Beta-Glucans and Beta-Glucanases

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CONCLUSIONS

This subcommittee is generally satisfied with the procedure; however, with results from only one year with eight laboratories reporting, and a rather high combined-laboratory coefficient of variation (c.v.), the subcommittee cannot recommend publication in the ASBC "Methods of Analysis" at this time.

RECOMMENDATIONS

The subcommittee feels that with one additional year of testing the c.v. can be reduced and confidence in the procedure will be gained.

β-Glucans make up the fabric of barley endosperm cell walls. They are high molecular weight, linear homopolysaccharides of D-glucose in β-1,3 and β-1,4 linkage. (It is important for this material to be broken down quickly during malt modification to allow effectual action of hydrolytic enzymes.)

The importance of β-glucans and β-glucanases to malting and brewing is exemplified by the numerous publications in this sphere of work. Major concern centers around the relatively high viscosity of solutions containing β-glucan. If sufficient β-glucanase activity is not present during mashing to lower this viscosity, slow filtration and lower extracts can result (2,3,4,5,7). Other areas in which β-glucans may be of significance include the formation of precipitates or gel-like deposits in beer (6,8,9), flavor, palate fullness, and foam stability (10,11,12).

To predict and control these effects, it is important to have a reliable means of quantifying endo-β-glucanase activity.

PROCEDURE

A viscometric procedure for determining endo-β-glucanase activity in malt, using barley β-glucan (BIOCON, Inc.) as the substrate, was evaluated. A substrate solution was carefully prepared by constantly stirring a weighed amount of β-glucan in near-boiling water for approximately 0.5 hr. It was then buffered to a specific pH for use in the assay.

The enzyme infusion used in this procedure was prepared by ASBC Method MALT-6 (1).

Enzyme activity was determined by measuring the reduction in viscosity over time as the enzyme degraded the β-glucan. β-Glucanase activity units (A.U.) are defined as the change in relative viscosity per minute times 1,000, on a dry basis. This is given by the formula:

$$\text{A.U.} = \frac{1{,}000\,(T_i - T_f)}{T(T_w)} \left(\frac{100}{100 - \%H_2O}\right)$$

where T = incubation time in minutes,
T_i = initial flow time,
T_f = final flow time, and
T_w = flow time for water.

The collaborative work had a Youden Block design (13) with two sample pairs plus a standard malt sample. The first pair consisted of two consecutive maltings of the 6-row barley, Morex. The second pair was taken fron consecutive maltings of the 2-row barley, Klages. Statistical calculations were performed with and without adjusting for the β-glucanase activity in the standard malt sample.

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In addition, the ruggedness of the method was examined by evaluating the effects of small changes in five of the procedural parameters.

RESULTS AND DISCUSSION

Ruggedness Testing

The ruggedness of the procedure was examined with a 2^3 factorial design replicated twice, and two simple replication experiments.

The factorial design, presented in Table I, was used to examine the effects of small changes in 1) the temperature at which the enzyme substrate mixture was incubated, 2) the concentration of β-glucan in the substrate preparation, and 3) the concentration of buffer in the substrate preparation. The various combinations of

TABLE I
Ruggedness Test: 2^3 Factorial Design (Replicated Twice)

	Factor[a]			Response (Enzyme Activity)[b] Replication	
Run	1	2	3	1	2
1	−	−	−	22.4	20.3
2	−	−	+	25.8	26.3
3	−	+	−	29.3	29.3
4	−	+	+	26.4	27.7
5	+	−	−	25.1	25.0
6	+	−	+	25.2	25.0
7	+	+	−	29.3	29.1
8	+	+	+	29.2	29.5

Analysis of Variance of Main Effects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	(7)	(108.634)		
Temperature	1		6.126	15.01[c]
β-Glucan	1		75.256	184.40[c]
Buffer	1		1.756	4.30
Error	8	3.265	0.408	
Total	15	111.899		

Critical $F_{0.05(1,8)} = 5.32$.

[a] Factors: 1, temperature (°C), − = 29, + = 31; 2, β-glucan concentration (%), − = 0.28, + = 0.32; 3, buffer concentration (%), − = 0.6, + = 0.8.
[b] A.U. as defined in the text.
[c] Significant effects.

TABLE II
Ruggedness Test: Variation Between Infusions and Between Batches of β-Glucan

	Enzyme Activity[a]	
Run	Infusions	β-Glucan Batches
1	27.2	28.0
2	28.2	27.8
3	28.4	25.2
4	28.1	30.6
$\bar{X}$	27.98	27.90
s^2	0.2825	4.8667
d.f.	3	3
F	0.692	11.925[b]

Critical $F_{0.05(3,8)} = 4.07$

[a] A.U. as defined in the text.
[b] Significant effect: The mean squared error from the factorial experiment (0.4081 with 8 degrees of freedom) was used to calculate the F-values.

TABLE III
β-Glucanase Activity Units[a] in Malt

Collaborator	Calculation Method 1[b]					Calculation Method 2[c]				
	6-row		2-row		Standard	6-row		2-row		Standard
	X	Y	X	Y	Z	X	Y	X	Y	Z
1	38.9	37.5	42.7	30.2	33.5	41.6	40.1	45.6	32.3	35.8
2	43.4	39.4	45.2	35.3	34.6	44.9	40.8	46.8	36.5	35.8
3	46.5	43.0	48.9	39.2	36.8	45.2	41.8	47.6	38.1	35.8
4	47.1	43.5	49.0	39.1	37.7	44.7	41.3	46.5	37.1	35.8
5	41.6	40.3	47.2	41.2	35.6	41.8	40.5	47.5	41.4	35.8
6	41.8	41.8	45.9	31.6	33.4	44.8	44.8	49.2	33.9	35.8
7	33.5	33.4	41.8	29.7	30.7	39.1	38.9	48.7	34.6	35.8
8	51.2	49.0	53.6	45.3	44.1	41.6	39.8	43.5	36.8	35.8
Mean	43.0	41.0	46.8	36.5	35.8	43.0	41.0	46.9	36.3	35.8
Grand mean	42.0		41.7			42.0		41.6		

[a] β-Glucanases activity units (A.U.) as defined in text.
[b] Calculated without adjustment for standard malt value.
[c] Calculated after adjustment for standard malt value.

TABLE IV
Statistical Summary

Method[a]	Sample Pair	No. of Labs.	Grand Mean[b]	Error			c.v.[e] (%)	Calculated F[c]	Critical F[f]
				Within-Laboratory[c]	Between-Laboratory[c]	Combined[d]			
A	1	8	42.0	1.04	4.92	5.03	11.9	45.53	3.787
	2	8	41.6	1.84	4.46	4.82	11.6	12.79	3.787
B	1	8	42.0	1.08	1.71	2.02	4.82	6.009	3.787
	2	8	41.6	2.39	0.00	2.39	5.74	0.945	3.787

[a] A = Data not adjusted for standard; B = data adjusted for standard.
[b] Grand mean = GM = $(\overline{X} + \overline{Y})/2$.
[c] Calculated per Youden and Steiner (13).
[d] Combined error (S_c) calculated from within-laboratory (S_r) and between-laboratory error (S_b); $S_c = \sqrt{S_r^2 + S_b^2}$.
[e] Coefficient of variation of S_c = c.v. = $100(S_c/GM)$.
[f] Critical F from table of F distribution at $P = 0.05$.

these factors result in eight different treatments which are referred to as "runs" in Table I. For example, run 4 represents the treatment with the lower level of temperature (20° C), and the higher levels of β-glucan (0.32%) and buffer (0.8%). Each run was performed in duplicate to give an estimate of the mean squared error (MSE) with 8 degrees of freedom (d.f.).

Data were analyzed using traditional analysis of variance techniques. Since there were eight different "runs" or treatments, the sum of squares for treatments had 7 d.f. and could be divided into seven different contrasts or comparisons between means. The three contrasts representing the main effects are presented in Table I. The mean squares for the effects are compared with the MSE to obtain the F-statistic which is a measure of the significance of the treatment effect. The β-glucan effect was large and significant. The temperature effect was also significant; the buffer effect was not significant.

Two factor interactions result in three additional contrasts and the three factor interaction results in the seventh contrast. These results are not presented here; however, it should be noted that the β-glucan, buffer interaction, and the three factor interaction were significant. This suggests that the buffer concentration may be more important than the main effect indicates.

Table II presents two simple replication experiments designed to analyze the variation between infusions and between batches of β-glucan. In testing four infusions of the same malt using one batch of β-glucan, the variance was not found to be significant when compared with the MSE estimate derived from the factorial experiment. Four different batches of β-glucan were compared using one of these fusions, and were found to contribute significantly to the error.

Collaborative Testing

Table III presents the results from the collaborative testing; a statistical summary of these results is presented in Table IV. Two calculation methods were used to analyze the data. Method A considers the sample pair data only, that is, it ignores the standard malt results. With Method A, the combined error (4.9) and c.v. (11.8) are large. Much of this error is contributed by between-laboratory variation.

Method B adjusts the raw data relative to the result for the standard malt of the individual laboratories. The average value for the standard malt was taken as its true value; in practice this value would be established more accurately through replication. Each individual laboratory results was multiplied by the average value of the standard malt and the product divided by the individual laboratory value for the standard malt. As a result of this adjustment, the between-laboratory error is substantially reduced and consequently the combined error and c.v. are also substantially improved. At this time, the subcommittee believes that such use of a standard malt will be necessary to obtain reproducible results between laboratories.

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