

Brix Factors for Determining the Solids in Starch Hydrolyzates

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The Brix hydrometer is widely used in the food industry for the measurement of solids in sugar sirups, but the readings obtained on corn sirups overestimate the solids. The amount of overestimation increases as the dextrose equivalent decreases. In the present work factors were obtained to correct Brix to actual solids. The ash of the usual starch hydrolyzates is largely sodium chloride, which affects the Brix reading more than an equal weight of sirup solids. This salt effect is compensated for by the results of a silver nitrate titration. A new, rapid, and accurate procedure for the determination of the dextrose equivalent is based on the dry substance value obtained by the Brix hydrometer, which has a precision comparable to the value based on a dry substance determination by the vacuum oven.

THE CONVENIENCE of the per cent by weight scale has prompted use of the Brix hydrometer in corn sirup produced in a wide range of dextrose equivalents (D.E., per cent reducing sugars calculated as dextrose and expressed on a dry substance basis). Fetzer and Evans (2) took advantage of this convenience to obtain correction factors to convert the Brix readings to actual solids.

Although the decision of Fetzer and Evans to base their factors on the assumption that the various products have a constant ash was justified at the time, conditions have changed. At present corn sirup and other starch hydrolyzates are often deionized. On the other hand, certain residual molasses resulting from the refining of dextrose and containing appreciable quantities of ash are commercial products. For this reason, it seemed highly desirable to redetermine the factors, basing them on the actual carbohydrate solids. Because the ash in these products is largely sodium chloride, its presence in the sirup can be determined volumetrically and corrected for its effect upon the observed Brix. A table for D.E. based on the two variables—Brix and Lane-Eynon titration—would be of great value not only within the laboratories of the wet milling industry but to the users of corn sirups.

The factors to correct the Brix reading to actual solids are based on the following considerations:

Determining the factors by specific gravity and actual solids, thus eliminating the reducing sugar determination.

Basing the factors for the various D.E.'s on ash-free hydrolyzates and determining the effect of the ash on the hydrometer by a volumetric determination of salt, with tables to convert the titration directly to the Brix correction necessary for the amount of salt present.

Corn Sirups and Corn Sugars

The starch hydrolyzates used in this research were, with one exception, commercial products produced by the acid hydrolysis of starch. The products in the D.E. range of 30 to about 58 are non-crystallizing and are termed corn sirups; those in the D.E. range of about 80 to 93 crystallize to a firm solid and are termed crude corn sugars (Nos. 70 and 80). No commercial acid-hydrolyzed product is produced in the range between corn

sirups and corn sugars. To provide such a product, a special factory conversion was made, employing the hydrolytic conditions for corn sirup. This product had a D.E. of 68.3 and is designated as a "special sirup." The usual analyses were made on hydrolyzates (Table I).

Effect of Ash on Brix

The effect of salt on the specific gravity at three Brix levels of sucrose was determined in the following manner:

Table I. Analysis of Starch Hydrolyzates

Type	Moisture, %	Dry Substance, %	Ash, %	N × 6.25, %	[α] _D ²⁰	Reducing Sugars, %	D.E.	Ash-Free D.E.
Corn sirup	22.35	77.65	0.248	0.0245	164.3	24.85	32.0	32.3
	18.58	81.42	0.334	0.0280	146.9	34.52	42.4	42.6
	18.79	81.21	0.410	0.0330	123.4	46.13	56.8	57.0
Special sirup	21.92	78.08	0.258	0.0440	103.3	53.33	68.3	68.6
Corn sugar	17.34	82.66	0.537	0.0530	65.7	69.68	84.3	84.9
	12.37	87.63	1.262	0.0742	53.7	80.00	91.3	92.6

Table II. Effect of Sodium Chloride on Specific Gravity

Grams/250 Ml.		NaCl, %	20°/20° C.	Increase	Increase per 1.00% NaCl
Sucrose	NaCl				
17.00 Brix					
45.338	0.000	0.000	1.06973	0.00000	0.00000
43.524	1.814	4.000	1.07195	0.00223	0.00056
42.618	2.720	6.000	1.07313	0.00341	0.00057
40.804	4.534	10.000	1.07521	0.00549	0.00055
20.00 Brix					
53.996	0.000	0.000	1.08302	0.00000	0.00000
53.456	0.540	1.000	1.08362	0.00060	0.00060
52.916	1.080	2.000	1.08431	0.00129	0.00065
51.836	2.160	4.000	1.08550	0.00248	0.00062
49.676	4.320	8.000	1.08805	0.00503	0.00063
48.596	5.400	10.000	1.08930	0.00628	0.00063
23.00 Brix					
62.869	0.000	0.000	1.09653	0.00000	0.00000
59.097	3.772	6.000	1.10093	0.00440	0.00073
56.582	6.287	10.000	1.10383	0.00730	0.00073

The quantity of sucrose (reagent grade) needed to produce, for example, 20.00 Brix was weighed, transferred to a 250-ml. Manufacturing Chemists' Association volumetric flask, dissolved in about 200 ml. of water, cooled to 20° C., and made to volume. The apparent specific gravity at 20°/20° C. was obtained in quadruplicate and the results were averaged. This value, zero ash, was checked against the published values to establish the accuracy of the procedure.

Then 99.00, 98.00, 96.00, 92.00, and 90.00% portions of the above weight of sucrose, with the corresponding weight of sodium chloride to assure 100.00% of the original solids, were transferred to the volumetric flask, dissolved in about 200 ml. of water, cooled, and made to volume, and the apparent specific gravity (20°/20° C.) was determined as before.

The increase in specific gravity indicates the effect of replacing a given weight of sucrose by sodium chloride; the increase divided by the per cent of salt used gives the increase in terms of 1.00% salt. The detailed results for levels of sucrose, 17.00, 20.00, and 23.00 Brix, are shown in Table II.

The data indicate that the effect of sodium chloride on the specific gravity is linear in amounts up to 10.00%. These data enable further calculations to be made in terms of Brix (Table III).

On the basis of the sucrose replaced by sodium chloride, the effect on the specific gravity is much greater. This is shown in Table IV, which is based on the experimental data given in Table II. The values in this table for 1.00% sodium chloride have been used for the calculations for determining Brix factors.

Because the ash in starch hydrolyzates is essentially sodium chloride, the amount present may be determined volumetrically. For this purpose, 0.0855*N* silver nitrate (1.00 ml. equals 0.00500 gram of sodium chloride) was employed for titrating 5.00 ml. of the sirup containing the salt. The milliliters of silver nitrate required for 10.00% salt, dry basis, for 20.00 Brix were obtained by substituting in the following equation:

$$\frac{\text{Ml. of 0.0855N silver nitrate} \times 0.00500}{5.00 \times 1.07991 (\bar{s}) \text{ Table 114}} \times 100 = 2.000\% \text{ salt}$$

Ml. = 21.60

Because the increase in Brix for this amount of salt is 1.41, the amount per milliliter of 0.0855*N* silver nitrate is 0.0653 Brix. Thus, the observed Brix may be corrected to true solids by subtracting the correction obtained by multiplying the milliliters of silver nitrate used for the titration by the factor of 0.0653. The above procedure was applied to the data obtained for the two other Brix values cited with results as follows:

17.00 Brix 18.18 ml. 0.0666 per ml.
23.00 Brix 25.15 ml. 0.0640 per ml.

Table V has been computed for the Brix range of 17.00 to 23.00 and for sufficient range in titration to cover all starch hydrolyzates and the usual residual sirups obtained in the manufacture of dextrose.

Brix Factors for Starch Hydrolyzates

The Brix reading can be converted to corn sirup or corn sugar solids by obtaining the ratio from the data obtained from two independent determinations on a given sirup:

$$\frac{A}{B} = \frac{\text{actual solids} - \text{vacuum oven moisture}}{\text{Brix solids} - \text{specific gravity}}$$

Ash-free products would have been ideal for this procedure, but they were not available. It is possible to place the results obtained on an ash-free basis from the data which have been reported.

Table III. Brix Corrections for Salt

Brix	Salt, %		Increase	
	Dry basis	As is	Brix	For 1.00% salt
17.00	10.00	1.700	1.21	0.121
20.00	10.00	2.000	1.41	0.141
23.00	10.00	2.300	1.61	0.161

Table IV. Effect of Salt on Specific Gravity in Terms of Sucrose Replaced

Brix, 20° C.	Grams/250 Ml.		Sp. Gr., 20°/20° C.		Increase in Sp. Gr.	
	Sucrose	Sucrose used, 90.00%	Calcd., Table 114 (5)	Found	10.00% salt	1.00% salt
17.00	45.338	40.805	1.06682	1.07521	0.00839	0.00084
20.00	53.996	48.995	1.08047	1.08930	0.00883	0.00088
23.00	62.869	56.658	1.09494	1.10383	0.00889	0.00089

Table V. Brix Subtractions for Effect of Salt Based on Silver Nitrate Titration

5.00-ml. sample. 0.0855*N* AgNO₃
20.0° C. 1.00 ml. = 0.00500 gram NaCl

Titn., Ml.	Observed Brix						
	17.00	18.00	19.00	20.00	21.00	22.00	23.00
1.00	0.07	0.07	0.07	0.07	0.06	0.06	0.06
1.50	0.10	0.10	0.10	0.10	0.10	0.10	0.09
2.00	0.13	0.13	0.13	0.13	0.13	0.13	0.13
2.50	0.17	0.17	0.16	0.16	0.16	0.16	0.16
3.00	0.20	0.20	0.20	0.20	0.19	0.19	0.19
3.50	0.23	0.23	0.23	0.23	0.23	0.22	0.22
4.00	0.27	0.26	0.26	0.26	0.26	0.26	0.25
4.50	0.30	0.30	0.30	0.29	0.29	0.29	0.28
5.00	0.33	0.33	0.33	0.33	0.32	0.32	0.32
5.50	0.37	0.36	0.36	0.36	0.36	0.35	0.35
6.00	0.40	0.40	0.39	0.39	0.39	0.38	0.37
6.50	0.43	0.43	0.42	0.42	0.42	0.42	0.41
7.00	0.47	0.46	0.46	0.46	0.45	0.45	0.44
7.50	0.50	0.50	0.49	0.49	0.48	0.48	0.47
8.00	0.53	0.53	0.53	0.52	0.52	0.51	0.51
8.50	0.57	0.56	0.56	0.55	0.55	0.54	0.54
9.00	0.60	0.60	0.59	0.59	0.58	0.58	0.57
9.50	0.63	0.63	0.62	0.62	0.61	0.61	0.60
10.00	0.67	0.66	0.66	0.65	0.64	0.64	0.63
10.50	0.70	0.69	0.69	0.69	0.68	0.67	0.66
11.00	0.73	0.73	0.72	0.72	0.71	0.70	0.70
11.50	0.77	0.76	0.76	0.75	0.74	0.74	0.73
12.00	0.80	0.79	0.79	0.78	0.78	0.77	0.76
12.50	0.83	0.83	0.82	0.82	0.81	0.80	0.79
13.00	0.87	0.86	0.85	0.85	0.84	0.83	0.82
13.50	0.90	0.89	0.89	0.88	0.87	0.86	0.85
14.00	0.93	0.93	0.92	0.91	0.90	0.90	0.89
14.50	0.97	0.96	0.95	0.95	0.94	0.93	0.92
15.00	1.00	0.99	0.99	0.98	0.97	0.96	0.95
15.50	1.03	1.03	1.02	1.01	1.00	0.99	0.98
16.00	1.06	1.06	1.05	1.04	1.03	1.02	1.01
16.50	1.10	1.09	1.08	1.08	1.07	1.06	1.05
17.00	1.13	1.12	1.12	1.11	1.10	1.09	1.08
17.50	1.16	1.16	1.15	1.14	1.13	1.12	1.11
18.00	1.20	1.19	1.18	1.17	1.16	1.15	1.14
18.50	1.23	1.22	1.22	1.21	1.20	1.18	1.17
19.00	1.26	1.26	1.25	1.24	1.23	1.22	1.21
19.50	1.30	1.29	1.28	1.27	1.26	1.25	1.24

Table VI. Brix Factors to Convert Brix Solids to Actual Solids

D.E.	Factor	D.E.	Factor
30.0	0.9685	69.0	0.9910
36.0	0.9720	75.0	0.9930
43.0	0.9760	79.0	0.9940
50.0	0.9800	85.0	0.9950
64.0	0.9880	93.0	0.9960

All of the six hydrolyzates shown in Table I were diluted to approximately 17.0, 20.0, and 23.0% solids and analyzed as follows:

A. Actual solids by the Filter Cel-vacuum oven procedure (7).

B. Brix solids from specific gravity obtained by a pycnometer, 20°/20° C.

The actual data obtained on one of the dilutions are shown in detail:

Corn Sirup. 56.8 D.E.; ash 0.41% D.E.; 57.0 ash-free D.E.

A. Dry substance on sirup. 19.658, 19.677, 19.677, 19.659%. Average 19.668% (each value shown average of duplicates).

Ash-free dry substance — 19.668 × 0.9959 = 19.587%

B. Specific gravity 20°/20° C. 1.08290; 1.08293; 1.08291; 1.08291. Average 1.08291 (each value shown average of duplicates).

Ash-free specific gravity. 1.08291 — (0.00088 × 0.41) = 1.08255

Corresponding Brix 20°/20° C. = 19.908

Ratio $\frac{19.587}{19.908} = 0.984$

This factor shows that if a corn sirup 56.8 D.E. were diluted to 20.00 Brix, the actual solids would be obtained by multiplying 20.00 by 0.984 or 19.68%.

At least two sets of data, as shown above, were obtained on each sirup; however, four sets were obtained on sirups with D.E.'s of 68.3, 84.3, and 91.3. The emphasis on the latter sirups was deemed necessary, as the plot of factor *vs.* ash-free D.E. showed a marked change in the slope of the curve in this area. The curve between 30 and 69 D.E. is essentially a straight line, which is in accord with unpublished chromatographic data on the composition of acid-converted starch hydrolyzates. Above 69 D.E. the curve levels off, showing little increase in slope above 90 D.E. This condition would be expected from our present knowledge of the hydrolysis of starch at this level of D.E. Essential points on the graph are shown in Table VI.

Table VII. Additions in D.E. to Compensate for Overestimation of Solids by Brix Hydrometer

D.E.	D.E. Addition	D.E.	D.E. Addition
30.0	0.98	70.0	0.63
35.0	1.02	72.0	0.57
40.0	1.06	74.0	0.54
45.0	1.05	76.0	0.51
50.0	1.02	78.0	0.49
55.0	0.96	80.0	0.47
60.0	0.86	82.0	0.45
62.0	0.82	85.0	0.43
64.0	0.77	87.0	0.41
66.0	0.72	90.0	0.40
68.0	0.66	93.0	0.37

Determination of Dextrose Equivalents

The Lane and Eynon (3, 4) procedure for reducing sugars is the accepted method in the wet milling industry.

The method for determining D.E., employing this procedure, the Brix hydrometer for the solids, and the factor for correction of the solids as developed here,

Table VIII. D.E. of Corn Sirups, Lane and Eynon Titration

(Brix 20° C., corrected for salt, sirup diluted 50.00 ml. to 500.0 ml.)

Titn., Ml.	Brix						
	17.00	18.00	19.00	20.00	21.00	22.00	23.00
10.0	67.0	63.2	59.7	56.6	53.8	51.2	48.8
10.5	63.9	60.3	56.9	54.0	51.3	48.8	46.5
11.0	61.1	52.6	54.4	51.6	49.0	46.6	44.4
11.5	58.6	55.2	52.2	49.4	46.9	44.6	42.6
12.0	56.2	53.0	50.0	47.4	45.0	42.8	40.8
12.5	54.0	50.9	48.1	45.5	43.2	41.1	39.2
13.0	52.0	49.0	46.2	43.8	41.6	39.6	37.7
13.5	50.1	47.2	44.6	42.2	40.1	38.2	36.4
14.0	48.3	45.6	43.0	40.8	38.7	36.8	35.1
14.5	46.7	44.1	41.6	39.4	37.4	35.6	33.9
15.0	45.2	42.7	40.2	38.1	36.2	34.5	32.8
15.5	43.8	41.3	39.0	36.9	35.1	33.4	31.8
16.0	42.5	40.1	37.8	35.8	34.0	32.4	30.8
16.5	41.3	38.9	36.7	34.7	33.0	31.4	29.9
17.0	40.1	37.7	35.6	33.7	32.1	30.5	29.1
17.5	39.0	36.6	34.6	32.8	31.2	29.7	28.3
18.0	37.8	35.6	33.7	31.9	30.3	28.9	27.6
18.5	36.8	34.7	32.8	31.1	29.5	28.1	26.8
19.0	35.9	33.8	32.0	30.3	28.8	27.4	26.1
19.5	35.0	33.0	31.2	29.5	28.1	26.7	25.5
20.0	34.1	32.0	30.4	28.8	27.4	26.1	24.8

Table IX. D.E. of Corn Sugar Sirups, Lane and Eynon Titration

(Brix 20° C., corrected for salt, sirup diluted 25.0 ml. to 500.0 ml.)

Titn., Ml.	Brix						
	17.00	18.00	19.00	20.00	21.00	22.00	23.00
10.2	94.1
10.4	92.3
10.6	90.6
10.8	93.3	88.9
11.0	91.6	87.3
11.2	90.0	85.8
11.4	93.0	88.4	84.3
11.6	91.4	86.9	82.9
11.8	94.7	89.9	85.5	81.6
12.0	93.1	88.4	84.1	80.2
12.2	91.6	86.9	82.7	78.9
12.4	90.2	85.6	81.4	77.6
12.6	93.8	88.7	84.3	80.1	76.4
12.8	92.3	87.4	83.0	78.9	75.2
13.0	90.9	86.1	81.7	77.7	74.1
13.2	...	94.9	89.5	84.8	80.5	76.5	73.0
13.4	...	93.4	88.2	83.5	79.3	75.4	71.9
13.6	...	91.9	86.9	82.3	78.1	74.3	70.9
13.8	...	90.7	85.6	81.1	77.0	73.3	69.9
14.0	...	89.5	84.5	80.0	75.9	72.3	68.9
14.2	93.7	88.2	83.3	78.9	74.9	71.3	68.0
14.4	92.4	87.0	82.2	77.8	73.9	70.3	67.1
14.6	91.2	85.8	81.0	76.7	72.8	69.4	66.7
14.8	90.0	84.7	80.0	75.7	71.9	68.5	65.3
15.0	88.8	83.6	78.9	74.7	71.0	67.6	64.5
15.5	86.8	80.9	76.4	72.4	68.8	65.5	62.5
16.0	82.3	78.4	74.1	70.2	66.7	63.5	60.6
16.5	80.8	76.1	71.9	68.1	64.8	61.7	58.8
17.0	78.5	73.9	69.8	66.2	62.9	59.9	57.2
17.5	76.2	71.8	67.9	64.4	61.2	58.3	55.6
18.0	74.3	69.5	66.1	62.7	59.6	56.7	54.1
18.5	72.2	68.1	64.4	61.0	58.0	55.2	52.7
19.0	70.4	66.3	62.8	59.5	56.6	53.8	51.4
19.5	68.6	64.7	61.2	58.0	55.1	52.5	50.0
20.0	67.0	63.1	59.7	56.6	53.8	51.2	48.8

is given in the equation which follows for corn sirup of 20 Brix with a dilution of the sirup, 50.0 ml. to 500.0 ml.

$$\frac{12.02/\text{ml. titration}}{50.0/500.0 \times 20.00 \times 1.08096 \times \text{factor}} \times 100$$

This calculation requires the use of a factor, which is specific for the D.E. before D.E. is known. This impasse is avoided by obtaining D.E. additions in advance and applying the required addition to a given calculation based on Brix solids. The additions to be applied were derived from the data which appear in Table VI and are shown in Table VII.

The additions of D.E. required to correct for the overestimation of solids in corn sirup and corn sugar by the Brix hydrometer are surprising and unexpectedly simple. The data are particularly useful in the analysis of such sirups as first and second greens obtained in the

manufacture of dextrose. Hydrol, corn sugar molasses, or second greens will vary in D.E. within the range of 68 to 75, and the salt within the range of 6 to 10% dry basis. Thus, the D.E. of the sugar itself with range from 75 to 88 D.E. obtained by the vacuum procedure for solids and the arbitrary addition of 0.5 D.E. to the D.E. obtained by the above method of analyses will be within 0.1 D.E., well within the tolerance of the procedure for the reducing sugar determination.

Laboratories in the corn wet milling industry determine a very large number of D.E.'s daily, particularly if refined corn sugar or dextrose is manufactured. To save time and expedite results to the plant, most laboratories have large tables with coordinates of Brix and "milliliters of titration," which give the D.E. for the specific condition involved. In the tables, the Brix values, 20° C., have been

adjusted for the effect of salt and the D.E. value obtained carries the correction for the overestimation of solids by the Brix hydrometer. Abbreviated forms of the two tables are shown (Table VIII for corn sirup and Table IX for corn sugar sirups).

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PLANT PECTIN ANALYSIS

Determination of Pectic Substances by Paper Chromatography

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Physical methods of purifying several fruit and vegetable pectinic acids resulted in polymers containing nonuronide sugar units. The sugars, α -D-galactose, β -L-arabinose, α -L-rhamnose, and α -D-xylose, were separated from the hydrolysis products of apricot pectin by thick-paper chromatography and identified by comparing their x-ray powder diagrams with those of authentic specimens. Two widely different molecular species of galacturonic acid-containing polymers were separated from apricot polysaccharides. Attempts to resolve copper-purified apricot pectinic acid by further copper precipitation, dialysis, paper electrophoresis, and fractionation of the acetate into different molecular species were unsuccessful. Nonuronide sugars appear to be incorporated into the galacturonan molecule of pectic substances from most plant materials.

THE PECTIC POLYSACCHARIDES exist in plants in close physical union with araban and galactan. This association is so firm that upon extraction of polysaccharides from plants, a triad containing galacturonan, araban, and galactan is obtained. Because of the facility of paper chromatography for detection of small amounts of sugars in complex polymers, researches on composition of pectic substances have been stimulated in recent years.

Reports of composition of most fruit and vegetable pectins indicate that they are mixtures of polysaccharides or molecules apparently containing sugar residues other than galacturonic acid (1, 4, 5, 7, 13, 16, 20, 24). Many attempts to isolate a pure galacturonan from a wide variety of plant materials were unsuccessful unless strong chemical procedures were applied, which were suspected of hydrolyzing glycosidic link-

ages and degrading one or more of the carbohydrate polymers.

The question of composition and structure of undegraded pectic substances appears to be open to further investigation. Mainly, the unanswered question is whether pectin is a pure galacturonan admixed with the carbohydrates araban and galactan, or whether the so-called galacturonan is a complex carbohydrate containing some nonuronide sugars as part of the molecule.

The experiments reported here indicate the difficulties involved in attempts to prepare pure galacturonan from some natural materials and suggest that families of soluble complex galacturonic acid-containing polysaccharides occur in some plants.

Experimental

Analyses. Pectic substances isolated by precipitation from aqueous solution

by ethyl alcohol were dried in vacuo at 60° C., ground to pass 60-mesh, humidified to an equilibrium moisture content of about 10%, and analyzed. Methods of analyses used were mainly those described by Owens *et al.* (27). Carbo-methoxy (ester methoxyl) analyses were made by saponification, moisture analyses by oven drying in vacuo, ash analyses by incineration at 600° C., anhydro-uronic acid analyses by a colorimetric carbazole method (74), and acetyl analyses by a hydroxamic acid color reaction (75). Rotations are specific rotations at 25° C. using the D-line of sodium. Intrinsic viscosities are those extrapolated to infinite dilution. Quantitative sugar analyses were done from reflection densities of colored spots on paper chromatograms (77).

Qualitative Chromatography. Polyuronides (1.0 gram in 50 ml. of 1N sulfuric acid) were boiled under reflux