.

Option	Description
Absorbance at 700 nm is ≤0.039 times absorbance at 430 nm	Beer is "free of turbidity". Refer to Procedure to measure the color.
Absorbance at 700 nm is >0.039 times absorbance at 430 nm	The turbidity is too high. Clarify the sample by centrifugation or filtration. Repeat the absorbance measurement at 430 nm and 700 nm. Report the filtration or centrifugation of the sample with the color value.

3. Fill the prepared sample in a 10-mm rectangular cuvette.
4. **Prepare the blank:** Fill distilled water in a 10-mm rectangular cuvette.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2020. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in degrees.

Principle

The sample must be free of turbidity to measure the beer color. The extinction is measured in a 10-mm rectangular cuvette at a wavelength of 430 nm and 700 nm. If absorbance at 700 nm is less or equal 0.039 times absorbance at 430 nm, beer is "free of turbidity" and color of beer is determined from the absorbance at 430 nm. Clarify the sample by centrifugation or filtration if the turbidity is too high. Report the filtration or

centrifugation of the sample with the color value. The color is reported as degrees to one decimal place.

Results specifications

Report beer color as degrees to one decimal place.

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07/2014, Edition 2

Bitter units

EBC procedure **Method 2001 and Method 2003**

Beer: 10 to 40 BU, wort: 20 to 60 BU **MEBAK[1] method**

Scope and application: For beer and wort.

[1] *MEBAK Wort, beer, beer-based beverages*, 1st edition 2012, P. 234 et seq

Test preparation

Items to collect

Reagents

- Hydrochloric acid, 6N
- Iso-octane (2,2,4-trimethylpentane), spectroscopic pure (extinction measured in 10-mm rectangular cuvette QS at 275 nm against distilled water less than 0.010)

Accessories

- Centrifuge glasses with solvent-proof seals, 35-mL
- Glass beads
- Shaking machine
- Centrifuge, 3000 rpm
- Spectrophotometer, 275 nm
- 10-mm rectangular cuvette QS

Sample preparation

1. Separate wort and cloudy beer with a centrifuge at 3000 rpm for 15 minutes.
   Note: *Do not use a filter.*
2. Remove carbon dioxide from the beer.
   Note: *Do not remove the foam.*
3. Modify the sample temperature to 20 °C (68 °F).
4. Add 10 mL of the sample (or 5 mL of wort and 5 mL of distilled water) in a centrifuge glass.
5. Add 0.5 mL 6N hydrochloric acid, 20 mL iso-octane and three glass beads to the sample.
6. Seal the centrifuge glass. Shake the centrifuge glass for about 15 minutes at 20 °C (68 °F).
7. Centrifuge the centrifuge glass for about 3 minutes at 3000 rpm.
8. Fill the prepared sample in a 10-mm rectangular cuvette.
9. **Prepare the blank**: Fill iso-octane in a 10-mm rectangular cuvette.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number. Push **OK**.

Number	Programm
2001	Bitter units beer
2003	Bitter units wort

4. Push **Start**.

5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in BU.

Principle

Bitter substances, mainly iso–α acids, are removed with iso-octane from the acidified sample. Measure the concentration of the iso-octane extract in 10-mm rectangular cuvette at 275 nm against iso-octane of the same quality (blank value).

Note: *Certain preservatives, such as n-heptyl p-hydroxybenzoate, sorbates and some brewing adjuncts or coloring agents, could contribute to absorbance at the wavelengths specified in this procedure. The possibility to catch ultraviolet-absorbing extraneous substances is greater in the BU procedure than in the IAA procedure. The possible effects of such materials must be examined before the measurement of bitter units.*

More information

Option	Description
Results specifications	Bitter units (BU) without decimals
Accuracy	r = –0.36 + 0.05 m; R = 0.72 + 0.14 m
Standard values	**Beer**: 10–40 BU, depending on nature, sort, type and origin; **Wort**: 20–60 BU, depending on beer and bitters yield

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07/2014, Edition 2

Bitter units

Manual isooctane extraction[1,2,3,4] (international method) **Method 2021 and Method 2022**

Beer: 10 to 100 IBU, wort: 20 to 200 IBU **ASBC[5,6] method: Beer-23, Wort-24**

Scope and application: For beer and wort.

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

Items to collect

Reagents

- Hydrochloric acid, 3N
- Iso-octane (2,2,4-trimethylpentane), spectroscopic pure (extinction measured in 10-mm rectangular cuvette QS at 275 nm against distilled water < 0.010)

Accessories

- Centrifuge glasses with solvent-proof seals, 35-mL
- Glass beads
- Shaking machine
- Centrifuge, 3000 rpm
- Spectrophotometer, 275 nm
- 10-mm rectangular cuvette QS

Sample preparation

1. Modify the sample temperature to 10 °C (50 °F).
2. Fill 10 mL of the sample in a 50-mL centrifuge glass.
3. Add 1 drop octyl alcohol, 1 mL 3N hydrochloric acid and 20 mL iso-octane to the sample.
4. Seal the centrifuge glass. Centrifuge the centrifuge glass for about 15 minutes.
5. Fill the upper, clear layer (prepared sample) in a 10-mm rectangular cuvette.
6. **Prepare the blank**: Add 1 drop octyl alcohol to 20 mL of iso-octane. Fill the solution (prepared blank) in a 10-mm rectangular cuvette.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number. Push **OK**.

Number	Programm
2021	ASBC Bitter units beer
2022	Bitter units wort

4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in BU.

Principle

Bitter substances, mainly iso–α acids, are removed with iso-octane from the acidified sample. Measure the concentration of the iso-octane extract in 10-mm rectangular cuvette at 275 nm against iso-octane of the same quality (blank value).

Note: *Certain preservatives, such as n-heptyl p-hydroxybenzoate, sorbates and some brewing adjuncts or coloring agents, could contribute to absorbance at the wavelengths specified in this procedure. The possibility to catch ultraviolet-absorbing extraneous substances is greater in the BU procedure than in the IAA procedure. The possible effects of such materials must be examined before the measurement of bitter units.*

Results specifications

International bitter units (IBU) to nearest one-half unit.

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07/2014, Edition 2

Free amino nitrogen (FAN)

Ninhydrin method according to EBC **Method 2007/8 and Method 2015/16**

0 to 400 mg/L **MEBAK[1] method**

Scope and application: For light and dark beer, light and dark wort.

[1] *MEBAK Wort, beer, beer-based beverages,* 1st edition 2012, P. 84 et seq

Test preparation

Items to collect

Reagents

- **Color reagent**
  1. Add 10.0 g of di-sodium-hydrogen phosphate (Na_2HPO_4 x 12 H_2O), 6.0 g of potassium di-hydrogen phosphate (KH_2PO_4), 0.5 g of ninhydrin and 0.3 g of fructose in a 100-mL volumetric flask.
  2. Fill the volumetric flask to the 100-mL mark with distilled water. Keep this solution for a maximum of 2 weeks in a dark flask. The pH value must be between 6.6–6.8.
- **Thinner**
  1. Dissolve 2 g of potassium iodate in 600 mL of distilled water and add 400 mL of 96 % ethanol. Keep this thinner solution at 5 °C (41 °F).
- **Parent solution**
  1. Dissolve 107.2 mg of glycine in 100 mL of distilled water. Keep this parent solution at 0 °C (32 °F).
- **Standard solution**
  1. Add 1 mL of parent solution in a 100-mL volumetric flask.
  2. Fill the volumetric flask to the 100-mL mark with distilled water. This standard solution contains 2 mg/L of amino nitrogen.

Accessories

- Spectrophotometer, 570 nm
- 10-mm rectangular cuvette OS
- Reagent flasks with ground-in stoppers, 16 × 150 mm
- Variable pipette 1.0–5.0 mL
- Pipette tips
- Boiling water bath
- Water bath at 20 °C (68 °F)
- Volumetric flask, 100-mL

Sample preparation for light beer and wort

1. Dilute the wort 100 times and the beer 50 times (1–3 mg/L amino nitrogen).
2. Analyze the sample, standard solution and the blank value three times each.
3. Add 2 mL of diluted sample or standard solution or distilled water into a reagent flask.
4. Add 1 mL of color reagent in each reagent flask. Swirl the flasks to mix.
5. Loosely seal the reagent flasks with glass stoppers to prevent loss due to evaporation.
6. Put the reagent flasks for exactly 16 minutes in a boiling water bath.

7. Put the reagent flasks for 20 minutes in a water bath of 20 °C (68 °F).
8. Add 5 mL of thinner in each reagent flask.
9. Fill the prepared sample, standard and blank in a 10-mm rectangular cuvette. Measure the extinction within 30 minutes at 570 nm.

Sample preparation for dark beer and wort

1. Dilute the wort 100 times and the beer 50 times (1–3 mg/L amino nitrogen).
2. Analyze the sample, correction solution, standard solution and the blank value three times each.
3. Add 2 mL of diluted sample or standard solution or distilled water into a reagent flask.
4. **Prepare sample, standard solution and blank value:** Add 1 mL of color reagent in each reagent flask. Swirl the flasks to mix.
5. **Prepare correction solution:** Add 1 mL of distilled water in each reagent flask and mix.
6. Loosely seal the reagent flasks with glass stoppers to prevent loss due to evaporation.
7. Put the reagent flasks for exactly 16 minutes in a boiling water bath.
8. Put the reagent flasks for 20 minutes in a water bath of 20 °C (68 °F).
9. Add 5 mL of thinner in each reagent flask.
10. Fill the prepared sample, correction solution, standard solution and blank value in a 10-mm rectangular cuvette. Measure the extinction within 30 minutes at 570 nm.

Procedure for light beer and wort

The procedure that follow shows a triplicate determination of blank values, standard solution and samples without correction of light beer and worts.

1. Prepare the zero solution (distilled water), blank value, standard solution and sample each three times.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number. Push **OK**.

Number	Programm
2008	FAN light beer
2007	FAN light wort

4. Push **Start**.
5. Install the zero solution. Push **Zero**.
   Z1 shows in the display.
6. Install the blank value. Push **Read**.
   R1 shows in the display.
   Note: *Do step 6 for the blank value cuvettes 2 and 3. R2 and R3 show in the display.*
7. Install the standard solution. Push **Read**.
   R4 shows in the display.
   Note: *Do step 7 for the standard solution cuvettes 2 and 3. R5 and R6 show in the display.*
8. Install the prepared sample. Push **Read**.
   R7 shows in the display.
   Note: *Do step 8 for the prepared sample cuvettes 2 and 3. R8 and the result show in the display.*
9. The FAN result shown in mg/L.

Procedure for dark beer and wort

The procedure that follow shows a triplicate determination of blank values, standard solution, correction and samples for dark beer and worts.

1. Prepare the zero solution (distilled water), blank value, standard solution, correction solution and sample each three times.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number. Push **OK**.

Number	Programm
2016	FAN dark beer
2015	FAN dark wort

4. Push **Start**.
5. Install the zero solution. Push **Zero**.
   Z1 shows in the display.
6. Install the blank value. Push **Read**.
   R1 shows in the display.
   Note: *Do step 6 for the blank value cuvettes 2 and 3. R2 and R3 show in the display.*
7. Install the standard solution. Push **Read**.
   R4 shows in the display.
   Note: *Do step 7 for the standard solution cuvettes 2 and 3. R5 and R6 show in the display.*
8. Install the correction solution. Push **Read**.
   R7 shows in the display.
   Note: *Do step 8 for the correction solution cuvettes 2 and 3. R8 and R9 show in the display.*
9. Install the prepared sample. Push **Read**.
   R10 shows in the display.
   Note: *Do step 9 for the prepared sample cuvettes 2 and 3. R11 and the result show in the display.*
10. The FAN results show in mg/L.

Principle

The examination solution must have the pH value 6.7 with ninhydrin. Measure the resultant color at 570 nm. The method records the amino acids, ammonia and also the terminal alpha amino groups of peptides and proteins. Prolin is partially co-determined at the applied wavelength. The method is not specific to alpha-amino-nitrogen because gammaamino-butanoic acid, which occurs in worts, also develops a color with ninhydrin.

Interferences

Since this test measures small quantities of amino acids, make sure to prevent contaminations. Clean the flasks carefully. Only touch the external surface of cleaned flaskes. Only use tweezers to move ground-in stoppers.

More information

Option	Description
Results specifications	In mg/L without decimals
Standard value	Cast wort (12 %): 200–250 mg/L; Beer (12 %): 10–120 mg/L Approximately 220–250 mg/L of free amino nitrogen in the engaged wort are necessary for a satisfactory primary and secondary fermentation.

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07/2014, Edition 2

Free amino nitrogen

International Ninhydrin method[1,2,3] **Method 2024 and Method 2025**

0 to 400 mg/L **ASBC[4,5] method: Beer-31, Wort-12**

Scope and application: For beer and wort.

1 European Brewery Convention. Analytica-EBC, 4th ed. *Schweizer Brauerei-Rundschau*, CH-8047, Zurich, Switzerland,1987.
2 Lie, S., *J. Inst. Brew.* 79:37. 1973.
3 Wylie, E. B., and Johnson, M. J. Biochim, *Biophys.* Acta 59:450, 1962.1975, rev. 1976, 2010
4 American Society of Brewing Chemists. *Report of Subcommittee on Free Amino Nitrogen.* Proc. 1974 , p. 34; Proc. 1975 (Vol.33, No. 3), p. 88.
5 *American Society of Brewing Chemists.* Reproduced with permission from the American Society of Brewing Chemists for use by purchasers of specified Hach instruments only. No other use or reproduction is permitted without written permission from the American Society of Brewing Chemists.

Test preparation

Before starting

Since the quantities of amino nitrogen reacting in this method are very small, it is necessary to prevent contaminations. Carefully clean glassware and only touch the external surfaces. Use suction bulbs for pipets and forceps to move glass marbles.

It is necessary to follow the times and temperatures for this procedure. A standard and blank sample must be included in each test to compensate for temperature variations in the boiling-water bath.

Suggested dilutions given for wort and beer are applicable for samples to contain 1–3 mg amino N/L after dilution. Examples given are for 65% malt and 12°P wort. For other malt-adjunct ratios and other °P values, make the necessary dilution adjustments.

Items to collect

Reagents

- **Color reagent**
  1. Add 10.0 g of di-sodium-hydrogen phosphate (Na_2HPO_4 x 12 H_2O), 6.0 g of potassium di-hydrogen phosphate (KH_2PO_4), 0.5 g of ninhydrin and 0.3 g of fructose in a 100-mL volumetric flask.
  2. Fill the volumetric flask to the 100-mL mark with distilled water. Keep this solution for a maximum of 2 weeks in a dark flask. The pH value must be between 6.6–6.8.
- **Thinner**
  1. Dissolve 2 g of potassium iodate in 600 mL of distilled water and add 400 mL of 96 % ethanol. Keep this thinner solution at 5 °C (41 °F).
- **Parent solution**
  1. Dissolve 107.2 mg of glycine in 100 mL of distilled water. Keep this parent solution at 0 °C (32 °F).
- **Standard solution**
  1. Add 1 mL of parent solution in a 100-mL volumetric flask.
  2. Fill the volumetric flask to the 100-mL mark with distilled water. This standard solution contains 2 mg/L of amino nitrogen.

Accessories

- Spectrophotometer, 570 nm

- 10-mm rectangular cuvette OS
- Reagent flasks with ground-in stoppers, 16 × 150 mm
- Variable pipette 1.0–5.0 mL
- Pipette tips
- Boiling water bath
- Water bath at 20 °C (68 °F)
- Volumetric flask, 100-mL

Sample preparation for light beer and wort

1. Dilute the wort 100 times and the beer 50 times (1–3 mg/L amino nitrogen).
2. Analyze the sample, standard solution and the blank value three times each.
3. Add 2 mL of diluted sample or standard solution or distilled water into a reagent flask.
4. Add 1 mL of color reagent in each reagent flask. Swirl the flasks to mix.
5. Loosely seal the reagent flasks with glass stoppers to prevent loss due to evaporation.
6. Put the reagent flasks for exactly 16 minutes in a boiling water bath.
7. Put the reagent flasks for 20 minutes in a water bath of 20 °C (68 °F).
8. Add 5 mL of thinner in each reagent flask.
9. Fill the prepared sample, standard and blank in a 10-mm rectangular cuvette. Measure the extinction within 30 minutes at 570 nm.

Procedure

The procedure that follow shows a triplicate determination of blank values, standard solution and samples without correction of light beer and worts.

1. Prepare the zero solution (distilled water), blank value, standard solution and sample each three times.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number. Push **OK**.

Number	Programm
2024	ASBC FAN beer
2025	ASBC FAN wort

4. Push **Start**.
5. Install the zero solution. Push **Zero**.
   Z1 shows in the display.
6. Install the blank value. Push **Read**.
   R1 shows in the display.
   Note: *Do step 6 for the blank value cuvettes 2 and 3. R2 and R3 show in the display.*
7. Install the standard solution. Push **Read**.
   R4 shows in the display.
   Note: *Do step 7 for the standard solution cuvettes 2 and 3. R5 and R6 show in the display.*
8. Install the prepared sample. Push **Read**.
   R7 shows in the display.
   Note: *Do step 8 for the prepared sample cuvettes 2 and 3. R8 and the result show in the display.*
9. The FAN result shown in mg/L.

Principle

Use the ninhydrin method to determine the quantity of free amino nitrogen in wort or beer. The method provides information about the quantity of amino nitrogen available to yeast during fermentation or the quantity of amino nitrogen that remains in beer after fermentation. The method measures amino acids, ammonia and to some extent end-

group α-amino nitrogen in peptides and proteins. The method is not specific for α-amino nitrogen since γ-aminobutyric acid, which is present in both wort and beer, yields substantial color with ninhydrin.

Result specifications

Wort: In mg/L without decimals

Beer: In mg/L with one decimals

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07/2014, Edition 2

Iron

Method 2017

0 to 1 mg/L **MEBAK[1] method**

Scope and application: For beer.

[1] *MEBAK Wort, beer, beer-based beverages*, 1st edition 2012, P. 423 et seq

Test preparation

Items to collect

Reagents
Prepare all solutions with iron-free distilled water.

- **Buffer solution**, pH 4.3

  1. Dissolve 75 g of ammonium acetate and 150 g of concentrated acetic acid in approximeately 800 mL distilled water.
  2. Control the pH value and fill the volumetric flask to the 1-L mark with distilled water.

- **Ferrozine reagent**

  1. Dissolve 0.257 g of ferrozine or ferrospectral in 50 mL buffer. Keep this solution for a maximum of 2 weeks.

- **Ascorbic acid**, 2.5 % (prepare fresh daily)
- **Hydrochloric acid**, concentrated
- **Iron(III) standard solution** for determination of the calibration curve

  1. Add 863.4 mg of ammonium iron(III) sulfate [$NH_4Fe(SO_4)_2$ x 12 H_2O] in a 1-L volumetric flask.
  2. Add 0.1 mL of concentrated hydraulic acid and fill the volumetric flask to the 1-L mark with distilled water.
  3. Add 50 mL of this solution in a 1-L volumetric flask. Fill the volumetric flask to the 1-L mark with distilled water. The standard solution contains 5 mg/mL Fe^{3+}.

Accessories

- Spectrophotometer, 560 nm
- 40-mm rectangular cuvette OS
- Weighing balances, reading precision 0.1 mg
- Pipettes, 0.1 mL, 2 mL, 5 mL

Determination of the calibration curve

The factor 1 = 0.037 is an empirical factor and must be individually determined through a calibration curve. The factor is the slope of the calibration curves.

1. Add 40 mL of beer into four 50-mL volumetric flasks.
2. Add 0.40 mL, 0.80 mL, 1.60 mL and 3.20 mL of the iron(III) standard solution into each volumetric flask.
3. Add 2 mL of ferrozine reagent and 1 mL of ascorbic acid solution into each volumetric flask .
4. Fill the volumetric flask to the 50-mL mark with distilled water.

5. Measure the extinction of the solution in a 40-mm rectangular cuvette at 560 nm against a corresponding blank value.
6. Subtract the results of the un-spiked sample from the spiked samples.

Sample preparation

1. Degas the beer and let the foam subside completely.
2. Add 40 mL of beer, 2 mL of ferrozine reagent and 1 mL of ascorbic acid solution into a 50-mL volumetric flask.
3. Fill the volumetric flask to the 50-mL mark with distilled water.
4. **Prepare the blank value:** Add 40 mL of beer and 1 mL of ascorbic acid solution into a 50-mL volumetric flask. Fill the volumetric flask to the 50-mL mark with distilled water.
   Note: *Use a separate blank for each individual beer.*
5. Measure the extinction of the solution in a 40-mm rectangular cuvette at 560 nm against a corresponding blank value.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2017. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in mg/L.

Principle

Iron can dissolve into beer through raw materials, filter additives and clarifiers. Iron can be absorbed from appliances, lines, containers and beer foam stabilizing agents. Iron negatively has an effect on the colloidal stability, the taste and the gushing tendency of the beer.

Bivalent iron forms a violet color with a very high molar extinction coefficient with the di-sodium salt of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine-4,4-disulfonic acids (ferrozine). It is necessary to reduce trivalent iron to bivalent ion iron before measurement. The color intensity is measured spectrometrically.

More information

Option	Description
Results specifications	In mg/L with three decimals
Accuracy	r = 0.0080
Target value	< 0.200 mg/L

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 07/2014, Edition 2

Iso-α and β acids

Method 2013

0 to 60 mg/L **MEBAK[1] method**

Scope and application: For beer and wort.

[1] *MEBAK Wort, beer, beer-based beverages*, 1st edition 2012, P. 237 et seq

Test preparation

Items to collect

Reagents

- **Hydrochloric acid**, 6N
- **Iso-octane** (2,2,4-trimethylpentane), spectroscopic purity
- **Sodium sulfate**, anhydrous
- **Methanol**
- **Hydrochloric acid**, 4N
- **Sodium hydroxide**, 6N, decarbonated
- **Acidic methanol solution** (prepare fresh daily)
  1. Mix 64 mL of methanol and 36 mL of 4N hydrochloric acid.
- **Alkali methanol solution** (prepare fresh daily)
  1. Add 0.2 mL of 6N sodium hydroxide into a 100-mL volumetric flask and fill to the 100-mL mark with methanol.

Accessories

- Spectrophotometer, 225 nm and 360 nm
- 10-mm rectangular cuvette QS
- Centrifuge, 3000 rpm
- Centrifuge flasks with solvent-proof screw closure, 100–110 mL capacity
- Shaking machine

Sample preparation

1. Separate wort and cloudy beer with a centrifuge at 3000 rpm for 15 minutes.
   Note: *Do not use a filter.*
2. Remove carbon dioxide from the beer.
   Note: *Do not remove the foam.*
3. Modify the sample temperature to 20 °C (68 °F).
4. Add 50 mL of the sample into a centrifuge flask.
5. Add 3 mL of 6N hydrochloric acid and 25 mL of iso-octane.
6. Seal the centrifuge flask and shake it mechanically for 30 minutes at optimal mixing intensity.
7. Centrifuge to separate the phases and break up the emulsion for 5 minutes at 3000 rpm.
8. Remove the lower aqueous phase through suction with a pipette and discard it. Displace the iso-octane phase with enough sodium sulfate that the solution is clear after brief vigorous shaking.
9. Add 10 mL of the iso-octane phase into a 25-mL volumetric flask.

10. Add 10 mL of acidic methanol solution.
11. Seal the flask and invert the flask 100 times.
12. Add 5 mL of the excess clear iso-octane phase into a 25-mL volumetric flask.
13. Fill the volumetric flask to the 25-mL mark with alkali methanol solution.
14. Measure the extinction of the iso-octane solution against a blank value at 255 nm and 360 nm.
15. **Prepare blank value:** Add 5 mL of iso-octane into a 25-mL volumetric flask. Fill the volumetric flask to the 25-mL mark with alkali methanol solution.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2013. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in mg/L.

Principle

Bitters are extracted with iso-octane from the acidified sample and certain disruptive substances removed through washing of the extract with acidic methanol. The concentration of iso-α acids and β acids is determined through measuring of the extinction in alkali methanol at 255 nm and 360 nm.

More information

Option	Description
Results specifications	In mg/L without decimals
Accuracy	V_{kr} = ±5%
Standard value	Beer: 10–40 mg/L iso-α acids, depending on nature, sort, type and origin; < 2mg/L β acids Wort: 15–50 mg/L iso-α acids, depending on beer and bitters yield; 1–15 mg/L β acids depending on degree of isomerization

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Photometric iodine sample

Method 2010

0 to 1 iodine value **MEBAK[1] method**

Scope and application: For wort and beer.

[1] *MEBAK Wort, beer, beer-based beverages,* 1st edition 2012, P. 52 et seq

Test preparation

Items to collect

Reagents

- **Ethanol**, 95%
- **Iodine solution**, 1N (parent solution)
- **Iodine solution**, 0.02N (prepare fresh daily from the parent solution)
- **Phosphate buffer solution**, 0.1M, pH 3.5

  1. Adjust 0.1M KH_2PO_4 solution with 0.1M phosphoric acid to pH 3.5.

Accessories

- Spectrophotometer, 578 nm
- 40-mm rectangular cuvette OS
- "Plumper" or plastic spatula
- Centrifuge
- Centrifuge flasks with ground-in stoppers, 100–110 mL capacity
- Shaking machine
- Pipettes, 0.5, 2, 10, 20 and 40 mL

Sample preparation

1. Add 10.0 mL of centrifuged wort or decarbonated beer into a centrifuge flask.
2. Add 40.0 mL of ethanol and shake mechanically for 10 minutes.
3. Centrifuge for 5 minutes at 2500 rpm.
4. Decant the clear phase as carefully and completely as possible.
5. Add 20.0 mL of phosphate buffer solution and shake mechanical for 10 minutes to loose the residue.
6. Centrifuge the solution for 5 minutes at 2500 rpm.
7. Add 2 mL of the excess and 8 mL of phosphate buffer solution into a 40-mm rectangular cuvette and measure at 578 nm against phosphate buffer solution.
8. Add 0.5 mL of 0.02N iodine solution and mix immediately the contents with the plumper. Measure after 30 seconds.
9. **Prepare iodine blank value:** Add 10 mL of phosphate buffer solution and 0.5 mL of 0.02N iodine solution into a 40-mm rectangular cuvette and mix. Measure the extinction at 578 nm against phosphate buffer solution.

Procedure

1. Prepare the blank value (phosphate buffer), the iodine blank value and the sample.
2. Select **STORED PROGRAMS>SELECT BY NUMBER**.
3. Enter the number 2010. Push **OK**.
4. Push **Start**.

5. Install the blank value (phosphate buffer). Push **Zero**.
   Z1 shows in the display.
6. Install the iodine blank value. Push **Read**.
   R1 shows in the display.
7. Install the prepared sample. Push **Read**.
   R2 shows in the display.
8. Remove the analysis cuvette and add 0.5 mL of 0.02N iodine solution to the analysis cuvette.
9. Mix the content immediately with the "plumper".
10. Install the analysis cuvette after 30 seconds. Push **Read**.

The result shown in the display.

Principle

High-molecular dextrines and starches are precipitated through addition of ethanol to wort and beer, centrifuged out, dissolved in phosphate buffer and displaced with iodine solution. Depending on molecular weight and branching factor of the erythrodextrine and starch, a red to blue color forms, that is read photometrically.

More information

Option	Description
Results specifications	Extinction to two decimals
Accuracy	V_{kr} = ±3%
Standard value	< 0.45 (wort)

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07/2014, Edition 2

Thiobarbituric acid number (TAN)

Method 2011 and Method 2012

0 to 100 **MEBAK[1] method**

Scope and application: For beer, wort, congress wort and malt extract.

[1] *MEBAK Wort, beer, beer-based beverages*, 1st edition 2012, P. 55 et seq

Test preparation

Items to collect

Reagents

- **Acetic acid**, 90 %
  1. Dilute 225 g of acetic acid 100 % (glacial acetic acid) with distilled water to 250 g.
- **Thiobarbituric acid**, 0.02 mol/L (prepare fresh daily)
  1. Add 0.288 g of 2-thiobarbituric acid (M = 144.15 g/mol) and 90% acetic acid in 100-mL volumetric flask. Increase the temperature with a water bath to dilute the suspension.
  2. Modify the solution temperature to 20 °C (68 °F).
  3. Fill the volumetric flask to the 100-mL mark with 90% acetic acid.
- **Diatomaceous Earth**

Accessories

- Spectrophotometer, 448 nm
- 10-mm rectangular cuvette OS
- Wather bath, 70°C (158°F)
- Brown reaction flasks with ground-in stopper, 20-mL or 25-mL

Sample preparation

For the best accuracy, prepare the sample as follows:

1. Clarify cloudy examination solutions through filtration over diatomaceous earth.
2. Dilute the sample.

Option	Description
Wort and beer	Dilute 10 times with distilled water.
Congress wort	Dilute 5 times with distilled water.

3. Prepare the empty value as follows:
   a. Add 10 mL of the diluted sample and 5 mL of 90% acetic acid in a reagent flask.
   b. Shake the solution and proceed as for the main value.
4. Prepare the main value as follows:
   a. Add 10 mL of the diluted sample and 5 mL of thiobarbituric acid in a reagent flask and shake.
   b. Put the reagent flasks in a water bath of 70 °C (158 °F) for 70 minutes, prevent exposure to direct sunlight. Make sure that the temperature of the reagent flasks in the bath only temporarily decreases by 1–2 °K.
   c. After reaction time, quickly decrease the temperature of the reagent flasks to 20 °C (68 °F) (fast-flowing cold water or cold bath).

d. Measure immediately the extinction of the solution in a 10-mm rectangular cuvette at 448 nm against distilled water.

Procedure

1. Prepare the zero solution (distilled water), empty value and sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number. Push **OK**.

Number	Programm
2011	TAN C-wort
2012	TAN beer/wort

4. Push **Start**.
5. Install the zero solution (distilled water). Push **Zero**.
   Z1 shows in the display.
6. Install the empty value. Push **Read**.
   R1 shows in the display.
7. Install the prepared sample. Push **Read**.
   The results show in the display.

Principle

The thiobarbituric acid number is a summary variable for the thermal load of malt and wort. It is an indicator that in addition to 5-hydroxymethylfurfural (HMF) records a multitude of products of the Maillard reaction and other organic compounds.

The examination sample is used with acetic thiobarbituric acid solution in reaction and the yellow coloration is measured spectrophotometrically.

More information

Option	Description
Results specifications	Thiobarbituric acid number (TAN), dimensionless number
Standard value	Light cast wort < 45 Light cold wort (after wort cooling) < 60

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07/2014, Edition 2

Total polyphenols

EBC procedure **Method 2002**

0 to 800 mg/L **MEBAK[1] method**

Scope and application: For beer and wort.

[1] *MEBAK Wort, beer, beer-based beverages,* 1st edition 2012, P. 223 et seq

Test preparation

Items to collect

Reagents

- **Carboxymethylcellulose-ethylenediaminetetraacetic acid solution** (CMC-EDTA-Na):
  1. Add 10 g of CMC (low-viscosity), 2 g of EDTA-Na_2 and 500 mL of distilled water in a 1-L volumetric flask.
  2. Stir the solution until complete dissolution.
  3. Fill the volumetric flask to the 1–L mark with distilled water.
  4. If necessary, use a centrifuge to clear the solution.
- **Ammonium iron(III) citrate**, 3.5%:
  1. Add 3.5 g of ammonium iron(III) citrate, green (16 % Fe) in a 100-mL volumetric flask.
  2. Fill the volumetric flask to the 100-mL mark with distilled water.
  3. Stir the solution until completely dissolved. The solution must be completely clear. Keep the solution for a maximum of 1 week.
- **Ammonia**, diluted:
  1. Dilute one part of concentrated ammonia (d = 0.91) with two parts of distilled water.

Accessories

- Spectrophotometer, 600 nm
- 10-mm rectangular cuvette OS
- Centrifuge
- Volumetric flask, 1-L
- Volumetric flask, 25-mL
- Volumetric flask, 100-mL

Sample preparation

1. Shake the beer to remove carbon dioxide.
2. Clarify the cloudy wort or beer with a centrifuge.
3. Add 10 mL of sample and 8 mL of CMC/EDTA reagent in a 25-mL volumetric flask. Fully mix the solution.
4. Add 0.5 mL of ferric reagent to the solution. Fully mix the solution.
5. Add 0.5 mL of ammonia, diluted to the solution. Fully mix the solution.
6. Fill the volumetric flask to the 25-mL mark with distilled water. Fully mix the solution.

7. Wait for 10 minutes. Then fill the prepared sample in a 10-mm rectangular cuvette.
8. Prepare the blank as follows:
   a. Add 10 mL of sample (clear and with no carbon dioxide) and 8 mL of CMC-EDTA-Na solution in a 25-mL volumetric flask. Fully mix the solution.
   b. Add 0.5 mL of ammonia, diluted to the solution. Fully mix the solution.
   c. Fill the volumetric flask to the 25-mL mark with distilled water. Fully mix the solution.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2002. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in mg/L.

Principle

Polyphenols react with iron(III) ions in alkali solution to a colored iron complexes. Measure the brown color in 10 mm rectangular cuvette at 600 nm against prepared blank value.

More information

Option	Description
Accuracy	r = 4.1
Standard values	**Beer**: 150—200 mg/L

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07/2014, Edition 2

Total polyphenols

Method 2026

0 to 800 mg/L **ASBC[1,2] method: Beer-35[3]**

Scope and application: For beer and wort.

[1] American Society of Brewing Chemists. *Report of Subcommittee-tee on Polyphenols in Beer*. Journal 36: 129, 1978

[2] *American Society of Brewing Chemists.* Reproduced with permission from the American Society of Brewing Chemists for use by purchasers of specified Hach instruments only. No other use or reproduction is permitted without written permission from the American Society of Brewing Chemists.

[3] European Brewery Convention. Analytica-EBC, 3rd ed. Method 7.7, p. E64. *Schweizer Brauerei-Rundschau*, Zurich, Switzerland, 1975

Test preparation

Items to collect

Reagents

- **Carboxymethylcellulose (CMC/EDTA) reagent** (1% solution of sodium salt of CMC (low viscosity) with addition of 0.2% ethylenediamine tetraacetic acid (EDTA))
  1. Add 10 g of CMC (low-viscosity), 2 g of EDTA-Na_2 and 500 mL of distilled water in a 1-L volumetric flask.
  2. Stir the solution until completely dissolved (1 to 3 hours).
  3. Fill the volumetric flask to the 1-L mark with distilled water.
  4. If necessary, use a centrifuge to clarify the solution.
  5. Keep the solution for a maximum of 1 month.
- **Ferric reagent**
  1. Add 3.5 g of ammonium iron (III) citrate, green (16 % Fe) in a 100-mL volumetric flask.
  2. Fill the volumetric flask to the 100-mL mark with distilled water.
  3. Stir the solution until completely dissolved. The solution must be completely clear.
  4. Keep the solution for a maximum of 1 week.
- **Ammonia**, diluted
  1. Dilute 1 part of concentrated ammonia (d = 0.91) with 2 parts of water.

Accessories

- Spectrophotometer, 600 nm
- 10-mm rectangular cuvette OS
- Centrifuge
- Volumetric flask, 25-mL, wide-necked, with ground glass stoppers.
- Volumetric flask, 100-mL
- Volumetric flask, 1-L
- Pipets

Sample preparation

1. Shake the beer to remove carbon dioxide.
2. Clarify the cloudy wort or beer with a centrifuge.
3. Add 10 mL of sample and 8 mL of CMC/EDTA reagent in a 25-mL volumetric flask. Fully mix the solution.
4. Add 0.5 mL of ferric reagent to the solution. Fully mix the solution.

5. Add 0.5 mL of ammonia, diluted to the solution. Fully mix the solution.
6. Fill the volumetric flask to the 25-mL mark with distilled water. Fully mix the solution.
7. Wait for 10 minutes. Then fill the prepared sample in a 10-mm rectangular cuvette.
8. Prepare the blank as follows:
   a. Add 10 mL of sample (clear and with no carbon dioxide) and 8 mL of CMC-EDTA-Na solution in a 25-mL volumetric flask. Fully mix the solution.
   b. Add 0.5 mL of ammonia, diluted to the solution. Fully mix the solution.
   c. Fill the volumetric flask to the 25-mL mark with distilled water. Fully mix the solution.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2026. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in mg/L.

Principle

Evidence that polyphenols are implicated in the complex phenomena of beer oxidation and haze formation prompted a collaborative study by the European Brewery Convention of a method for their evaluation, resulting in subsequent publication in *Analytica* III (Ref. 2). The same method was subjected to a collaborative investigation by ASBC using ales, regular lager beers and low-calorie beers, resulting in acceptance of the procedure as an International Method.

The polyphenols are reacted with ferric iron in alkaline solution and the red color produced is measured photometrically.

More information

Option	Description
Results specifications	mg/L without decimals
Accuracy	Sr = 2.17 to 3.28; Sc = 5.58 to 11.61 (with the highest values in each instance being for the high-polyphenol ales)
Standard values	Beer: 150–200 mg/L

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07/2014, Edition 2

Vicinal diketones

Diacetyl, 2,3-pentanedione **Method 2014**

0.00 to 1.00 mg/kg **MEBAK[1] method**

Scope and application: For beer.

[1] *MEBAK Wort, beer, beer-based beverages*, 1st edition 2012

Test preparation

Items to collect

Reagents

- **Hydrochloric acid**, 4N
- **1,2-phenylenediamine**, 1 % in 4N hydrochloric acid
  Note: *Prepare solutions daily and keep in the dark. 1,2- phenylenediamine is toxic and an allergen; it must be handled carefully, work with gloves.*
- **Anti-foam emulsion** (no diketones)

Accessories

- Spectrophotometer, 335 nm
- 20-mm rectangular cuvette QS
- Appliance for nitrogen determination with heating jacket, macro execution. Replace the supplied cooler by a larger one if the distillate is not sufficiently cooled. Other similar appliances are also applicable.

Test preparation

1. Add 100 g of non-decarbonated beer into a pre-heated distillation appliance.
2. Add a drop of anti-foam emulsion.
3. Control the steam supply so that within 2 minutes, approximately 25 mL of distillate transfers.
4. Collect the distillate in a 25-mL volumetric flask.
5. Pipette 10 mL of the mixed distillate into two 50-mL Erlenmeyer flasks (main value, blank value).
6. **Prepare blank value:** Add 2.5 mL of 4N hydrochloric acid.
7. **Prepare main value:** Add 0.5 mL of 1,2-phenylenediamine solution, mix and put in the dark for 30 minutes. Add 2 mL of 4N hydrochloric acid.
8. Measure the extinction of the main value against the blank value within 20 minutes at 335 nm in a 20-mm rectangular cuvette.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2014. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in mg/kg.

Principle

During yeast metabolism, fermentation causes 2-acetolactate and 2-acetohydroxy butyrate to emerge. Oxidation causes a conversion into the vicinal diketones diacetyl (2,3 butanedione) and 2,3-pentanedione. Diacetyl can also occur as a characteristic metabolic product of certain micro-organisms. When there is more than the threshold value, the beer has a bad aroma.

The photometric determination method in operation control is recommended as an alternative over the gas chromatographic methods, because photometric determination is performed quickly and without great equipment expense. This method does not let the preferable differentiation between diacetyl and pentanedione.

The method uses the reaction between diacetyl and/or 2,3- pentanedione and 1,2-phenylenediamine under formation of 2,3- dimethylquinoxaline, which shows a specific absorption at 335 nm.

More information

Option	Description
Results specifications	In mg/kg with two decimals
Accuracy	r = 0.03
Target value	For light full beer <0.15 mg/kg
Comments	It is not necessary to clean or purge the distillation appliance between tests. The distillation appliance can be immediately refilled between tests. After testing is complete, remove the adherent residues with diluted sodium hydroxide or another suitable cleaning agent. Acetohydroxy acids present in bottled beer are oxidized in the presence of oxygen to diketones. Analyze the total diketone content before this analysis. Keep the beer sample for a maximum of 1.5 hour at 70 °C (158 °F) for diketone content analysis.

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07/2014, Edition 2

Diacetyl

Broad spectrum method for VDK **Method 2023**

0 to 1 mg/L **ASBC [1]method: Beer-25B**

Scope and application: For beer.

[1] *American Society of Brewing Chemists.* Reproduced with permission from the American Society of Brewing Chemists for use by purchasers of specified Hach instruments only. No other use or reproduction is permitted without written permission from the American Society of Brewing Chemists.

Test preparation

Items to collect

Reagents

- **α-Naphthol solution** ($C_{10}H_7OH$)
  1. Dissolve 4 g of α-Naphthol solution in 100 mL of isopropanol, 99.6%.
  2. Add ca. 0.5 g vegetable carbon and shake mixture for about 0.5 hours, then filter. Keep the filtrate in the dark in an amber bottle.
- **KOH-creatine solution**
  1. Dissolve 0.3 g of creatine in 80 mL 40% KOH solution (aqueous) and filter. Keep in a polyethylene container under refrigeration.
- **Diacetyl**, stock solution
  1. Prepare an aqueous solution containing 500 mg/L. Keep the stock solution in an amber bottle in refrigerator.
- **Diacetyl**, working solution
  1. Prepare immediately before use. Dilute 1 mL of stock solution to 100 mL with distilled water; concentration: 5.0 mg/L diacetyl.

Accessories

- Spectrophotometer, 530 nm
- 10-mm rectangular cuvette OS
- Distillation equipment, preferably all glass
- Volumetric flasks, 10-mL
- Graduated cylinders, 50-mL
- Heat mantle for boiling flask
- Boiling flask, two-neck, 500-mL
- Distilling tube, mounted vertically on boiling flask
- Condenser, water-cooled, connected (by a 75° adapter if necessary) to distilling tube so that it slopes downward
- Curved tapered tube adapter, connected to condensator, delivery tip to dip below liquid level in receiver
- Receiver, such as 50-mL beaker, marked at 15 and 33-mL levels

Sample preparation

1. Distill 100 mL of decarbonated beer into a 50-mL graduated cylinder that contains 5 mL of distilled water.
2. Collect ca. 15 mL distillate and fill to the 25-mL mark with distilled water.
3. Pipet a 5 mL aliquot into a 10-mL volumetric flask.

4. Add 1 mL of α-Naphthol solution to each flask and swirl.
5. Add 0.5 mL of KOH-creatine solution to a maximum of 4 or 5 flasks at a time.
6. Fill the volumetric flasks to the 10-mL mark with distilled water.
7. Shake vigorously for exactly 1 minute.
8. Wait 5 minutes. Then measure absorbance at 530 nm against the reagent blank.
9. Do step 6 for all samples to measure.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2023. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Push **Read**.
   The results show in mg/L.

Principle

Spectrophotometric methods determine vicinal diketones (VDKs). All methods are affected by sample treatment before and during analysis. VDK precursors will convert to free compounds, partially or completely, depending on the pH, extent of exposure to air and temperature.

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07/2014, Edition 2

Reducibility

Spectrophotometric method **Method 2004**

0 to 100% **MEBAK[1] method**

Scope and application: For beer.

[1] *MEBAK Wort, beer, beer-based beverages,* 1st edition 2012, P. 204 et seq

Test preparation

Items to collect

Reagents

- **2,6-dichlorophenol-indophenol**, 0.005 M (DPI solution, molecular weight of the sodium salt 290.08):
  1. Weigh into a beaker approximately 100 mg of DPI solution and add approximately 25 mL of distilled water. Use heat to dissolve the solution (approximately 60 °C (140 °F)).
  2. Let the temperature of the solution decrease and rinse the contents into a 50-mL volumetric flask. Fill the volumetric flask to the 50-mL mark. Filter through a fiberglass filter.
  3. Add 10 mL of filtrate, 1 g of KJ and 2 mL of H_2SO_4 (1+6) into a 150-mL volumetric flask and titrate with 0.01 N sodium thiosulfate until the color changes to starch paste.
  4. Calculate the indicator content: used mL × 14.5 = mg indicator in 100 mL
  5. Dilute the remaining filtrate so that 100 mL solution contains 145 mg indicator.
  6. Keep the solution in brim-full brown bottles at 4 °C (39 °F) for approximately 1 week.
- **Phosphate-citrate buffer**, pH =4.35
  1. Dissolve 31.60 g of di-sodium hydrogen phosphate (Na_2HPO_4 x 12 H_2O) and 11.75 g of citric acid ($C_6H_8O_7$ x H_2O) in distilled water and dilute to 1 L.

Accessories

- Spectrophotometer, 520 nm
- 10-mm rectangular cuvette OS
- Stop watch
- Water jet pump
- Breaker, 100-mL
- Volumetric flask, 50-mL
- Volumetric flask, 150-mL

Test preparation

1. Keep the beer at 20 °C (68 °F) and remove the carbon dioxide under vacuum (water jet pump).
2. Add the decarbonated beer into a 10-mL reagent flask with glass stopper. Pour 0.25 mL of (0.005 M) DPI solution slowly into the flask.
3. Seal the reagent flask immediately and invert the flask two times. Start the stop watch after the first inversion.
4. Fill the prepared sample in a 10-mm rectangular cuvette immediately.
5. **Prepare the blank**: Decarbonated beer without the added reagent.

Procedure

1. Prepare the blank and the sample.
2. Select STORED PROGRAMS>SELECT BY NUMBER.
3. Enter the number 2004. Push **OK**.
4. Push **Start**.
5. Install the prepared blank. Push **Zero**.
6. Install the prepared sample. Wait 60 seconds. Push **Read**.
   The results show in the display.

Principle

Reducibility is a measure for the fast-reducing substances give in the beer. Reducers occur in the beer in relatively small quantities, but have great significance in the chemical, physical, and biological properties as well as the taste stability of the beer.

Reducers decrease a certain amount of Tillmann's reagent (2,6- dichlorophenol indophenol, DPI) within a certain time span. The decoloration of the reagent is spectrophotometrically measured and calculated.

More information

Option	Description
Results specifications	Proportion of the supplied DPI quantity in %, which is reduced by 10 mL beer in 60 seconds
Accuracy	V_{kr} = ±1%
Standard values	> 60 very good; 50–60 good; 45–50 satisfactory; < 45 poor

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