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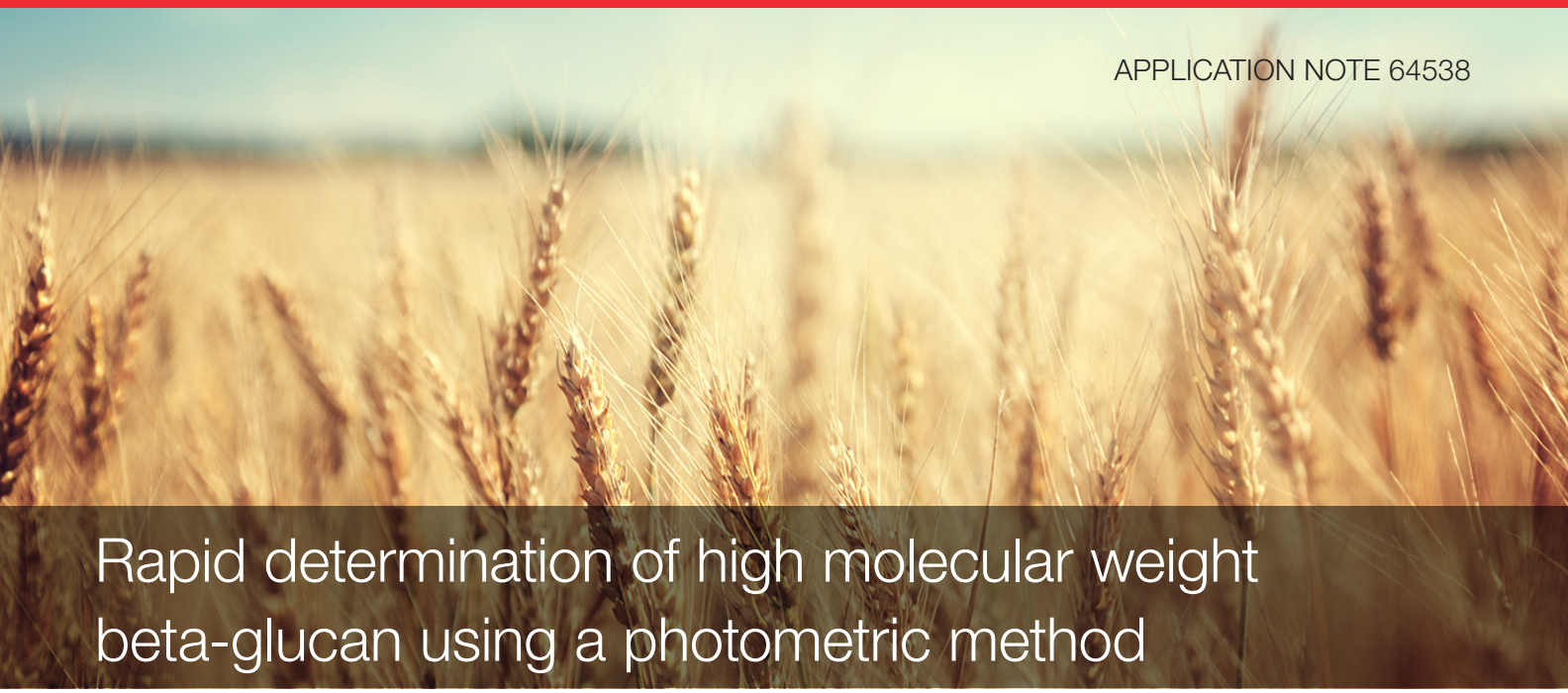

Rapid determination of high molecular weight beta-glucan using a photometric method

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Keywords

Automated Photometric Analysis, Beer Analysis, Malt Analysis

Goal

To demonstrate that an automated method for analysis of beta-glucan provides comparable results to traditional manual methods.

Introduction

In the malting and brewing process used for beer production, one important analyte is beta-glucan (β-glucan). β-glucans are polysaccharides of D-glucose monomers linked by beta-glycosidic bonds as shown in Figure 1. β-glucans are present in the cell walls of various cereals and are capable of clogging process filters; therefore, an excess of β-glucans may cause haze in the end product and thus impact appearance of the beer. For this reason, it is important to determine the concentration of β-glucan, particularly the portion of the polymer with a molecular size of 10,000 daltons (Da) or more.

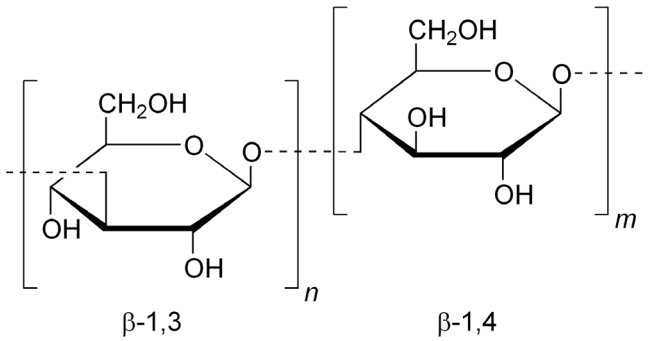

Figure 1. The barley b-D-glucan molecule with a 1,3/1,4-linkage.

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A novel method for analyzing β-glucans from wort and beer samples is presented. This rapid two reagent method was developed for a multipurpose discrete analyzer as well as for manual spectrophotometer use. The method was designed with the use of a blank buffer to eliminate possible sample color interference.

Method automation, repeatability, reproducibility, linearity, molecular weight studies and a correlation to the Calcofluor method are presented in this study. A fully automated benchtop photometric analyzer which enables simultaneous analysis of multiple parameters, like β-glucan, color, SO_2, pH, and free amino nitrogen (FAN) from the same sample is also described.

Experimental

Materials and methods

Instrument

The β-glucan method was validated using a Thermo Scientific™ Gallery™ discrete analyzer. Similar results were obtained using the Thermo Scientific™ Gallery™ Plus, Thermo Scientific™ Arena™ and Thermo Scientific™ Gallery™ Plus Beermaster discrete analzyers.

Reagents

A Beta-Glucan (High MW) test kit from Thermo Fisher Scientific was used. The kit provides ready-to-use non-hazardous reagents for up to 350 tests when performed by the automated analyzer.

Method principle

In this novel method high molecular weight barley (1,3/1,4)-β-glucan forms a complex with reagent R2 in buffered conditions at pH 8 that is proportional to the concentration of high molecular weight β-glucan in the sample. The reaction is measured photometrically at a 405 nm wavelength using a side wavelength of 600 nm. Side wavelengths are measured from the area of the spectrum where no reaction occurs and are used to eliminate variations in the readings.

Samples

Beer and wort samples were analyzed for method comparison and performance studies.

Method calibration and quality control

The method was calibrated using barley β-D-glucan from Sigma-Aldrich (Cat. No. G6 513). For the quality control sample, purified barley β-glucan from Scandinavian Brewery Laboratory (Cat. No. #01) was used. Several barley and oat β-glucan standard samples from Megazyme (Cat. No. P-BGBL, P-BGBM, P-BGBH and P-MWBGS) were tested to verify method performance for different molecular weight samples.

The calibrator solution was prepared by weighing 0.525 g of pure β-glucan standard at a purity of 95% into a 120 mL Pyrex® beaker and reconstituting with 5 mL of ethanol and 85 mL deionized water by simultaneously stirring and heating the mixture on hot plate at 120 °C. After the β-glucan was completely dissolved, the solution was allowed to cool to room temperature. Then the solution was adjusted to 100 mL with deionized water. The solution had a β-glucan concentration of 500 mg/L and was manually diluted to a final concentration of 300 mg/L. Molecular weight samples were prepared with the same process as that used for the calibrator solution.

Sample preparation

Turbid wort samples were centrifuged. Beer samples were degassed by manual shaking for ten minutes.

Application for automation

The automated β-glucan application for the Gallery discrete analyzer consists of two reagents (R1 and R2), an end-point measurement with a sample blank, and a linear calibration curve for result calculation. First, 120 µL of β-glucan reagent R1 and 20 µL of sample are dispensed and incubated for 200 seconds and the reaction is blanked. After the addition of 18 µL reagent R2 and a 200 second incubation, the reaction is measured at 405 nm with a side wavelength of 600 nm. The analysis is performed at 37 °C. With this application design the total analysis of nine samples with ten replicates, a total of 90 results, was less than 40 minutes. Reagent open on-board stability (in the instrument) was determined to be at least 30 days.

Method calibration

The results were calculated automatically by the analyzer using a linear calibration curve. A calibrator solution containing 300 mg/L of β-glucan and deionized water was used for calibrating the test and was automatically diluted by the analyzer. An example of the calibration curve is shown in Figure 2.

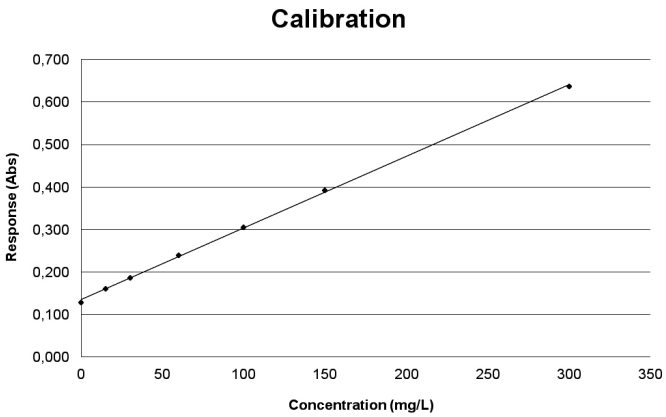

Figure 2. A calibration curve example. Calibration was performed with water and automatically diluted stock calibrator. Responses, reported as absorbance (Abs), ranged from 0.128 to 0.638.

Results and discussion

Repeatability and reproducibility

Repeatability was verified by analyzing β-glucan samples with the Gallery discrete analyzer for two days. Three different wort samples were analyzed with the number of measurements at *n* = 20. The test was calibrated at the beginning of each day.

The total repeatability for the samples was between 0.4 and 1.1%. Results are shown in Table 1.

Table 1. Method repeatability (*n* = 20).

	Wort 1		Wort 2		Wort 3	
	SD	CV%	SD	CV%	SD	CV%
Within run	0.79	0.4	1.34	0.5	0.96	1.1
Between run	0.17	0.1	0.55	0.2	0.13	0.1
Total	**0.81**	**0.4**	**1.45**	**0.6**	**0.97**	**1.1**

The reproducibility study was performed using three Gallery discrete analyzers for four days in different environmental conditions. Results are shown in Table 2. Beer samples with a β-glucan concentration of 130 mg/L were used. Fresh sample was added for each run and the test was calibrated at the beginning of the day. The daily coefficient of variation (CV%) ranged from 1.5 to 2.3%; the total CV% ranged from 2.4 to 2.6% with the total number of results at approximately *n* = 300. Environmental conditions tested were: ambient temperature from 18 to 30 °C (the analyzer incubator was always set to 37 °C) and relative humidity (% RH) from 40 to 80%.

No significant difference was noted among the three analyzers or as a consequence of different environmental conditions.

Table 2. Method reproducibility (*n* = 297–318).

		Gallery 1	Gallery 2	Gallery 3
30 °C / 80 % RH	*n*	84	81	84
	Average (mg/L)	126	125	124
	SD	2.42	1.86	2.20
	CV%	1.9	1.5	1.8
18 °C / 80 % RH	*n*	72	72	60
	Average (mg/L)	132	130	130
	SD	2.42	1.88	2.00
	CV%	1.8	1.4	1.5
30 °C / 40 % RH	*n*	66	81	69
	Average (mg/L)	130	131	130
	SD	3.04	1.98	2.32
	CV%	2.3	1.5	1.8
	Average (mg/L)	131	128	131
	SD	2.41	2.12	1.98
	CV%	1.8	1.7	1.5
Total		*n* = 306 2.6%	*n* = 318 2.4%	*n* = 297 2.6%

% RH = relative humidity (%)

Linearity

Method linearity was tested with pure chemicals dissolved in deionized water. The primary measurement was determined to range from 15 to 300 mg/L and was extended with an automatic secondary dilution (1:3) up to 500 mg/L. All linearity samples were measured in triplicate. A method linearity example is shown in Figure 3.

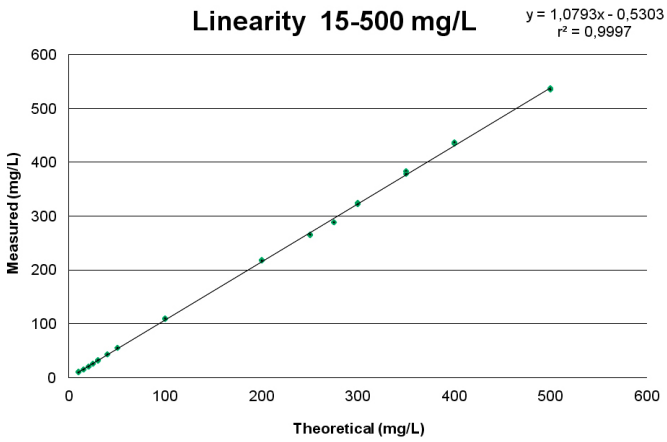

Figure 3. Method linearity.

Molecular weight study

Water based standards with different molecular weights were also analyzed to show the recovery rates of different molecules. Tested samples were prepared from barley β-glucan standards with molecular sizes between 165 kilodalton (kDa) and 650 kDa. The method was calibrated using a Sigma Aldrich β-glucan standard solution as described above. Two concentration levels for each sample were analyzed in three replicates.

Barley β-glucan standards from molecular weights 165, 391, 495 and 650 kDA showed recoveries between 102 to 113% proving the method is capable of analyzing several high molecular weight β-glucan molecules. Results are shown in Table 3.

Table 3. Barley b-glucan standards.

Sample	Average (mg/L)	Theoretical (mg/L)	Recovery%
MW 165 kDa	104	100	104
MW 165 kDa	52	50	103
MW 391 kDa	104	100	104
MW 391 kDa	51	50	101
MW 495 kDa	113	100	113
MW 495 kDa	54	50	107
MW 650 kDa	51	50	102
MW 650 kDa	111	100	111

In addition to barley β-glucan molecular weight samples, oat β-glucan samples between 35.6 kDa and 391 kDa were analyzed at three concentration levels for each sample. For all tested oat β-glucan standards, the recoveries were in the range of 99 to 114%. The method was calibrated with a Sigma Aldrich β-glucan standard solution as described above. Samples were analyzed in ten replicates and the CV% was below 1.89% for all concentration levels and molecular weights. Results are shown in Table 4.

Table 4. Oat b-glucan standards (n = 10).

	Oat MW 35.6 kDa			Oat MW 70.6 kDa			Oat MW 265 kDa			Oat MW 391 kDa		
	50 mg/L	100 mg/L	200 mg/L	50 mg/L	100 mg/L	200 mg/L	50 mg/L	100 mg/L	200 mg/L	50 mg/L	100 mg/L	200 mg/L
Average (mg/L)	55	107	203	57	110	213	53	101	197	53	105	207
SD	0.63	1.61	2.68	1.00	1.95	3.61	0.61	1.23	2.33	1.01	1.47	2.82
CV%	1.14	1.50	1.32	1.75	1.77	1.69	1.16	1.22	1.18	1.89	1.39	1.36
Recovery%	110	107	102	114	110	107	105	101	99	107	105	104

Method comparison to calcofluor

For the method comparison study, six beer samples and five wort samples were analyzed and compared to the Calcofluor method that uses flow injection analysis (FIA). A similar correlation was found for both sample types. Based on this preliminary study, the automated Beta-Glucan (High MW) method shows good correlation when compared to the Calcofluor method (r^2 = 0.96). The method comparison curve is shown in Figure 4.

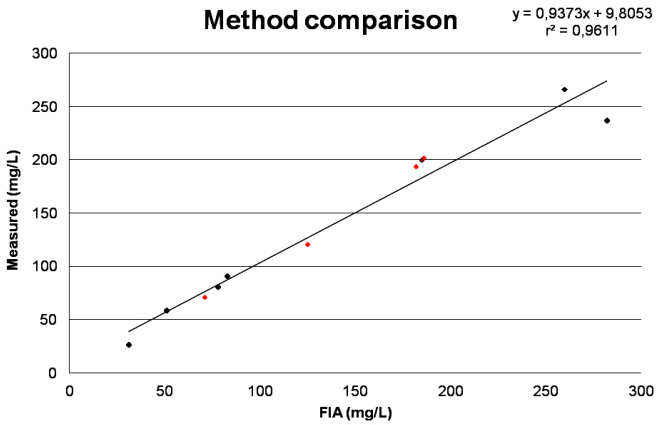

Figure 4. Method comparison. (Note that wort samples are marked in red and beer samples are marked in black).

Manual method

The Thermo Scientific Beta-Glucan (High MW) method can also be performed using a manual spectrophotometer at 405 nm with a one cm cuvette path length. The baseline is measured against air or deionized water at 37 °C. The method was designed as an end-point method with a reaction time of 10 minutes. The sample / R1 / R2 ratio was 1 / 6 / 0.9. Manual method linearity was determined to fall between 50 to 300 mg/L and correlation results compared to the Gallery discrete analyzer are shown in Figure 5.

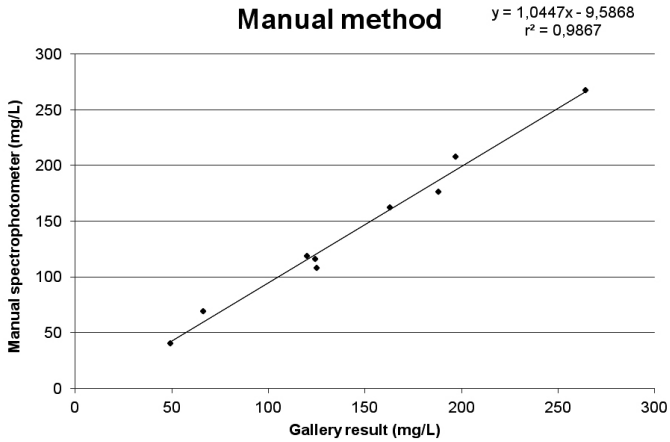

Figure 5. Manual method correlation to the Gallery discrete analyzer.

Conclusion

The β-glucan method performance study was done using Thermo Scientific Gallery discrete analyzers at a wavelength of 405 nm. Method linearity was determined to range from 15 to 500 mg/L with aqueous β-glucan standard solutions. Beer and wort samples showed excellent repeatability and reproducibility with a typical variation of 2% or less. Total analysis time for nine samples with ten replicates, a total of 90 results, was less than 40 minutes.

In preliminary analysis of the beer and wort samples, this method correlates well with results obtained by fluorometry using Calcofluor fluorescence dye as recommended in EBC 8.13.2, 4.16.2, 3.10.2[1] and ASBC Wort-18[2]. An improvement over the existing fluorometric methods, the open on-board stability of these novel non-hazardous reagents was determined to be at least 30 days. In addition, this β-glucan method is versatile enough to be used to analyze other β-glucan containing cereals, such as those derived from oats. This study demonstrates that the novel β-glucan method is precise and a suitable alternative method for routine use.

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