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Determination of the high molecular weight β -glucan content of malt wort by a spectrophotometric method - determination of the accuracy, repeatability and reproducibility

Submitted on behalf of the EBC Analysis Committee

Descriptors: Analytics, Analytica-EBC, spectrophotometric method, β -glucan

A new spectrophotometric method for the determination of the soluble high molecular weight mixed linkage (1,3)(1,4) β -D glucan (β -glucans) fraction present in malt was collaboratively tested by the Analysis Committee of the European Brewery Convention according to ISO Standard 5725-2 in order to determine its suitability for publication in Analytica-EBC. At the same time and by using the same samples the fluorimetric method used currently was also collaboratively tested. Accuracy, repeatability $r(95)$ and reproducibility $R(95)$ values are presented.

The new spectrophotometric and the fluorimetric method in current use were found to have comparable and acceptable within and between laboratory precision for the determination of β -glucans.

For the new spectrophotometric method, repeatability $r(95)$ and reproducibility $R(95)$ were found to be independent of the mean value and were 28,2 mg/l and 59,4 mg/l respectively over the range of β -glucan tested (88 to 308 mg/l).

The new spectrophotometric method using a β -glucan assay kit from NovaBiotec® Dr. Fechter GmbH, Berlin, Germany, is recommended for inclusion in Analytica-EBC (Method 4.16.3).

1 Introduction

The Analysis Committee of the European Brewery Convention (EBC) decided to carry out an inter-laboratory collaborative trial to determine the accuracy and precision of a new spectrophotometric method for the determination of β -glucans in malt wort. The new method is based on the photometric assay of a β -glucan-dye-complex of the soluble malt β -glucans.

2 Experimental

The organisation of the trial and the statistical treatment of the data was carried out according to the procedure given in the International Standard ISO 5725-2.

Samples of six commercial pale malts, each from a single homogeneous batch, covering the approximate β -glucan range 80-310 mg/l were sent to 14 participating laboratories.

Participants were asked to make malt wort using the EBC congress mashing method 4.5.1. Participants had to perform the β -glucans analysis as real duplicate determinations from two undiluted congress wort preparations both by spectrophotometry using the new EBC method and by using the fluorimetric EBC method 8.13.2, using their own choice of β -glucan analysis equipment. Participants were asked to complete a short questionnaire that requested details of the type equipment (make and model) and reagents used in the fluorimetric method.

3 Results and discussion

The basic data received from the participating laboratories are shown in tables I and II.

Analysis of the data

The statistical evaluation included the following steps:

1. analysis of the homogeneity of the within laboratory variance,
2. analysis of the outliers among the within laboratory means,
3. calculation of repeatability $r(95)$ and reproducibility $R(95)$,
4. determination of the dependence of the precision on the mean.

Numerical consistency testing using Cochran's and Grubb's test was used in the statistical evaluation of the data to identify stragglers and outliers. For the spectrophotometric method the Cochran's test detected three outliers and by Grubbs' test two outliers were detected. In addition 8 stragglers were found. For the fluorimetric method the Cochran's test detected two outliers and by Grubbs' test one outlier was detected. Furthermore 11 stragglers were found by both statistical tests. The results of the statistical treatment are also indicated in tables I and II. Based on the results of the statistical treatment, it was decided to reject the outliers from the calculation of the precision data, and to retain the stragglers.

Precision

The precision data using the remaining analytical results are summarized in tables III and IV.

Table I Spectrophotometric method: raw results for β -glucan mg/l in undiluted malt wort

Participant ID No.	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
1	200°	207°	203	198	119°	136°	347	345	154°°	143°°	244**	283**
2	179	173	194	184	75	90	314	312	127	118	251	246
3	178	160	210	186	85	109	308	339	116	124	256	270
4	168	172	167	176	87	93	301	323	107	122	241	225
5	148	140	160	156	72	70	250°	240°	92	92	246	224
6	165	179	151**	202**	106	99	279	293	106	109	246	242
7	164	159	189	185	101	99	304	302	116	119	223	219
8	156	159	176	174	73	72	317	316	88	94	257	248
9	197*	170*	201	177	80	81	268	278	91	113	282	272
10	175	181	172	186	109	111	318	342	114	118	252	244
11	163*	136*	164	172	79*	49*	297	319	79**	119**	264	286
12	186	187	210°	211°	60	59	364°	364°	113	112	314°°	313°°
13	150	155	166	161	85	101	264	293	97	109	241	239
14	152	137	163	173	80	75	295	317	118	111	248	234

* detected as straggler by Cochran's test; retained
 ** detected as outlier by Cochran's test; rejected
 ° detected as straggler by Grubbs' test; retained
 °° detected as outlier by Grubbs' test; rejected

Table II Fluorimetric method: raw results for β -glucan mg/l in undiluted malt wort

Participant ID No.	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
1	191	196	221	218	93°°	108°°	386	386	147*	133*	279	313
2	159	155	201	191	63	71	344	343	118	110	260	256
3	162*	136*	210	183	64	83	332	356	102	112	269	285
4	151	161	190	198	80	82	405	436	112	122	311	282
5	159*	137*	224**	175**	67*	88*	399	378	115	114	358**	298**
6	129	138	124*	166*	84	83	269	282	95	96	222	234
7	145	141	204	202	80	79	374	378	104	102	255	259
8	164	162	204	203	79	77	382	385	112	110	313	312
9	154	156	221	223	80	82	375	380	102	105	320	322
10	155	167	197	192	80	86	377	356	118	121	267	275
11	162	157	117°	109°	80	61	215°	188°	109	102	257	273
12	199°	198°	242	249	90	87	357	362	143°	145°	330	332
13	120	119	140	136	68	74	196°	202°	90	100	183°	184°
14	-	-	-	-	-	-	-	-	-	-	-	-

* detected as straggler by Cochran's test; retained
 ** detected as outlier by Cochran's test; rejected
 ° detected as straggler by Grubbs' test; retained
 °° detected as outlier by Grubbs' test; rejected

Table III Precision data - spectrophotometric method

	A	B	C	D	E	F
n	14	13	14	14	12	12
sr2	88,0	68,5	84,7	151,4	46,6	80,1
SL2	255,2	216,3	313,1	846,7	85,4	238,8
sr	9,4	8,3	9,2	12,3	6,8	8,9
sR	18,5	16,9	19,9	3,6	11,5	17,9
m	167,7	181,3	87,7	307,5	109,4	248,2
r95	28,7	25,5	28,1	37,6	21,2	27,9
R95	56,6	52,0	60,9	96,5	35,8	55,6
CVSr	5,6	4,6	10,5	4,0	6,2	3,6
CVSR	11,0	9,3	22,7	10,3	10,5	7,2

Table IV Precision data - fluorimetric method

	A	B	C	D	E	F
n	13	12	12	13	13	12
sr2	60,7	117,9	55,1	131,9	24,7	115,0
SL2	421,0	1403,9	11,7	4923,8	210,0	1658,4
sr	7,8	10,9	7,4	11,5	5,0	10,7
sR	21,9	39,0	8,2	71,1	15,3	42,1
m	156,7	189,2	77,8	340,1	113,0	274,7
r95	24,0	33,8	23,1	35,4	15,3	33,4
R95	67,6	121,4	25,4	219,1	47,2	131,1
CVSr	5,0	5,7	9,5	3,4	4,4	3,9
CVSR	14,0	20,6	10,5	20,9	13,6	15,3

Table V Fluorimetric method: raw results for (β -glucan mg/l) from the 7 SBL/FIA users

Participant ID No.	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
1	191 ^o	196 ^o	221	218	93 ^o	108 ^o	386	386	147* ^o	133* ^o	279**	313**
2	159	155	201	191	63	71	344	343	118	110	260	256
3	162**	136**	210	183	64*	83*	332	356	102	112	269	285
6	129	138	124 ^{oo}	166 ^{oo}	84	83	269 ^{oo}	282 ^{oo}	95	96	222	234
7	145	141	204	202	80	79	374	378	104	102	255	259
8	164	162	204	203	79	77	382	385	112	110	313	312
10	155	167	197	192	80	86	377	356	118	121	267	275

* detected as straggler by Cochran's test; retained

** detected as outlier by Cochran's test; rejected

^o detected as straggler by Grubbs' test; retained

^{oo} detected as outlier by Grubbs' test; rejected

In case of the spectrophotometric method repeatability $r(95)$ and reproducibility $R(95)$ were found to be independent of the mean value and were 28,2 mg/l and 59,4 mg/l respectively.

For the fluorimetric method repeatability $r(95)$ was found to be independent of the mean value and equal to 27,5 mg/l, whereas a strongly significant relationship of 0,69m - 30,3 mg/l was found between the reproducibility $R(95)$ and the mean value m.

Questionnaire results

The questionnaire (table VII) showed that 9 of the 13 participants of the fluorimetric test used a flow injection analysis (FIA) system (system Carlsberg or equivalent equipment) as prescribed in EBC methods 4.16.2 and 8.13.2. Most of these laboratories have also used reagents (β -glucan standard and Calcofluor reagent) of Scandinavian Bryggeri Laboratorium (SBL) Denmark (also prescribed in the EBC methods). Only one participant of the 8 laboratories using a Carlsberg FIA-system has used reagents of Foss Sweden. Two participants have used a Continuous Flow Analyzer (CFA) system of Skalar with reagents of Megazyme.

Because the use of both SBL reagents and a FIA-system is prescribed in the fluorimetric EBC methods, the precision data of the 7 participants using both SBL reagents and a FIA-system are also calculated separately. Using Cochran's and Grubb's tests in the statistical evaluation of the fluorimetric data of these 7 participants in total 4 outliers were found. Tables V and VI give the basic data and precision calculations respectively.

Table VI Precision data - fluorimetric method (from the 7 SBL/FIA users)

	A	B	C	D	E	F
n	6	6	7	6	7	6
sr ₂	23,8	72,3	49,4	86,9	27,0	41,4
SL ₂	411,9	50,2	84,2	315,5	189,6	756,9
sr	4,9	8,5	7,0	9,3	5,2	6,4
sR	20,9	11,1	11,6	20,1	14,7	28,3
m	158,5	202,2	80,7	366,6	112,9	267,3
r_{95}	17,7	30,9	24,3	33,9	18,0	23,4
R_{95}	75,9	40,2	40,0	72,9	50,9	102,7
CVS _r	3,1	4,2	8,7	2,5	4,6	2,4
CVS _R	13,2	5,5	14,3	5,5	13,0	10,6

Both repeatability $r(95)$ and reproducibility $R(95)$ were found to be independent of the mean value and are equal to 24,6 and mg/l 63,8 mg/l respectively.

Although 7 laboratories is usually considered insufficient to enable a robust statistical evaluation of the comparative precision between the fluorimetric FIA-system (using SBL reagents) and the spectrophotometric method, the precision figures of both methods indicate that there is no significant difference in their accuracy or precision.

Also the fluorimetric analysis results of the two laboratories using a continuous flow injection analyser of Skalar (participant ID No 4 and 9) indicate a good correlation with the corresponding fluorimetric mean values. Especially in the range 0-300 mg/l there is a good correlation with the mean values mentioned in table VI.

4 Conclusion

Repeatability, reproducibility and accuracy results for the determination of β -glucans in malt wort by a spectrophotometric method, using a β -glucan assay kit from NovaBiotec® Dr. Fechter GmbH, Berlin, Germany, are acceptable. The EBC Analysis Committee has recommended the inclusion of the method in Analytica-EBC.

Table VII Questionnaire results

Participant ID No.	Fluorimetric system	Supplier reagents
1	Carlsberg flow injection analyzer	SBL
2	Carlsberg flow injection analyzer	SBL
3	Carlsberg flow injection analyzer	SBL
4	Skalar continuous flow analyzer	Megazyme
5	Carlsberg flow injection analyzer	Foss Sweden
6	Carlsberg flow injection analyzer	SBL
7	Carlsberg flow injection analyzer	SBL
8	Carlsberg flow injection analyzer	SBL
9	Skalar continuous flow analyzer	Megazyme
10	Carlsberg flow injection analyzer	SBL
11	HPLC system	-
12	Custom build system	Megazyme/ Foss
13	Gilson flow injection system	Sigma/ Biocon
14	no fluorimetric system available	