Brewing Sugars and Syrups

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CONCLUSIONS

1. The total laboratory error was lowest for glucose and maltose using method 2, gas-liquid chromatography (GLC) of trimethylsilyl (TMS) derivatives, when considering data for both types of syrups.
2. The total laboratory error was lowest for maltotriose (triose) using method 3, high-pressure liquid chromatography (HPLC), when considering data for both types of syrups.
3. The total laboratory error was lowest using method 3, HPLC, when considering a combination of all three sugars and both types of syrups.
4. The coefficients of variation for total laboratory error using method 2, GLC of TMS derivatives, ranged from 3.7 to 13.6 for the individual sugars and from 2.0 to 5.5 for total fermentable sugars.
5. The coefficients of variation for total laboratory error using method 3, HPLC, ranged from 3.6 to 7.7 for the individual sugars and from 3.5 to 3.8 for total fermentable sugars.

RECOMMENDATIONS

1. It is recommended that method 2, GLC of TMS derivatives (Appendix A), and method 3, HPLC (Appendix B), be accepted for publication in the ASBC *Methods of Analysis*.
2. It is recommended that the Subcommittee on Brewing Sugars and Syrups be retired.

The evaluation of instrumental methods for determining fermentable carbohydrates in brewing syrups was assigned to this Subcommittee in 1975. A collaborative study was undertaken in 1975-76 to evaluate two gas chromatographic methods and a high-pressure liquid chromatographic method for determining glucose, maltose, and triose concentrations in a pair of corn syrup samples. The collaborative results (1) indicated that the least total error resulted from the use of the HPLC method. The highest error for all three methods was associated with the determination of triose concentration.

Based on recommendations evolving from the first collaborative study, a second collaborative study was initiated to include the following:

1. The same three methods, with minor revisions, would be evaluated.
2. Standard sugars would be sent to collaborators.
3. Two pairs of syrups, one with high glucose and low maltose and the other with low glucose and high maltose concentrations, would be included in the study.

COLLABORATIVE STUDY

Two pairs of corn syrup samples were sent to each collaborator for the determination of glucose, maltose, and triose

concentrations. Glucose and maltose standards were also supplied. Since pure triose was not available commercially, collaborators were instructed to use the response obtained for the maltose standard modified by a factor unique to each method for calculating triose concentrations in the syrups. The syrups were to be analyzed by one HPLC and two GLC methods, which were briefly described in the 1976 subcommittee report (1). Revisions submitted by the authors of the methods were included.

TABLE I
Carbohydrate Analysis by Method 1 (GLC of TMS-Oximes)
(Expressed in g/100 g as-is)

	Glucose				Maltose				Maltotriose			
Collaborator/Syrup	A	B	C	D	A	B	C	D	A	B	C	D
1	20.2	22.0	7.4	9.1	25.8	19.7	31.4	36.4	16.5	18.3	19.8	17.0
2	20.6	19.8	6.4	8.0	24.1	19.0	28.8	35.2	13.3	14.9	18.2	15.9
3	20.9	22.5	6.6	7.9	21.6	17.8	29.7	34.0	...[b]	...[b]	...[b]	...[b]
4	18.4[a]	21.1[a]	5.8	7.5	22.5	18.0	23.6[a]	28.1[a]	5.6	8.2	12.5	11.3
5	20.4	21.1	6.4	7.3	24.7	18.7	31.0	31.7	13.3	13.3	16.9	13.6
6	20.0	19.4	6.5	6.6	24.4	17.9	29.8	29.1	22.5	20.3	23.8	23.8
Mean[c]	20.42	20.96	6.52	7.73	23.85	18.52	30.14	33.28	14.24	15.00	18.24	16.32
Grand mean[c]	20.7		7.1		21.2		31.7		14.6		17.3	

[a]Rejected as an outlier according to Dixon's test (p = 0.05) (2).
[b]No data reported.
[c]The mean does not include outliers.

TABLE II
Carbohydrate Analysis by Method 2 (GLC of TMS)
(Expressed in g/100 g as-is)

	Glucose				Maltose				Maltotriose			
Collaborator/Syrup	A	B	C	D	A	B	C	D	A	B	C	D
1	20.5	21.2	6.8	8.4	26.4	20.4	31.0	36.8	17.3	17.9	19.5	17.3
2	21.6	22.9	7.2	9.0	24.8	19.5	32.0	37.9	12.7	14.4	18.4	16.3
3	20.8	20.7	6.7	8.1	25.2	18.7	30.6	36.1	16.1	15.9	19.8	18.1
4	22.5	23.6	7.6	9.4	26.5	21.0	31.0	35.4	17.3	17.3	22.9[a]	19.4[a]
5	21.1	21.6	6.8	8.3	26.8	19.0	31.4	36.9	27.1[a]	25.0[a]	31.6[a]	29.1[a]
6	20.3	22.4	6.9	8.4	25.9	20.9	31.5	36.6	15.9	15.7	18.4	17.5
7	21.3	22.8	7.1	8.7	25.3	20.2	32.7	38.3	14.1	14.3	17.0	15.6
12	22.4	23.1	7.2	8.8	26.8	20.6	32.2	38.9	16.3	17.2	18.7	18.0
Mean[b]	21.31	22.29	7.04	8.64	25.96	20.04	31.55	37.11	15.67	16.10	18.63	17.13
Grand mean[b]	21.8		7.8		23.0		34.3		15.9		17.9	

[a]Rejected as an outlier according to Dixon's test (p = 0.05) (2).
[b]The mean does not include outliers.

TABLE III
Carbohydrate Analysis by Method 3 (HPLC)
(Expressed in g/100 g as-is)

	DP1				DP2				DP3			
Collaborator/Syrup	A	B	C	D	A	B	C	D	A	B	C	D
1	20.7	21.9	6.7	8.2	26.7	20.7	31.9	37.6	16.5	17.3	19.3	16.8
2	22.9	22.6	7.4	9.6	24.3	18.0	25.3[a]	32.1[a]	16.1	14.9	15.7	17.5
3	20.9	21.7	6.5	8.3	26.6	20.7	31.8	37.5	15.6	15.9	18.2	16.6
6	19.3	21.0	6.2	7.7	24.6	19.7	30.2	35.4	15.1	15.6	18.0	16.1
7	20.0	20.6	7.1	8.3	27.1	21.6	33.7	38.7	16.9	17.4	18.7	16.1
8	20.1	21.7	6.6	8.0	25.8	19.2	30.4	36.4	15.7	16.4	18.6	15.9
9	19.0	20.1	6.9	8.2	25.1	20.1	30.3	35.9	14.9	15.6	18.0	16.7
10	19.9	22.5	7.3	8.4	20.1[a]	20.6[a]	33.2	39.0	13.2[a]	14.4[a]	16.2	16.8
11	21.2	22.2	6.9	8.8	26.8	20.8	31.7	38.1	15.2	15.1	17.9	16.6
13	20.1	22.1	6.6	8.3	25.0	20.3	30.6	36.4	15.3	17.5	18.2	16.8
Mean[b]	20.41	21.64	6.82	8.38	25.78	20.12	31.53	37.22	15.70	16.19	17.88	16.59
Grand mean[b]	21.0		7.6		23.0		34.4		15.9		17.2	

[a]Rejected as an outlier according to Dixon's test (p = 0.05) (2).
[b]The mean does not include outliers.

RESULTS AND DISCUSSION

The data reported by collaborators are given in Tables I, II, and III. Six collaborators reported data for method 1, GLC of TMS-oxime derivatives. Some of the data from Collaborator 4 were rejected as outliers, while Collaborator 3 could not obtain triose results. Eight collaborators reported data for method 2, GLC of TMS derivatives. Three pairs of data were rejected as outliers for this method. Ten collaborators reported data for method 3, HPLC. Three pairs of data were also rejected as outliers for this method. While the number of outliers appears to be excessive, it should be noted that, except for one case, only one outlier pair was present in each unit block.

An analysis of variance was performed on the data according to Youden (3). The results are given in Table IV for syrups A and B, which were high glucose-low maltose, and in Table V for syrups C and D, which were low glucose-high maltose. The columns in the tables show the grand means of sample pairs, the within- and between-lab errors, total error and F ratio, the magnitude of which indicates the proportion of variability due to between-laboratory error. By comparing the F ratio to the critical F, shown in the last columns of the tables, it can be seen that, in many cases, the critical F is exceeded by the calculated F, indicating that between-laboratory error is significant. It should be noted, however, that high F ratios are sometimes due to the very low within-laboratory variances rather than excessively high between-laboratory variances.

The data can be better evaluated by considering coefficients of variation for within-laboratory, between-laboratory, and total errors. These values are tabulated in Table VI for syrups A and B and Table VII for syrups C and D. It is immediately obvious that there was a problem in measuring triose concentration using method 1 (GLC-TMS-Oxime). Comments from collaborators indicated this method was the least preferred because triose peaks were either too small, too broad, or absent.

Further study of Tables VI and VII shows there may also be a problem with triose measurements using method 2 (GLC-TMS), as indicated by the relatively high between-laboratory and total errors obtained on analysis of the high glucose-low maltose syrups, A and B. These syrups contained slightly less triose than did the low glucose-high maltose syrups, C and D.

The use of method 3 (HPLC) resulted in the least total error when all three sugars are considered. While method 2 showed slightly lower error terms for the determination of glucose and maltose in both types of syrups, method 3 was consistent in showing considerably less error for the determination of triose. Thus, in choosing between methods 2 and 3, the advantage of slightly better precision for glucose and maltose provided by method 2 must be weighed against the considerably better precision for triose provided by method 3.

A comparison of the error terms for the two different types of syrups used in the collaboration shows that, for methods 2 and 3, the higher the sugar concentration, the lower the coefficient of variation for total error. However, except for triose determined by

TABLE IV
Analysis of Variance
High Glucose-Low Maltose Syrups A and B

Method	Sugar	Grand Mean	Within-Lab Error (S_r)	Between-Lab Error (S_b)	Total Error (S_t)	No. of Labs[a]	F Ratio	Critical F (95%)
	Glucose	20.7	0.854	0.49	1.098	5	1.65	6.388
1	Maltose	21.2	0.741	0.95	1.540	6	4.32	5.050
(GLC-TMS-O)	Triose[b]	14.6	1.347	5.28	7.593	5	31.78	6.388
	Glucose	21.8	0.478	0.79	1.212	8	6.44	3.787
2	Maltose	23.0	0.657	0.50	0.967	8	2.17	3.787
(GLC-TMS)	Triose	15.9	0.491	1.49	2.161	7	19.35	4.284
	Glucose (DP1)	21.0	0.569	0.79	1.256	10	4.88	3.179
3	Maltose (DP2)	23.0	0.471	0.93	1.417	9	9.07	3.438
(HPLC)	Triose (DP3)	15.9	0.630	0.58	1.035	9	2.70	3.438

[a]Outliers excluded.
[b]"Triose" is maltotriose.

TABLE V
Analysis of Variance
Low Glucose-High Maltose Syrups C and D

Method	Sugar	Grand Mean	Within-Lab Error (S_r)	Between-Lab Error (S_b)	Total Error (S_t)	No. of Labs[a]	F Ratio	Critical F (95%)
	Glucose	7.1	0.444	0.53	0.876	6	3.90	5.050
1	Maltose	31.7	2.125	0.53	2.251	5	1.12	6.388
(GLC-TMS-O)	Triose	17.3	0.937	4.33	6.20	5	43.76	6.388
	Glucose	7.8	0.100	0.35	0.508	8	25.82	3.787
2	Maltose	34.3	0.466	0.85	1.284	8	7.60	3.787
(GLC-TMS)	Triose	17.9	0.436	0.89	1.329	6	9.30	5.050
	Glucose (DP1)	7.6	0.241	0.38	0.586	10	5.92	3.179
3	Maltose (DP2)	34.4	0.290	1.24	1.776	9	37.42	3.438
(HPLC)	Triose (DP3)	17.2	1.021	0	0.629	10	0.38	3.179

[a]Outliers excluded.

method 2, the coefficients of variation for the sugars analyzed by each method are not vastly different. Thus, it appears that method 3 could be used for both types of syrups, whereas method 2, while suitable for relatively wide ranges of glucose and maltose concentrations, might be troublesome with syrups of low triose concentrations. This observation can be substantiated by a review of the 1976 collaborative data (1) for the determination of triose in syrups averaging only 7.2% triose by method 2, which showed a 23.6 coefficient of variation for between-laboratory error.

Several collaborators determined isomaltose concentration in the syrups by method 2 (GLC-TMS). The concentration of isomaltose in these syrups was low enough to have a negligible effect on the DP2 concentration determined by method 3 (HPLC). If, however, syrups were encountered having a high isomaltose concentration, the DP2 concentration would reflect the concentration of both maltose and isomaltose. Thus, method 2 has a distinct advantage over method 3 in that isomaltose could be separately measured. It, therefore, appears that both methods 2 and

TABLE VI
Coefficients of Variation[a]
High Glucose-Low Maltose Syrups (A and B)

Method	Sugar	Within-Lab Error (S_r)	Between-Lab Error (S_b)	Total Error (S_t)
	Glucose	4.1	2.4	5.3
1	Maltose	3.5	4.5	7.3
(GLC-TMS-O)	Triose	9.2	36.0	51.9
	Glucose	2.2	3.6	5.6
2	Maltose	2.6	2.2	4.2
(GLC-TMS)	Triose	3.1	9.4	13.6
	Glucose (DP1)	2.7	3.8	6.0
3	Maltose (DP2)	2.0	4.1	6.2
(HPLC)	Triose (DP3)	4.0	3.6	6.5

[a]Coefficient of variation = (S × 100)/grand mean.

TABLE VII
Coefficients of Variation[a]
Low Glucose-High Maltose Syrups (C and D)

Method	Sugar	Within-Lab Error (S_r)	Between-Lab Error (S_b)	Total Error (S_t)
	Glucose	6.2	7.4	12.3
1	Maltose	6.7	1.7	7.1
(GLC-TMS-O)	Triose	5.4	25.1	35.9
	Glucose	1.3	4.5	6.5
2	Maltose	1.4	2.5	3.7
(GLC-TMS)	Triose	2.4	5.0	7.4
	Glucose (DP1)	3.2	5.0	7.7
3	Maltose (DP2)	0.8	3.6	5.2
(HPLC)	Triose (DP3)	5.9	0	3.6

[a]Coefficient of variation = (S × 100)/grand mean.

TABLE VIII
Total Fermentable Carbohydrate
(Expressed as g/100 g as-is)

	Method 2 (GLC-TMS)				Method 3 (HPLC)			
Collaborator	A	B	C	D	A	B	C	D
1	64.2	59.5	57.3	62.5	63.9	59.9	57.9	62.6
2	59.1	56.8	57.6	63.2	63.3	55.5	48.4[a]	59.2[a]
3	62.1	55.3	57.1	62.3	63.1	58.3	56.5	62.4
4	66.3	61.9	61.5[a]	64.2[a]	...[b]	...[b]	...[b]	...[b]
5	75.0[a]	65.6[a]	69.8[a]	74.3[a]	...[b]	...[b]	...[b]	...[b]
6	62.1	59.0	56.8	62.5	59.0	56.3	54.4	59.2
7	60.7	57.3	56.8	62.6	64.0	59.6	59.5	63.1
8	...[b]	...[b]	...[b]	...[b]	61.6	57.3	55.6	60.3
9	...[b]	...[b]	...[b]	...[b]	59.0	55.7	55.2	60.8
10	...[b]	...[b]	...[b]	...[b]	53.2[a]	57.5[a]	56.7	64.2
11	...[b]	...[b]	...[b]	...[b]	63.2	58.1	56.5	63.5
12	65.5	60.9	58.1	65.7	...[b]	...[b]	...[b]	...[b]
13	...[b]	...[b]	...[b]	...[b]	60.4	59.9	55.4	61.5
Mean[c]	62.86	58.67	57.28	63.13	61.94	57.84	56.41	61.96
Grand mean[c]	60.8		60.2		59.9		59.2	

[a]Rejected as an outlier according to Dixon's test (p = 0.05) (2).
[b]No data reported.
[c]The mean does not include outliers.

TABLE X
Coefficient of Variation[a]
Total Fermentable Carbohydrates

Method	Syrups	Within-Lab Error (S_r)	Between-Lab Error (S_b)	Total Error (S_t)
	A and B	1.7	3.7	5.5
2 (GLC-TMS)	C and D	1.0	1.2	2.0
	A and B	2.3	2.1	3.8
3 (HPLC)	C and D	1.5	2.2	3.5

[a]Coefficient of variation = (S × 100)/grand mean.

TABLE IX
Analysis of Variance
Total Fermentable Carbohydrates

Method	Syrups	Grand Mean	Within-Lab Error (S_r)	Between-Lab Error (S_b)	Total Error (S_t)	No. of Labs[a]	F Ratio	Critical F (95%)
	A and B	60.8	1.027	2.25	3.346	7	10.60	3.787
2 (GLC-TMS)	C and D	60.2	0.632	0.75	1.236	6	3.83	4.284
	A and B	59.9	1.389	1.28	2.285	9	2.71	3.438
3 (HPLC)	C and D	59.2	0.871	1.33	2.068	9	5.63	3.438

[a]Outliers excluded.

3 should be considered for publication in the ASBC *Methods of Analysis*.

Since the coefficients of variation for total error in determining the individual sugars by methods 2 and 3 ranged from 3.6 to 13.7, the question arises as to whether or not these methods have the reproducibility required for acceptance as ASBC methods. Possibly this can best be answered by showing that the reproducibility of the methods is excellent when used to determine the total amount of fermentable carbohydrate in syrups. Table VIII presents the sum of glucose (DP1), maltose (DP2), and triose (DP3), expressed as total fermentable carbohydrate for each syrup and each collaborator using methods 2 and 3. An analysis of variance of the data is given in Table IX, and coefficients of variation for the error terms in Table X. The data show that the methods have low within-laboratory, between-laboratory, and total errors for determining total fermentable carbohydrate. This, coupled with the fact that total errors for the individual sugars are not excessive, supports the recommendation that the methods be accepted.

Literature Cited

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APPENDIX A

Brewing Sugars and Syrups—Glucose, Maltose, and Maltotriose by Gas Chromatography of Trimethylsilyl Derivatives

Sugars or syrups are reacted with hexamethyldisilazane to form their trimethylsilyl ethers which are volatile enough to be gas chromatographed. Glucose, maltose, and triose are separated and quantitatively measured.

Reagents

a) *Pyridine,* reagent or spectro grade, dried over potassium hydroxide pellets or silylation grade (see **Note**).
b) *Hexamethyldisilazane,* silylation grade.
c) *Trifluoroacetic acid,* 99 + % purity.
d) *Phenyl beta-D-glucopyranoside,* Nutritional Biochemicals Co., Division of ICN Pharmaceuticals, Life Sciences Group, 26201 Miles Rd., Cleveland, OH 44128, or equivalent.
e) *Glucose,* 99.9% purity, Baker analyzed reagent, or equivalent.
f) *Maltose,* 99% purity, dry solids basis, 5.9% moisture, Grade HHH, Hayashibara Biochemical Laboratories, Inc., 2-3.1-Chome, Shimoishi, Okayama, Japan, or equivalent.
g) *Drierite* (anhydrous calcium sulfate), 10–20 mesh, W. A. Hammond Drierite Co., Xenia, Ohio.
h) *Ammonium hydroxide,* concentrated.
i) *Silicone OV-17*
j) *Chromosorb W,* High Performance, 80–100 mesh.

Apparatus

a) *Gas chromatograph* with flame ionization detector and recorder.
b) *3% OV-17 on Chromosorb W,* high performance, 80–100 mesh, packed in 6-ft × 1/8-in. (i.d.) stainless-steel columns, cured at 340°C (reagents i and j). Dual columns are required.
c) *Syringe,* 10-μl.
d) *Serum bottles,* 10-ml with caps or other appropriate vials.
e) *Shaker,* wrist action.

Method

a) Internal standard: Weigh exactly 166.7 mg of phenyl beta-D-glucopyranoside (reagent d) into a 100-ml volumetric flask and dilute to 100 ml with pyridine (reagent a).
b) Calibration standard: Weigh 1.0000 g glucose (reagent e) and 1.0730 g maltose (reagent f) into a 50-ml volumetric flask. Add 10 ml H_2O and 2 drops of ammonium hydroxide (reagent h), mix, and dilute to volume with distilled water. Freeze dry 0.5 ml aliquots in 10-ml serum bottles. To the freeze-dried materials add about 40 mg of Drierite (reagent g), 3 ml of internal standard solution, 0.9 ml of hexamethyldisilazane (reagent b), and 0.1 ml of trifluoroacetic acid (reagent c). Shake for 1 hr on wrist action shaker. Allow Drierite to settle and transfer supernatant to another vial.
c) Syrups: Weigh about 30 mg of syrup into a 10-ml serum vial and record exact weight. Add about 40 mg Drierite (reagent g), 3 ml internal standard solution, 0.9 ml hexamethyldisilazane (reagent b), and 0.1 ml of trifluoroacetic acid (reagent c). Shake for 1 hr on wrist action shaker. Allow Drierite to settle and transfer supernatant to another vial.
d) Chromatograph the calibration standard three times and the syrup samples once using the following conditions:

Injection port temperature:	325°C
Detector temperature:	350°C
Column temperature:	150° to 325°C at 10°C/min, hold 10 min
Carrier gas flow rate:	40 ml/min
Sample size:	2 μl

e) Measure peak areas for α- and β-glucose, internal standard, and α- and β-maltose for the standard chromatograms. For syrups, measure the previous and, in addition, α- and β-isomaltose and α- and β-triose. Approximate retention times are shown on the attached sample chromatogram. Sum the areas of α- plus β-glucose, α- plus β-maltose, etc.

Calculations

a) Determine the ratio response per mg sugar in the calibration mixture as follows:

$$\text{Ratio to internal standard} = \frac{\text{Area glucose or maltose}}{\text{Area internal standard}}$$

$$\text{Ratio response per mg glucose or maltose} = \frac{\text{Ratio to internal standard for glucose or maltose}}{10}$$

Average the ratio responses/mg glucose or maltose from the three chromatograms of the calibration mixture.

b) Determine the mg each sugar present in syrup sample as follows:

$$\text{Ratio for each sugar} = \frac{\text{Total area each sugar}}{\text{Area internal standard}}$$

$$\text{mg glucose or maltose} = \frac{\text{Ratio glucose or maltose}}{\text{Av ratio response/mg glucose or maltose (calculated in a)}}$$

$$\text{mg isomaltose} = \frac{\text{Ratio isomaltose}}{\text{Av ratio response/mg maltose}}$$

$$\text{mg triose} = \frac{\text{Ratio triose}}{\text{Av ratio response/mg maltose} \times 0.82}$$

c) Determine the % each sugar present in syrup as follows:

$$\% \text{ each sugar as-is} = \left(\frac{\text{mg each sugar}}{\text{mg syrup as-is}}\right) \times 100$$

Report to one decimal place.

Example

a) Peak area α-glucose + β-glucose standard = 157925 integrator counts
Peak area α-maltose + β-maltose standard = 136170 integrator counts
Peak area internal standard = 101566 integrator counts

$$\text{Ratio glucose} = \frac{157925}{101566} = 1.5549$$

$$\text{Ratio response/mg glucose} = \frac{1.5549}{10} = 0.15549$$

Repeat above calculation on two additional standard chromatograms and average the three results.

$$\text{Ratio maltose} = \frac{136170}{101566} = 1.3407$$

$$\text{Ratio response/mg maltose} = \frac{1.3407}{10} = 0.13407$$

Again obtain average from three chromatograms.

Assuming above are averages, the ratio response/mg glucose = 0.15549 and/mg maltose = 0.13407. Ratio response/mg triose = 0.13407 × 0.82 = 0.10994.

b) Peak area α-glucose + β-glucose from syrup = 143700 integrator counts
Peak area α-maltose + β-maltose from syrup = 98975 integrator counts
Peak area α-isomaltose + β-isomaltose from syrup = 1844 integrator counts
Peak area α-triose + β-triose from syrup = 26060 integrator counts
Peak area internal standard = 96875 integrator counts
wt syrup as-is = 28.4 mg

$$\text{Ratio glucose} = \frac{143700}{96875} = 1.4833$$

$$\text{mg glucose} = \frac{1.4833}{0.15549} = 9.54$$

$$\% \text{ glucose as-is} = \left(\frac{9.54}{28.40}\right) \times 100 = 33.6$$

$$\text{Ratio maltose} = \frac{98975}{96875} = 1.0217$$

$$\text{mg maltose} = \frac{1.0217}{0.13407} = 7.62$$

$$\% \text{ maltose as-is} = \left(\frac{7.62}{28.40}\right) \times 100 = 26.8$$

$$\text{Ratio isomaltose} = \frac{1844}{96875} = 0.0190$$

$$\text{mg isomaltose} = \frac{0.0190}{0.13407} = 0.1420$$

$$\% \text{ isomaltose as-is} = \left(\frac{0.142}{28.400}\right) \times 100 = 0.5$$

$$\text{Ratio triose} = \frac{26060}{96875} = 0.2690$$

$$\text{mg triose} = \frac{0.2690}{0.10994} = 2.45$$

$$\% \text{ triose as-is} = \left(\frac{2.45}{28.400}\right) \times 100 = 8.6$$

Notes

1. It is essential that pyridine by anhydrous. If in doubt, prepare as follows:
   For every 500 ml pyridine, add 30 g KOH pellets.
   Mix well, allow KOH to settle, and wait 24 hr before using.
2. Isomaltose can be detected using this method (see sample chromatogram). Since isomaltose will probably be in very low concentration in the syrup samples provided, and since the α- and β- anomers are not completely separated, an integrator will be required to determine isomaltose peak area.
3. Isomaltose and triose responses were obtained from K. Brobst, A. E. Staley Manufacturing Co., since these compounds are not commercially available.

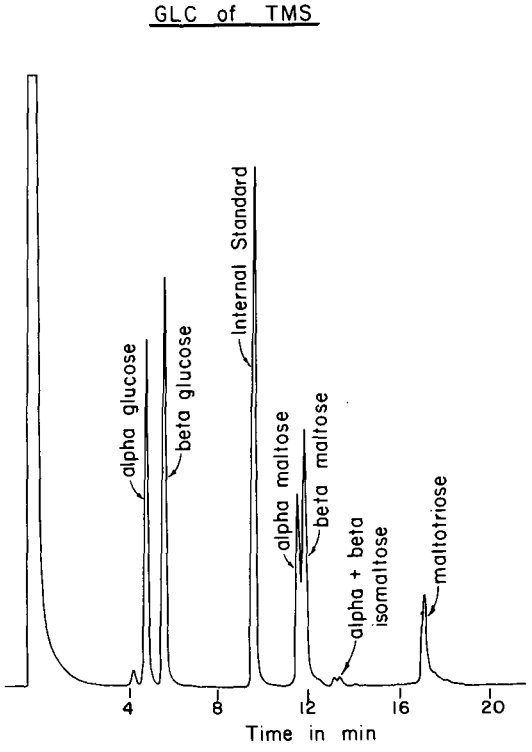

Fig. A, 1. Sample chromatogram for GLC-TMS derivatives.

References

1. AMERICAN SOCIETY OF BREWING CHEMISTS. Report of subcommittee on brewing sugars and syrups. *Journal 34*(3): 103 (1976).
2. BROBST, K. M., and LOTT, C. E., Jr. *Cereal Chem. 43*: 35 (1966).

APPENDIX B

Sugars and Syrups—Sugars of Degree of Polymerization 1, 2, and 3 by High-Pressure Liquid Chromatography

An aqueous solution of sugars or syrups containing approximately 15% dry solids is prepared and injected directly into a high-pressure liquid chromatograph. Sugars of degree of polymerization 1, 2, and 3 are separated from higher molecular weight carbohydrates and are quantitatively measured.

Reagents

a) *Calcium chloride dihydrate,* 50 g, Mallinckrodt Cat. No. 4160, or equivalent.
b) *Aminex Q15S,* 19–25 μ, 25 g, Bio Rad Laboratories, Richmond, Calif., Cat. No. 147-2202.
c) *Distilled water,* deionized, for reagents and column preparation
d) *HCl, 2N,* one l.
e) *Amberlite MB-1* (or equivalent) mixed bed ion-exchange resin. Predry by placing in a glass beaker and dry overnight at 40°C in an air oven.
f) *Glucose,* 99.9% purity, Baker Analyzed Reagent, or equivalent.
g) *Maltose,* 99% purity dry solids basis, 5.9% moisture, Grade HHH, Hayashibara Biochemical Laboratories, Inc., 2-3.1-Chome, Shimoishi, Okayama, Japan, or equivalent.

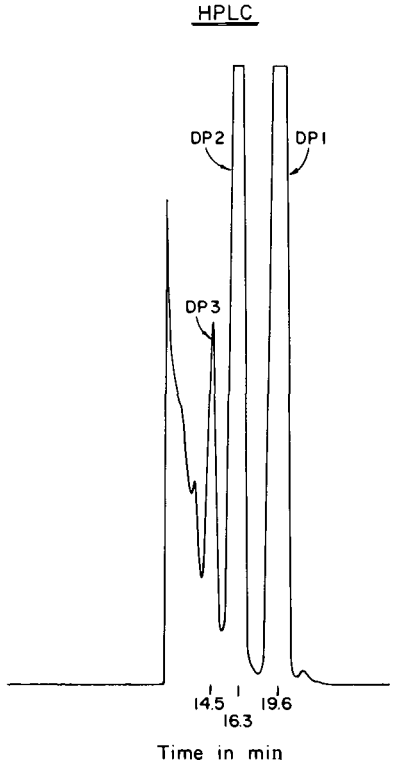

Fig. B, 1. Sample chromatogram for HPLC.

Apparatus

a) *High-pressure liquid chromatograph* with refractive index detector.
b) *Recorder.*
c) *Syringe,* 10 μl.
d) *Aminex Q15S (calcium form) column,* 20 in. × 0.305 in. i.d.
e) *Shaker,* wrist action.

Method

a) Column preparation: See **Column Preparation**, below.
b) Calibration standard: Dissolve 1.000 g glucose (reagent f) and 1.073 g maltose (reagent g) in water and dilute to 25 ml.
c) Sugars or syrups: Dissolve about 18 g in water (record exact weight) and dilute to 100 ml. To 10 ml of dilute solutions, add 100 mg of predried Amberlite MB-1 (reagent e) and shake for 15 min on wrist action shaker.
d) Operating conditions for high-pressure liquid chromatograph:
Column temperature: 85°C.
Solvent: deionized, distilled, degassed water.
Flow rate: 0.6 ml/min.
Refractometer temperature: 45°C.
Sample size: 10 μl.
Refractometer attenuation: 4× (this may vary with type of instrument).

Chromatograph the calibration standard three times, injecting 10 μl each time. Chromatograph each syrup once, again injecting 10 μl of each. Measure the peak areas for glucose and maltose from the three chromatograms of the calibration mixture. Average the area values from the three chromatograms for each sugar. Measure the peak areas for glucose, maltose, and triose from the syrup chromatograms. Approximate retention times for the sugars are given below:

Triose	14.5 min.
Maltose	16.3 min.
Glucose	19.6 min.

Calculations

a) Determine the responses for the glucose and maltose standards as follows:

$$\text{Response per 1\% sugar w/v} = \frac{\text{Peak area glucose or maltose}}{4}$$

b) Determine the response for triose by multiplying the maltose response by 1.03.
c) Determine the concentration of sugars in test samples as follows:

$$\text{Sugar, \% w/w in sample as-is} = \left(\frac{\text{Area sugar}}{\text{Sugar response}}\right)\left(\frac{1}{\text{wt syrup g}}\right) \times 100$$

d) Report glucose as DP1, maltose as DP2, triose as DP3, each as % as-is in syrup to one decimal place.

Example

Weight of corn syrup as-is = 18.000 g
Peak area glucose standard = 20 cm^2 (av of three chromatograms)
Peak area maltose standard = 20 cm^2 (av of three chromatograms)
Peak area glucose from syrup = 27 cm^2
Peak area maltose from syrup = 25 cm^2
Peak area triose from syrup = 7 cm^2

$$\text{Glucose response} = \frac{20}{4} = 5$$

$$\text{Maltose response} = \frac{20}{4} = 5$$

$$\text{Triose response} = 5 \times 1.03 = 5.15$$

% Glucose (DP1) as-is in syrup =

$$\left(\frac{27}{5}\right)\left(\frac{1}{18}\right) \times 100 = 30.0$$

% Maltose (DP2) as-is in syrup =

$$\left(\frac{25}{5}\right)\left(\frac{1}{18}\right) \times 100 = 27.8$$

% Triose (DP3) as-is in syrup =

$$\left(\frac{7}{5.15}\right)\left(\frac{1}{18}\right) \times 100 = 7.6$$

Note

No pure triose standard was commercially available. Hence, the triose response factor is based on maltose × 1.03. This factor was obtained from K. Brobst, A. E. Staley Manufacturing Co.

References

1. AMERICAN SOCIETY OF BREWING CHEMISTS. Report of subcommittee on brewing sugars and syrups. *Journal 34*(3): 103 (1976).
2. BROBST, K. M., and SCOBELL, H. D. Personal Communication.
3. BROBST, K. M., SCOBELL, H. D., and STEELE, E. M. *Amer. Soc. Brew. Chem., Proc. 1973,* p. 43.

COLUMN PACKING PROCEDURE FOR HPLC METHOD

(AMINEX Q15S CALCIUM FORM)

Materials

a) *Two 3/8 in. o.s. × 0.305 in. i.d. × 24-in. columns* complete with 10-μ end fittings. Waters Associates Cat. No. 26976.
b) *Milton Roy 1000 No. pump*—use A1C-201 unit or an auxiliary unit. Waters Cat. No. 98534.
c) *Circulating water bath*—use one of system units or an auxiliary unit.
d) *One 2-in. o.d. × 1 7/8-in. i.d. × 18 1/4-in. S. S. column jacket.*
e) *Rubber stoppers,* 2 No. 10.
f) *Graduate cylinder,* 2 l.
g) *Filter flask,* 2 l.
h) *K/D evaporator flask or equivalent resin reservoir,* 250 ml.
i) *12-ft, 1/2-in. o.d. × 3/8-in. i.d. Tygon tubing.*
j) *Tubing union,* 3/8 in.

Reagents

a) *Calcium chloride dehydrate,* 50 g, Mallinckrodt Cat. No. 4160.
b) *Aminex Q15S*—19–25 μ, 25 g, Bio Rad Labs Cat. No. 147-2202.
c) *Deionized distilled water.*
d) *HCl,* 2*N*, one l.

Tools

a) *Adjustable wrenches,* two 6 in.
b) *Tubing cutter.*
c) *Cork bore,* one 3/8 in.
d) *File,* 6 in.
e) *Small vise.*

Resin Preparation

NOTE: Use deionized distilled water in all of the following steps.

a) Add one l. of 2*N* HCl to a 2-l. filter flask.
b) Slurry 25 g Aminex Q15S in 100 ml of water and add to filter flask.
c) Stopper flask and apply aspirator vacuum for 10 min.
d) Transfer contents of filter flask to a 2-l. graduate cylinder and allow resin to completely settle.
e) Decant off liquid, add 200 ml of water, and swirl resin back into a slurry. Add water to the 2-l. mark and allow resin to settle.
f) Repeat step "e" two additional times.
g) Dissolve 50 g $CaCl_2 \cdot 2H_2O$ in one l. water in a 2-l. filter flask.
h) Decant liquid from 2-l. graduate, add 200 ml of water, reslurry resin, and transfer into the $CaCl_2 \cdot 2H_2O$ solution.
i) Bring the solution in the filter flask to 80° C and hold for 30 min.
j) Transfer contents to a 2-l. graduate cylinder and allow resin to settle.
k) Decant liquid, add 200 ml of water, reslurry resin, fill to the 2-l. mark with water, and allow resin to settle.
l) Repeat step "k" two additional times.

Column Preparation

a) Using the tubing cutter, cut one of the 3/8-in. o.d. columns to 20 in.
b) File the cut end until flat and ream. Refile until smooth.
c) Cut the remaining 4-in. piece of 3/8-in. tubing in half.
d) From the bottom, bore two 3/8-in. holes in each No. 10 stopper as far apart as possible.
e) Insert the 20-in. piece of 3/8-in. tubing into one of the holes in the large end of a No. 10 stopper. Push the tubing through until the connecting nut is flush with the end of the stopper.
f) Force this stopper into the end of the 2-in. o.d. column jacket.
g) Force the small end of the remaining stopper over the other end of the 3/8-in. column and into the 2-in. column jacket.
h) Using a 3/8-in. tubing union, lock a nut and ferrules on the bare end of the 3/8-in. column.
i) Force the 2-in. pieces of 3/8-in. tubing into the remaining holes on either end of the column assembly. Leave 3/4 in. of these short tubes protrude.
j) Remove the end fittings from both ends of the remaining 3/8-in. o.d. × 24-in. column.
k) Connect this column to the 3/8-in. tubing union.
l) Wash the inside of this two-column assembly with 100 ml of acetone, 100 ml of ethanol, and 200 ml of distilled water.
m) Put a column end fitting on the jacketed end of the assembly. Tighten the fitting and fitting plug.
n) Clamp the assembly in a vertical position, jacketed end down.
o) Slip a 1/2-in. piece of 1/2-in. o.d. × 3/8-in. i.d. Tygon tubing over the upper end of the column assembly.
p) Force the 250 ml KD evaporator flask down over the Tygon sleeve.
q) Fill the column assembly with deionized distilled water, until approximately 10 ml of water is in the bottom of the KD flask.

Column Packing Procedure

a) Decant liquid from settled resin, add 200 ml of water, reslurry resin, and transfer to the 250-ml KD flask atop the column assembly.
b) Allow to settle until all resin disappears into prepacker column.
c) Siphon liquid from KD flask and remove it from assembly.
d) Put a 10-μ end fitting on the upper end of the prepacker column

and tighten.

e) Attach a line from the solvent pump to the upper end fitting, remove the lower cap plug, and start pumping at the rate of 0.6 ml per min, *i.e.*, 20% full stroke.

f) Attach the column jacket to the circulating water bath with the two 6-ft pieces of Tygon tubing.

g) Turn on the water bath and set temperature to 80° C.

h) When temperature reaches 80° C, increase pump stroke to 40%, *i.e.*, 1.2 ml/min.

i) Continue pumping at this rate for a minimum of 2 hr.

j) Study the following steps so they may be performed in rapid succession.

k) Turn off pump, remove tubing from top of column, and place plug on lower column end-fitting.

l) Remove end-fitting from top of column assembly and lay aside.

m) Remove prepacker from column assembly at the point where the 3/8-in. tubing union joins the jacketed column.

n) Using a small spatula, remove some resin from the 3/8-in. tubing union and transfer it to the upper end-fitting. Continue to do this until 1/4 in. of resin is deposited in the fitting.

o) Remove the water from the resin by applying aspirator vacuum to the 1/16-in. end of the fitting.

p) Put this fitting on the upper end of the jacketed column, tighten, and cap.

q) If the column is to be put into the chromatograph, turn off the water bath, drain the jacket, and transfer the column to chromatograph immediately.

r) If the column is to be stored, reconnect the column to the pump, remove the plug from the lower end-fitting, and pump at the rate of 0.3 ml/min, *i.e.*, 10% of full stroke.

s) Turn off water bath, drain column jacket, and allow column to cool to room temperature; approximately 3 hr required.

t) Cap both ends of column and store.

u) Remove resin from prepacker column and save.