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Free Amino Nitrogen (FAN) Measurement in Beer using an Eppendorf BioSpectrometer®

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Introduction

Objective
Free amino nitrogen (FAN) measurements assess the level of amino acids, ammonia and end group amino nitrogens in a brew. Total FANs indicate the bioavailability of nitrogen in beer. As a diagnostic test, low FAN measurements indicate slow or incomplete fermentation, while high FAN measurements may indicate haze issues and/or diacetyl formation.

Principle
This is a ninhydrin-based dying method where absorbance is measured at 570 nm against glycine.

Material and Methods

Preparation
Ninhydrin Color Reagent:
Dissolve 10 g Na_2HPO_4, 6 g KH_2PO_4, 0.5 g ninhydrin and 0.3 g fructose in a total of 100 mL of distilled water. The pH must be between 6.6-6.8. Store under refrigeration in an opaque container for max. 2 weeks.

Dilution solution:
Dissolve 2 g potassium iodide in 600 mL distilled water and then add 400 mL of 96 % ethanol.
Glycine stock solution: Dissolve 107.2 mg glycine in a total of 100 mL of distilled water. Store this solution at 0 °C.

Glycine Standard Solution:
Dilute 1 mL of the glycine standard stock in 99 mL of distilled water. This standard solution contains 2 mg/L amino nitrogen.

Protocol
1. Dilute 1 mL of beer in 49 mL (or 1 mL of wort in 99 mL) of distilled water. Use 50 mL distilled water for a blank.
2. Perform analysis of the sample, standard solution and H_2O (blank value) in triplicates.
3. Pipette 2 mL of the diluted sample, the standard solution and the H_2O each into separate test tubes.
4. Add 1 mL of Ninhydrin Color Reagent and mix.
5. Close each test tube loosely in order to avoid evaporative losses and heat the solutions at 100 °C for 16 minutes.
6. Cool the solutions at 20 °C for 20 minutes.
7. Add 5 mL of Dilution Solution and measure within 30 minutes the absorbance in a 10 mm cuvette at 570 nm against distilled water.
8. On the BioSpectrometer: Under MAIN GROUPS select the ABSORBANCE folder. Under SUB GROUPS select SINGLE λ. Under METHODS select SINGLE λ.
9. Select the soft key EDIT to change the parameters.
   Parameters:
   Cuvette: 10 mm
   Wavelength: 570 nm

10. Save parameters by pressing the soft key SAVE.
11. Select the soft key NEXT.
12. Transfer blank (distilled water) to cuvette, insert the cuvette and measure by pressing the round Blank key.
13. Transfer analysis samples (beer, glycine standard, H_2O) to cuvette, insert cuvette in the same orientation as the blank and measure by pressing the round SAMPLE key.

Optional:
Correction for dark wort and beer samples
(> 100 EBC) units:
> Transfer 2 mL of the diluted sample to a test tube.
> In place of Ninhydrin Color Reagent, add 1 mL distilled water and continue with steps 5-7.

FAN Calculation:

$$\text{Free amino nitrogen [mg/L]} = \frac{A_S - A_B - A_C}{A_G - A_B} \times 2 \times F$$

A_S = average absorbance of the sample
A_G = average absorbance of the glycine standard solution
A_B = average absorbance of the blank value (H_2O)
A_C = average absorbance of the correction for dark wort and beer
F = dilution factor of the sample
2 = concentration of the glycine standard solution in mg/L

Literature

[1] 2.6.4.1.1 Ninhydrin Method (Photometric Method, EBC). In: MEBAK Wort, Beer, Beer-based Beverages: Collection of Brewing Analysis Methods of the Mitteleuropäische Brautechnische Analysenkommission (MEBAK). Freising-Weihenstephan: Self-published by MEBAK; 2013.

Ordering information

Description	Order no. international	Order no. North America
Eppendorf BioSpectrometer® basic 230 V / 50-60 Hz, mains/power plug Europe, 120 V / 50-60 Hz, mains/power plug North America	6135 000.009	6135000017
Eppendorf BioSpectrometer® kinetic 230 V / 50-60 Hz, mains/power plug Europe, 120 V / 50-60 Hz, mains/power plug North America	6136 000.002	6136000010
Eppendorf BioSpectrometer® fluorescence 230 V / 50-60 Hz, mains/power plug Europe, 120 V / 50-60 Hz, mains/power plug North America	6137 000.006	6137000014
Eppendorf® macro Vis Cuvette plastic cuvette for measurements in the Vis range, max. filling volume 4,500 µL, 10 x box of 100	0030 079.345	0030079345
Eppendorf® semi-micro Vis Cuvette plastic cuvette for measurements in the Vis range, max. filling volume 3,000 µL, 10 x box of 100	0030 079.353	0030079353

Verified by: Research Center Weihenstephan for Brewing and Food Quality

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