A RAPID METHOD TO DETERMINE PERCENT FERMENTABLES[1]

YEE S. KIM

ABSTRACT

A new method to determine percent fermentables (% FE) of high-fermentable corn syrup was investigated. In this procedure a colorimetric method was used to estimate unfermentable carbohydrate residues. Optimum conditions for complete fermentation of 10% sugar solutions were established, and under these conditions the percent fermentability of various sugars was determined. The percent fermentable equivalent values (% FE = fermentable sugars as a percentage of total sugars) thus obtained were compared with the method of the Corn Industries Research Foundation. The two methods agreed within ± 1% FE values.

Throughout the food-processing industry the need for accurate determination of fermentable sugars arises frequently, and various methods appropriate for each type of industry are available for the determination of percent fermentables (% FE) of carbohydrate material (fermentable sugars as a percentage of total sugars). The ASBC method (1) is widely used in the brewing industry, and the CIRF method (2) is employed by all the corn-refining industries. Both of these methods are, of course, variations of the AOAC method (3), and use Plato's table and specific gravity to estimate the dry substance in the unfermented residues.

The AACC method, developed by Sandstedt and Blish (4), is a manometric method to determine gassing power of fermentable sugars in dough, and is often used in the baking industries. Recently, Kleber *et al.* (5) described a paper-chromatographic method to determine mono-, di-, and tri-saccharides and thereby to calculate percent fermentables of wort.

Although the paper-chromatographic methods (5) or column chromatographic methods (6,7) could offer theoretically accurate means to determine fermentable sugars, separation of similar sugars, such as maltose from isomaltose or maltotriose from panose, etc., is very difficult; and furthermore, correlation of oligosaccharides with actual fermentability has yet to be established. In addition, paper chromatography is somewhat difficult to adapt in a production plant as a routine analytical method.

[1]Manuscript received July 20, 1965. Contribution from the Biology Department, State University of New York at Buffalo, Buffalo, N.Y. This work was performed largely in the laboratory of the Union Starch and Refining Co., Granite City, Ill.

The ASBC method and CIRF method have been critically examined and a number of shortcomings of each method have been brought up.[2] When the ASBC method was directly applied to corn syrup, Plato's table was found quite inadequate, especially when inorganic salts are present. Because of the variation in strains of yeast used by brewers, the percent fermentability values of a sugar vary from brewer to brewer. On the other hand, the CIRF method has been seriously criticized for its evaporation step to remove alcohol and other fermented products. Theoretically, alcohols form azeotropes, and complete removal by evaporation is impossible. Furthermore, identities of the residue after evaporation have not been established.

Aside from the above theoretical drawbacks, these methods are very time-consuming and require the utmost analytical skill to accomplish an accurate determination. Generally, reproducibilities of ± 2% FE were claimed by a skilled analyst and often ± 5 to 10% FE's were reported by unskilled technicians using either the ASBC or the CIRF method.

In this paper, a simple and efficient method to determine percent fermentables of corn syrup is described. The procedure is very similar to the CIRF method, but in order to improve the accuracy and to simplify the method the amount of unfermentable sugar residue is determined by the phenol-sulfuric acid method (8) instead of specific gravities and Plato's table. An unskilled technician could easily analyze 20 samples a day with reproducibility of less than ± 2% FE's using this method, while the same technician could analyze five or six samples a day with the same or less accuracy using the CIRF method.

Materials and Methods

Materials.

1. Fleischmann's active dry yeast obtained from Standard Brands, Inc., New York, N.Y.: 10% (w./v.) suspension in distilled water prepared as instructed by the manufacturer on the label.

2. Nutrient solution as described in (2): dissolve 32 g. of potassium orthophosphate monohydrogen (K_2HPO_4), 40 g. of ammonium orthophosphate dihydrogen ($NH_4H_2PO_4$), and 20 g. of DIFCO Yeast Extract in approximately 500 ml. of distilled water, and dilute to 1,000-ml. volume. Store in refrigerator and discard if mold growth appears.

3. Phenol sulfuric acid, and apparatus described in reference 8.

4. Anhydrous dextrose, DIFCO maltose, and corn syrups of known percent fermentabilities and known dry substance weights.

[2]Analytical Subcommittee Discussions. Corn Industries Research Foundation, Washington, D.C., 1959 (unpublished).

5. Casein: reagent grade.

6. Shaker with constant-temperature water bath (100 strokes per min., about 3.5 cm. per stroke and 35°C.).

Methods. In a 100-ml. volumetric flask, 10.0 g. (d.s.b.) of sugar, an appropriate amount of yeast suspension and nutrient solution were placed, and the volume was made up with distilled water. The entire mixture was then transferred to a 250-ml. Erlenmeyer flask fitted with a rubber stopper with small holes, and incubated at 35°C. on a shaker for an appropriate length of time.

To establish optimum conditions to achieve complete fermentation of the 10% sugar solution at 35°C., the amounts of yeast suspension and nutrient solution, and length of fermentation time were varied. For determination of the amount of yeast for the complete fermentation, 3 ml. of nutrient solution was used and fermentation was carried out for 4 hr. In experiments to determine the nutrient solution requirement, 10 ml. of the yeast suspension was fermented for 4 hr. In the experiments to determine optimum length of fermentation time, 1 ml. of nutrient solution and 10 ml. of yeast suspension were used and the fermentation was stopped at 1-hr. intervals up to 5 hr.

After each fermentation, the reaction mixture was centrifuged for 30 min. at 500 × *g*. The supernatant substance was diluted appropriately and treated with phenol and sulfuric acid as described by Dubois *et al.* (8). Absorbance was read at 490 mμ against a blank prepared by an identical method except that distilled water was used in place of sugar solution. From a standard curve, the unfermented sugar left in the supernatant was estimated, and % FE was calculated as follows:

$$\%\ \text{FE} = 100 - \left(\frac{\text{unfermented sugar}}{\text{total sugar at beginning}} \times 100\right)$$

To determine the degree of interference by protein impurities in the phenol-sulfuric acid method, various amounts of casein were added to a known amount of sugar solution; then fermentation and dilution procedures were carried out as above, and differences in absorbance values of the sugar alone and the sugar in the presence of casein were determined.

In experiments designed to compare the new method with the CIRF method and to check the reproducibility of the new method, various sugars of known % FE's were dissolved in distilled water to make about a 30% solution. About 30 g. of the solution was accurately weighed out, and fermentation was carried out under the optimum condition established by the preceding experiments; i.e., 9–10 g. of sugar with 10 ml. of yeast suspension and 1 ml. of nutrient solution per 100 ml. final volume of the reaction mixture were fermented on a shaker at 35°C. for 4 hr. In this case the initial total sugar was estimated by both the oven-filter aid method (2) and the phenol-sulfuric acid method.

Results and Discussion

Optimum Conditions to Achieve Complete Fermentation. Anhydrous dextrose and 85.2% FE corn syrup were fermented under various conditions. Anhydrous dextrose should be theoretically 100% FE. The value of % FE of the syrup was determine by both the CIRF method and paper-chromatographic method (5).

Tables I, II, and III show % FE values calculated from the unfermented sugars left after the fermentation under the various conditions.

TABLE I

AMOUNT OF YEAST REQUIRED FOR COMPLETE FERMENTATION[a]

AMOUNT OF YEAST	ANHYDROUS DEXTROSE	85.2% FE CORN SYRUP
ml.	*% FE*	*% FE*
10.0	100.0	85.3
7.5	100.0	86.2
5.0	94.5	81.7
2.5	86.3	74.0

[a] The final volume of 100 ml. reaction mixture contained 3 ml. of nutrient solution and 10.0 g. of sugar. The fermentation was carried out on a shaker at 35°C. for 4 hr.

Table I indicates that 7.5 ml. of the yeast suspension fermented 100% of the theoretically fermentable sugars present in 10.0 g. of each sample within 4 hr. at 35°C.

TABLE II

AMOUNT OF NUTRIENT SOLUTION REQUIRED TO KEEP 10 ML. OF YEAST ACTIVE[a]

NUTRIENT SOLUTION	ANHYDROUS DEXTROSE	85.2% FE CORN SYRUP
ml.	*% FE*	*% FE*
3.0	100.0	85.5
2.0	99.5	86.2
1.0	99.8	85.2

[a] The final volume of 100 ml. reaction mixture contained 10 ml. of yeast solution and 10.0 g. of sugar. Other conditions were the same as in Table I.

Table II indicates that 1 ml. of nutrient solution was sufficient to keep 10 ml. of yeast suspension active for 4 hr.

Similarly, Table III shows that with 10 ml. of yeast suspension and 1 ml. of nutrient solution, anhydrous dextrose was completely fer-

TABLE III
TIME REQUIRED FOR COMPLETE FERMENTATION[a]

FERMENTATION TIME	ANHYDROUS DEXTROSE	85.2% FE CORN SYRUP
hr.	*% FE*	*% FE*
1	98.0	...
2	100.0	75.0
3	100.0	85.6
4	100.0	85.2
5	100.0	85.4

[a] The final volume of 100 ml. reaction mixture contained 1.0 ml. of nutrient solution, 10.0 ml. of yeast suspension and 10.0 g. of sugar. The fermentation temperature was 35°C.

mented within 2 hr. and 85.2% FE corn syrup within 3 hr. From these results, optimum conditions were established as follows: 10 g. of sugar, 10 ml. of yeast suspension, and 1 ml. of nutrient solution were mixed and made up to 100 ml. with distilled water. The reaction mixture was fermented for 4 hr. at 35°C. with shaking, 100 strokes per min.

Effect of Protein Contamination on the New Method. Usually, high-fermentable sugars are made by enzymatic methods, and consequently contain a certain amount of proteinaceous material.

In a typical experiment, 0.5 and 1.0% casein (which is much higher than the actual protein levels of average corn syrup) increased absorbance reading by 0.007 and 0.013, respectively. An absorbance reading of 0.013 corresponded to less than 0.2% FE value in a typical run of the method. Hence, the normal amount of proteinaceous material present in corn syrup should not interfere with the method. As for the possible interference by other material, the original authors of the phenol-sulfuric acid method discussed superiority of the method in comparison with the anthrone method, etc.

Comparison with CIRF Method and Reproducibility of the New Method. Weighing out exactly 1 g. of a sugar is very tedious and is inaccurate, especially with viscous corn syrup. In experiments for comparison with the CIRF method and in reproducibility tests, about 30 g. of 30% sugar solution (about 9 to 10% in the final volume) were fermented under the optimum condition established in the preceding section. Dry-substance weights in the 30 g. of sugar solution were determined by both the oven-filter acid method (2) and the phenol-sulfuric acid method. Dry-substance values obtained by both methods agreed very well.

As shown in Tables IV and V, % FE values obtained by the CIRF method and the new method agreed within ± 2% FE, and reproducibilities for five separate runs were ± 1% FE.

TABLE IV

COMPARISON BETWEEN THE NEW METHOD AND THE CIRF METHOD[a]

TEST SAMPLES	CIRF METHOD	NEW METHOD
	% FE	*% FE*
Anhydrous dextrose	98.0	99.2
Difco maltose	97.4	96.7
Corn syrup No. 1	85.2	85.3
Corn syrup No. 2	80.0	83.7
Corn syrup No. 3	69.7	69.5

[a] The fermentation was carried out under the established conditions: 10% sugar, 1 ml. nutrient solution 10 ml. yeast suspension, final volume 100 ml., incubation at 35°C. for 4 hr.

In Table IV, it is interesting to note that % FE values of anhydrous dextrose were 98.0 by the CIRF method and 99.2 by the new

TABLE V

REPRODUCIBILITY TEST[a]

TEST RUN No.		TEST SAMPLES: Anhydrous Dextrose	TEST SAMPLES: 85.2% FE Corn Syrup
		% FE	*% FE*
1		100.0	85.3
2		100.0	85.5
3		99.5	85.6
4		99.2	85.2
5		99.8	86.0
	Average	99.7	85.5
	Standard Deviation	0.4	0.3

[a] The fermentation conditions were the same as in Table IV. Each test run represents an independent performance of the entire procedure.

method. As shown in Table V, % FE values of anhydrous dextrose by the new method were invariably above 99. In Table IV, % FE values of maltose were considerably lower than the theoretical value. However, the Difco maltose used here showed maltotriose, maltotetraose, and higher oligosaccharide spots on paper chromatogram. These high-molecular-weight sugars are not readily fermentable, and this can account for the low % FE values.

Applicability of the New Method. Although the new method was as good as or better than the CIRF method for accuracy and reproducibility, when the new method was applied to high-feregmentable sugars the degree of accuracy decreased with low-fermentable sugars. With low-fermentable sugars (40% or below), the levels of unfermented sugars were very high and had to be diluted to greater ratios before a reasonable range of the absorbance reading could be obtained. Consequently, errors may have been magnified by the dilution factors. An inherent source of error was the difference in absorbance characteristics of various species of saccharides in the phenol-sulfuric acid method. However, there is no practical value in determining fermentability of low-FE corn syrup.

Finally, the new method was developed for high-fermentable corn syrup. However, this method with slight modifications should be applicable to other types of sugars, such as low-FE syrup or wort in the brewing industry.

Acknowledgment

The author wishes to thank T. Reid for his expert aid in performing the experiments.

Literature Cited

1. American Society of Brewing Chemists. Methods of analysis (6th ed.). The Society: Madison, Wis. (1958).
2. Corn Industries Research Foundation. Standard analytical methods (1st ed.). The Foundation: Washington D.C. (1961).
3. Association of Official Agricultural Chemists. Official methods of analysis (8th ed.). The Association: Washington, D.C. (1955).
4. Sandstedt, R. M., and Blish, M. J. Yeast variability and its control in flour gassing-power tests. Cereal Chem. **11:** 368–383 (1934).
5. Kleber, I. W., Runkel, U. D., and Seyfarth, I. Determination of fermentable sugars by paper chromatography. Brauwissenschaft **14:** 65–70 (1961).
6. Patterson, S. J., and Savage, R. I. Selective desorption from carbon columns for the determination of dextrose and maltose in starch conversion products. Analyst **82:** 812–818 (1957).
7. Patterson, S. J., and Buchan, J. L. The use of selective desorption from carbon columns for determination of maltotriose in starch conversion products. Analyst **86:** 160–163 (1961).
8. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:** 350–356 (1956).