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Rapid Automated Method to Measure Alpha-Amylase Activity in Malt

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Introduction

Alpha-Amylase is responsible for rapid degradation of starch during mashing and promotes fast conversion. α-Amylase is synthesized during the malting process and is influenced by variety and the degree of modification. Low levels of α-amylase can lead to long conversion times and poor extract yields in the brewery.

In modern malt quality laboratories, α-amylase activity is measured by monitoring the color change of the reaction of a buffered extract of malt with a dextrinized starch substrate and iodine using segmented flow analysis to increase sample throughput, however these systems are expensive and require large amounts of reagents.

Method principle

Method is adapted from chemistries described in ASBC method collection Malt 7-A and 7-C using fixed reaction time and temperature. Alpha-amylase activity is measured by monitoring the color change of the reaction of a buffered extract of malt with a dextrinized starch substrate and iodine. Reactions are performed at 37 °C and a photometric endpoint measurement at 660 nm.

Materials

Instruments

Analysis was performed using Thermo Scientific™ Gallery™ Plus Beermaster discrete photometric analyzer where all analysis steps are fully automated, such as sample and reagent dispensings, mixing, incubation and photometric reading at the selected wavelength. The instrument is capable of performing multiple parameters simultaneously without any method changeover time or system priming. Samples with α-amylase levels outside the calibration range are automatically reanalyzed with a dilution.
For the method comparison studies a segmented flow analyzer (SFA) was used to perform the analysis according to the ASBC Malt-7C method.

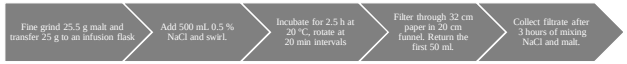
Figure 1. Thermo Scientific™ Gallery™ Plus Beermaster discrete photometric analyzer and a disposable Gallery Decacell™ cuvette.

Reagents

Substrate solution was prepared otherwise as described in the ASBC method collection, method Malt-7 A. Iodine working solution was prepared as described in the ASBC method collection, method Malt-7 C. All reagents were prepared fresh daily.

Samples

Samples were typical North American style malts, such as Lacey and Tradition, and craft malts extracted according to ASBC Malt-7C:



Results

Calibration

The results were calculated automatically by the analyzer using a 2nd order calibration curve. Megazyme EMAST Malt Amylase standard is used as calibrator. 6.6 g (6 mL) of EMAST standard was diluted to 100 mL in volumetric flask with 0.5 % NaCl solution. Assigned value of this stock solution was 240 DU. Calibration points were diluted automatically by the analyzer from the stock solution. All calibration points were measured as duplicate. Example of the calibration curve is shown in Figure 2.

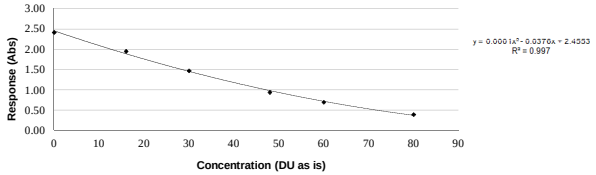


Figure 2. Example of an α-amylase calibration curve with Gallery analyzer

Method comparison

A method comparison study was performed by analyzing a series of malt samples using a range of α-amylase. Samples were selected to cover a wide range of α-amylase activity. The comparison included the automated method and the ASBC Malt-7C as a reference method. The novel method was well correlated with the reference method over the range of activity normally encountered. With Gallery analyzer, a zero point is included in the calibration which enables accurate measurement of low AA values as well.

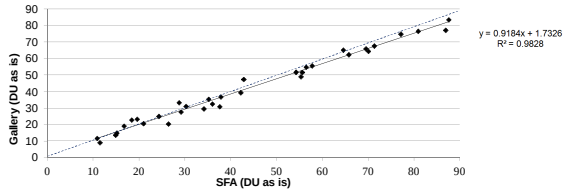


Figure 3. Method comparison Gallery vs. segmented flow analyser (SFA)

Repeatability and reproducibility

Method repeatability was tested with ten malt samples measured in ten replicates each. Tested samples were typical North American style malts. Same samples were analyzed in two different laboratories to verify the method reproducibility. Repeatability results are shown in table 1. The repeatability standard deviation (within-lab) was 1.4 DU. The reproducibility standard deviation (between lab) was 3.8 DU.

Table 1. Method repeatability and reproducibility (n=10)

		AVG	SD	RSD %
Sample 1	Lab 1	56	1.17	2.1 %
	Lab 2	54	0.84	1.6 %
Sample 2	Lab 1	52	1.18	2.3 %
	Lab 2	50	1.08	2.2 %
Sample 3	Lab 1	18	0.41	2.3 %
	Lab 2	19	0.48	2.5 %
Sample 4	Lab 1	41	0.69	1.7 %
	Lab 2	45	1.06	2.4 %
Sample 5	Lab 1	68	1.00	1.5 %
	Lab 2	71	1.17	1.7 %
Sample 6	Lab 1	77	0.77	1.0 %
	Lab 2	71	1.17	1.7 %
Sample 7	Lab 1	73	0.74	1.0 %
	Lab 2	81	2.33	2.9 %
Sample 8	Lab 1	77	0.83	1.1 %
	Lab 2	84	2.76	3.3 %
Sample 9	Lab 1	60	0.77	1.3 %
	Lab 2	68	2.27	3.4 %
Sample 10	Lab 1	59	0.49	0.8 %
	Lab 2	66	2.82	4.3 %

Conclusions

Method showed excellent repeatability and reproducibility and good correlation to segmented flow analysis. Discrete analyzer technology enables multiple samples and parameters to be analyzed simultaneously. Unlike in the traditional flow injection analysis, each measurement takes place in an individual reaction cuvette cell. Cuvettes are disposable which enables a contamination free analysis. Total reaction volume of the α-amylase method is only 180 µL which significantly decreases the reagent consumption compared to traditional methods. Benefits include automation of sample dispensing, standardized analysis conditions, and use of micro liter volumes of reagents that reduces both analysis time and costs without compromising method performance.

Reference

ASBC method collection, method Malt-7

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