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Thiobarbituric Acid Index (TBI) Analysis in Beer using an Eppendorf BioSpectrometer®

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Introduction

Objective
The thiobarbituric acid index is used to monitor thermal stress in wort and malt resulting from the processing. The number represents the amount of Maillard reaction products as well as 5-hydroxymethylfufural (HMF) and other organic compounds.

Principle
Wort or beer reacts with a solution of thiobarbituric acid and acetic acid which results in a yellow color that can be read on using the Eppendorf BioSpectrometer at an absorbance of 448 nm to determine the TBI.

Material and Methods

Protocol

1. Clarify beer or wort by filtration if necessary.
2. Dilute samples in order to achieve absorbance values within the range of 0.1 – 0.5 after reaction with thiobarbituric acid.
3. Blank: Add 5 mL 90% acetic acid to 10 mL of the diluted beer or wort sample.
4. Sample: Add 5 mL 20 mM thiobarbituric acid in 90% acetic acid to 10 mL of the diluted beer or wort sample.
5. After shaking the tubes place blank and samples in a 70 °C water bath for 70 minutes. (Avoid direct sunlight and take care that the temperature only decreases 1-2 °C for a short time after placing the tubes in the water bath).
5. Immediately cool the blank and samples to room temperature and read on the BioSpectrometer.
6. On the BioSpectrometer: Under MAIN GROUPS select the ABSORBANCE folder. Under SUB GROUPS select SINGLE λ. Under METHODS select SINGLE λ.
7. Select the soft key EDIT to change the parameters.
   Parameters:
   Cuvette: 10 mm
   Wavelength: 448 nm
   Factor: 10 x dilution factor
8. Save parameters using the soft key SAVE or SAVE AS.
9. Select the soft key NEXT.
10. Transfer blank to cuvette (e.g. Eppendorf Vis Cuvettes), insert the cuvette and measure by pressing the round BLANK key.
11. Insert the cuvette containing the sample in the same orientation as the blank and measure the sample by pressing the round SAMPLE key.

TBI Calculation:
The calculation can be implemented in the parameters of the BioSpectrometer via "Factor" (as described in step 7).
$TBI = (A_S - A_B) \times 10 \times F$
TBI = thiobarbituric acid index (dimensionless number)
A_S = average absorbance of the sample
A_B = average absorbance of the blank
F = dilution factor

Literature

[1] 2.4 Thiobarbituric Acid Index (TBI). 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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