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Novel Automated Method to Measure Malt Diastatic Power

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Introduction

Diastatic power refers to the ability of malt enzymes to break down starch into fermentable sugars. A new automated method to measure diastatic power in malted cereal grains is presented. Traditionally, the diastatic power of malt has been determined by measuring the reducing substances (primarily reducing sugars) produced from a controlled diastasis of starch under standardized conditions. Older manual titrimetric methods for reducing sugars have been largely replaced by automated measurements using continuous flow analysis systems to increase sample throughput; however these systems are expensive and require large quantities of reagents.

In this novel method, diastatic power is determined by measuring the formation of D-glucose using a specific enzymatic reaction through automation with the Thermo Scientific™ Gallery™ Plus Beermaster Automated Discrete Analyzer. A method comparison study was performed by analyzing a series of malt samples over a range of diastatic powers using both the novel method and ASBC Malt-6C as a reference method. The repeatability of the new method was also determined.

Method principle

The process involves extraction of enzymes by malt infusion with dilute salt solution, followed by reaction with ASBC special starch substrate under the controlled conditions of time, temperature, pH, and substrate concentration. The resulting sugars, primarily maltose, are further hydrolyzed with α-glucosidase to produce D-glucose. D-glucose is subsequently measured using the Thermo Scientific™ D-Glucose kit, which includes ready-to-use system reagents including hexokinase and glucose-6-phosphate dehydrogenase. Reactions are performed at 37 °C with a photometric endpoint measurement of 340 nm.

Materials

Instruments

Analysis was performed using the Gallery Plus Beermaster Automated Discrete Photometric Analyzer.

Figure 1. Gallery Plus Beermaster Automated Discrete Photometric Analyzer

Reagents

Starch solution (1 %) was prepared otherwise according to ASBC Malt-6, but with 1 g of starch instead of 2 g and with acetate buffer replaced by citrate buffer, pH 6.
Alpha-Glucosidase (Sigma, product code G0660) was first dissolved to 6 ml with deionized water and further diluted to 1:8 with citrate buffer, pH 6.
NaCl (0.5 %) solution was prepared according to ASBC Malt-6.
For the final D-Glucose measurement Thermo Scientific D-Glucose kit, product code 984304, was used.

Samples

Samples were typical North American style malts and craft malts extracted according to ASBC Malt-6C.

Results

Calibration

Calibration was performed using a Megazyme Malt Amylase standard (E-MAST). A series of dilutions were prepared and the results were calculated automatically by the analyzer using a 2nd order calibration curve. All calibration points were measured as duplicate. Example of the calibration curve is shown in Figure 2.

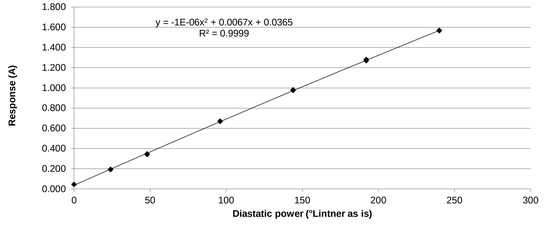

Figure 2. Calibration example

Repeatability

Method repeatability was tested with four malt samples measured in eight replicates each. Samples were selected to cover a wide range of DP levels. Repeatability results are shown in table 1.

Table 1. Method repeatability

	Sample 1	Sample 2	Sample 3	Sample 4
Rep 1	72.7	116.9	116.3	197.5
Rep 2	76.1	117.1	116.0	194.9
Rep 3	78.5	121.1	110.8	195.9
Rep 4	74.9	116.8	113.2	191.5
Rep 5	75.1	115.4	113.2	194.6
Rep 6	75.2	116.0	116.7	191.9
Rep 7	74.6	114.1	111.0	198.8
Rep 8	75.6	112.3	114.4	199.1
Average	75.3	116.2	114.0	195.5
SD	1.6	2.6	2.3	2.9
CV %	2.2 %	2.2 %	2.0 %	1.5 %

Method comparison

The newly developed method was compared against ASBC Malt-6 C method (SFA, Segmented Flow Analysis). A clear correlation between the two methods can be seen, despite the different chemistries and specificities. The results from the Gallery Plus Beermaster Automated Discrete Photometric Analyzer were slightly lower than the reference values. This may be due to the difference in detection mechanisms between the two methods. The Gallery method is specific to D-glucose, while the SFA method measures reducing substances. It should be noted that some of the samples measured were below the calibration range of the SFA method. With the Gallery Plus Discrete Analyzer, a zero point is included in the calibration, enabling accurate measurement of low DP values as well.

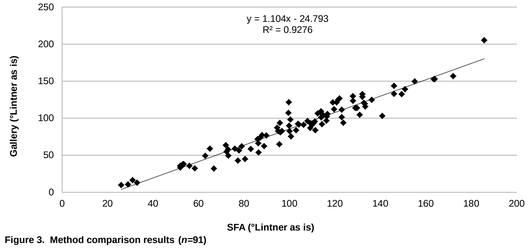

Figure 3. Method comparison results (*n*=91)

Discussion

A new approach to measure malt diastatic power that is specific to D-glucose fractions resulting from starch degradation by malt enzymes is presented. Method verification continues with a ring-test trial in order to test the method reproducibility under different laboratory conditions.

Discrete analyzer technology also has the additional benefit of enabling simultaneous analysis of multiple parameters, such as malt alpha-amylase, beta-glucan and alpha-amino nitrogen. Disposable cuvettes enable contamination free analysis.Together, the fully automated test procedure and microliter-scale reaction volumes provide cost efficiency.

Acknowledgements

Dr. Sherman Chan is acknowledged for the method concept.

Reference

ASBC method collection, method Malt-6

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