World Brewing Congress 2012

July 28 – August 1, 2012
Oregon Convention Center
Portland, Oregon, U.S.A.

105

Rapid determination of high molecular weight 1,3/1,4-Beta-D-Glucan by a novel photometric method

Otama, L.[1], Tikanoja, S[1], Hartikainen, S.[1] and Suoniemi-Kähärä, A.[1,2]
[1]Thermo Fisher Scientific, CDD Finland Diagnostics R&D, Ratastie 2, 01620 Vantaa Finland.
[2]Corresponding author: annu.suoniemi-kahara@thermofisher.com

Introduction

In the beer malting and brewing process, one important analyte is beta-glucan. Beta-glucans are polysaccharides of D-glucose monomers linked by beta-glycosidic bonds (Figure 1). Beta-glucans are present in the cell walls of cereals and are capable of clogging process filters. Excessive amounts of beta-glucans may cause haze in the end product and thus impair the taste of the beer. For this reason it is important to determine the concentration of beta-glucan, in particular the part of the beta-glucan polymer which has a molecular size of about 10,000 Da or more.

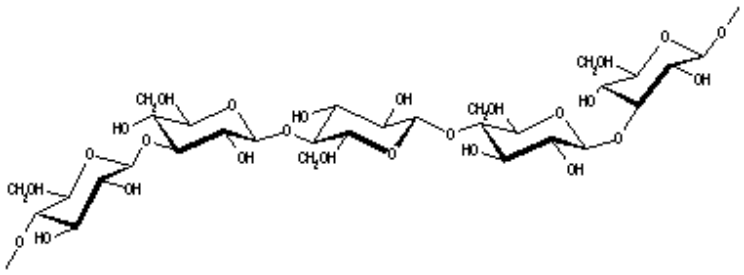


Figure 1. Barley beta-D-glucan molecule with a 1,3/1,4-linkage.

Here we present a novel method for analyzing beta-glucans, e.g., from wort and beer samples. A rapid two reagent method was developed for a multipurpose discrete analyzer and for a manual spectrophotometer use. The method was designed to use a blank buffer to eliminate possible sample color interference.

Method automation, repeatability, reproducibility, linearity, molecular weight studies and method correlation to Calcofluor is presented in this study. We also describe here a fully automated bench-top photometric analyzer concept which enables simultaneous analysis of multiple parameters, like beta-glucan, color, SO2, pH and FAN from the same sample.

Materials and Methods

Instrument

Beta-glucan method was validated using a Thermo Scientific Gallery discrete photometric analyzer. We also obtained similar results with Thermo Scientific Gallery Plus, Arena and Gallery Plus Beermaster analyzers (Figure 2).

Reagents

Beta-Glucan (High MW) test kit from Thermo Fisher Scientific (Cat. No. 984305) was used. Kit provides ready-to-use non-hazardous reagents for 350 tests when performed by the automated analyzer.

Method principle

In this novel method high molecular weight barley (1,3/1,4)-beta-glucan forms a complex with a reagent R2 in buffered conditions at pH 8. Complex forming is proportional to the concentration of high molecular weight beta-glucan in the sample. Reaction is measured photometrically at 405 nm wavelength, side wavelength being at 600 nm.

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

Method calibration and quality control
Method was calibrated using barley beta-D-glucan from Sigma-Aldrich (Cat. No. G6513). For the quality control sample, purified barley beta-glucan from Scandinavian Brewery Laboratory (Cat. No. #01) was used. Several barley and oat beta-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.

Calibrator solution was prepared by weighting 0.525 g of pure beta-glucan standard (purity 95 %) into a 120 ml pyrex beaker and reconstitute to 5 ml of ethanol and 85 ml deionized water simultaneously stirring and heating the mixture to 120 °C. After the beta-glucan was completely dissolved, solution was allowed to settle room temperature. Solution was then adjusted to 100 ml volume with deionized water. The solution had a beta-glucan concentration of 500 mg/L and it was manually diluted to final concentration of 300 mg/L. Molecular weight samples were prepared as the calibrator solution.

Sample preparation
Turbid wort samples were centrifugated. Beer samples were degassed by shaking for ten minutes.

Application for automation
Automatic Gallery beta-glucan application consists of two reagents (R1 and R2), end-point measurement with sample blank and linear calibration curve for result calculation. First 120 µL of beta-glucan R1 reagent and 20 µL of sample is incubated for 200 seconds and reaction is blanked. After addition of 18 µL R2 reagent and 200 s incubation, the reaction is measured at 405 nm with side wavelength of 600 nm. Method is performed at 37 °C. With this application design the total analysis time of nine samples with ten replicates (total 90 results) was less than 40 minutes. Reagent open on-board stability of these reagents was tested to be at least 30 days.

Method calibration
The results were calculated automatically by the analyzer using a linear calibration curve. Calibrator solution with 300 mg/L of beta-glucan and deionized water were used for calibrating the test. Calibrator solution was automatically diluted by the analyzer. Example of the calibration curve is shown in Figure 3.

Results and discussion

Repeatability and reproducibility
Repeatability was performed by analyzing beta-glucan samples with the Gallery analyzer for two days. Three different wort samples were analyzed with the number of measurements being *n*=20. Test was calibrated at the beginning of each day.

The total repeatability of the measurement for the samples was between 0.4 and 1.1 %. Repeatability results are shown in Table 1.

Reproducibility study was performed using three Gallery analyzers for four days in different environmental conditions. Result details are shown in Table 2. Sample used was beer sample with beta-glucan concentration of 130 mg/L. Fresh sample was used for each run and test was calibrated at the beginning of the day. Daily CV's were 1.5 to 2.3 % and total CV's were from 2.4 to 2.6 % with the total number of results being approximately *n*=300. Environmental conditions tested were ambient temperature from 18 to 30 °C (the analyzer incubator was always set to 37 °C) and humidity from 40 to 80 %rH.

As a test result, we saw no significant difference between the three analyzers or in different environmental conditions.

Linearity
Method linearity has tested with pure chemicals dissolved in deionized water. Primary measurement was designed from 15-300 mg/l and it was extended with a automatic secondary dilution (1+2) to up to 500 mg/l. All linearity samples were measured as triplicates. Example of the method linearity is shown in Figure 4.

Molecular weight study
Water based standards with different molecular weight were also analyzed to show the recovery of different molecules. Molecular weights tested were prepared from barley beta-glucan standards and the molecular sizes were between 165 kDa and 650 kDa. Method was calibrated with Sigma Aldrich beta-glucan standard solution as described above. Two concentration levels of each sample were analyzed in three replicates.

This study showed that barley beta-glucan standards from molecular weights 165, 391, 495 and 650 kDA showed recoveries between 102-113 %. This proves that the method is capable to analyze several high molecular weight beta-glucan molecules. Results are shown in Table 3.

Additionally to barley beta-glucan molecular weight samples, oat beta-glucan samples between 35.6 kDa and 391 kDa were tested. Three concentration levels of each sample were analyzed. For all tested oat beta-glucan standards the recoveries were 99-114 %. Method was calibrated with Sigma Aldrich beta-glucan standard solution as described above. Samples were analyzed in ten replicates and the CV's were below 1.89% with all concentration levels and molecular weight sizes. Results are shown in Table 4.

Method comparison to Calcofluor
For method comparison study, 6 beer samples and 5 wort samples were analyzed. Results analyzed by this method were compared against Calcofluor method by flow injection analysis (FIA). Data shows similar correlation with both sample types enabling the use of one application. Based on this preliminary study, our method shows good correlation to Calcofluor (R^2=0,96). Method comparison curve is shown in Figure 5.

Manual method
Thermo Scientific Beta-glucan (High MW) method can also be performed with manual spectrophotometer at 405 nm with 1 cm cuvette path length. Baseline is done against air or deionized water at 37 °C. Method was designed as an end-point method, reaction time being 10 minutes. Sample/R1/R2 ratio was 1/6/0.9. Manual method linearity was determined between 50-300 mg/L. Manual method correlation to Gallery is shown in Figure 6.

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 %

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

		Gallery 1	Gallery 2	Gallery 3
30 °C / 80 % rH	*n*	84	81	84
	Average	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	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	130	131	130
	SD	3.04	1,98	2.32
	CV %	2.3 %	1.5 %	1.8 %
22 °C / 40 % rH	*n*	84	84	84
	Average	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 huminidity (%)

Table 3. Barley beta-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

Table 1. Oat beta-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	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

Figure 2. Thermo Scientific Gallery Plus Beermaster discrete photometric analyzer

Figure 3. Calibration curve example. Calibration was performed with water and with automatically diluted stock calibrator (1+1, 1+2, 1+4,1+ 9,1+19). Responses (Abs) were from 0.128 to 0.638.

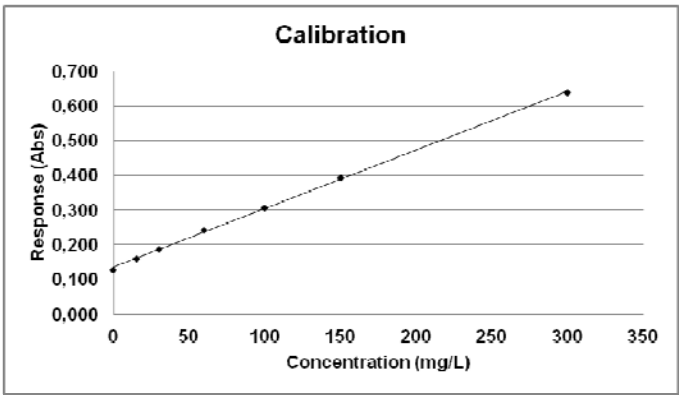


Figure 4. Method linearity

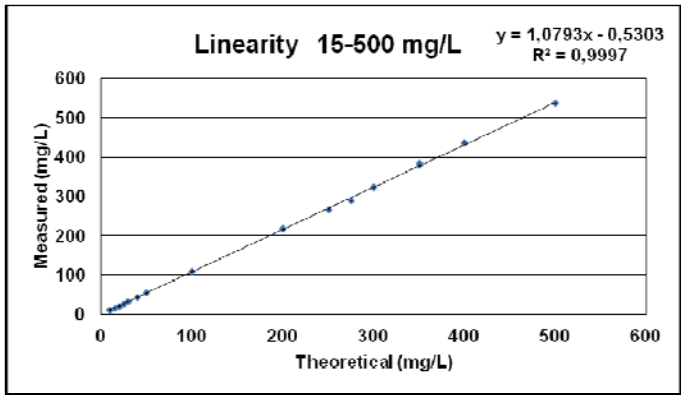


Figure 5. Method comparison. Note that wort samples are marked with red color and beer samples are marked with black color.

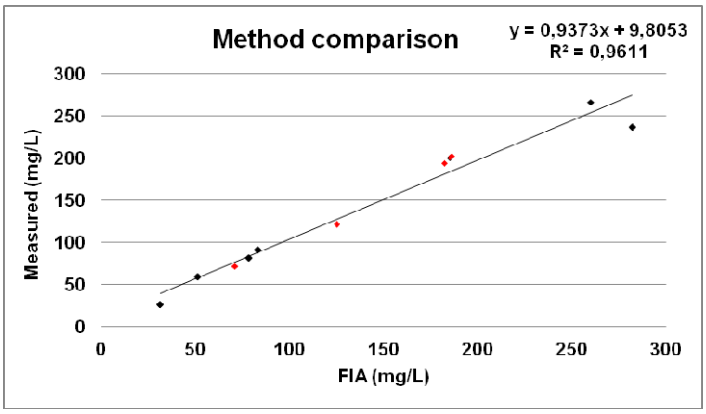
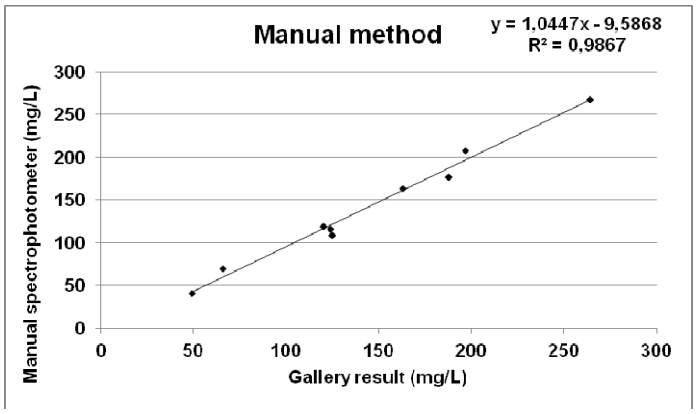


Figure 6. Manual method correlation to Gallery.



Conclusion

Beta-glucan method performance study was done by Thermo Scientific Gallery discrete analyzers at wavelength 405 nm. Method linearity was determined between 15-500 mg/L with aqueous beta-glucan standard solutions. Beer and wort samples tested showed excellent repeatability and reproducibility with typical variation being 2 % or less. Total analysis time of nine samples with ten replicates (total 90 results) was less than 40 minutes.

In preliminary analysis of beer and wort samples, this method correlates well with the 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]. As an improvement for the existing fluorometric methods, open on-board stability of these novel non-hazardous reagents was tested to be at least 30 days. In addition, the studied novel beta-glucan method can be used to analyze other cereal origin beta-glucan, such as derived from oat. This study demonstrates that a novel beta-glucan method is precise and as an alternative method well suitable for routine use.

References

1. Analytica-EBC, Method collection from European Brewery convention (2008)
2. ASBC (The American society of brewing chemists) methods of analysis, 2009 edition