

## Dextrose Determination in Honey: A Rapid Photometric Determination

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**The granulating tendency of honey may be predicted by the dextrose-water ratio. The reducing disaccharides in honey complicate the determination of dextrose by routine procedures. True glucose can be determined in honey with a glucose oxidase reagent in which the contaminant *alpha*-glucosidase activity is inhibited. Accuracy and precision of the method are comparable with those of the selective adsorption procedure, and it is more suitable for routine control use.**

The tendency for many types of honey to granulate causes considerable vexation and some economic difficulty in honey marketing. Most honeys are supersaturated with dextrose and will precipitate it as the monohydrate; considerable variation in the speed and completeness of granulation is noted among different honey types (1). Such granulation can be sufficiently delayed (not prevented) by suitable processing of honey, but recognition of granulation-prone honey

before processing would allow more intelligent blending of lots for the desired properties. The texture of the commercial product known as "honey spread" or finely granulated honey must be within acceptable limits during its shelf-life. A knowledge of the dextrose content of commercial lots of honey would be of considerable value for this use.

Problems encountered in the determination of dextrose in honey have been discussed previously (2-5). The presence of 3-10% of reducing aldose and ketose disaccharides (6) eliminates any non-specific methods for determination of reducing sugar without class separation of the sugars, even though they may differentiate between ketose and aldose. Chromatographic methods have been described which separate the

This paper was presented at the Seventy-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 14-17, 1963, at Washington, D.C.

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monosaccharides from each other and other sugars prior to microanalysis (7), or separate the monosaccharides as a group from other sugars followed by relatively specific semi-micro analysis. The latter is the first action AOAC selective adsorption (SA) method for honey analysis. Recently we showed (5) the essential accuracy of dextrose and levulose determination in honey by this procedure.

Even if this procedure was used only for dextrose determination, it is too time-consuming and complex for routine control work. The paper chromatographic method described by McDonald (7) for determination of dextrose (and levulose) in honey requires considerable time for paper chromatography before the analysis. No data are available concerning its accuracy in honey analysis.

Glucose oxidase has been used as a specific reagent for glucose determination, particularly in clinical work and also in agricultural analysis. It had not been realized until recently, however, that commercial preparations of glucose oxidase may be significantly contaminated with invertase or  $\alpha$ -glucosidase; this may in some cases lead to considerable error in glucose estimation, especially where carbohydrate-splitting enzymes are assayed. Glucose oxidase reagents have recently been described (8-10) in which the contaminant enzyme activities are inhibited by tris-(hydroxymethyl) aminomethane (Tris), which permits their use to assay glucose-producing carbohydrases.

We have applied such a reagent to the direct determination of dextrose in honey. By suitable replication and inclusion of standards, the true dextrose content of honey may be determined photometrically with an accuracy and reproducibility comparable to that of the SA method, and much more rapidly. There is some indication that it is more accurate than the SA method.

### Experimental

#### Apparatus

Photoelectric photometer with 500  $m\mu$  interference filter, accepting test tubes or 1 cm cuvettes. (Lumetron Model 402-E, Photovolt Corp.,<sup>2</sup> New York, N.Y., or equivalent.)

#### Reagents

(a) *Tris-(hydroxymethyl)aminomethane*.—“Sigma 7-9” (Sigma Chemical Company, St. Louis, Mo.).

(b) *Glucose oxidase*.—Crude. (Sigma Chemical Company.)

(c) *Peroxidase*.—Type I, Horseradish. (Sigma Chemical Company.)

(d) *o-Tolidine dihydrochloride*.—White label. (Eastman Organic Chemicals Dept., Distillation Products Industries, Rochester, N.Y.)

(e) *Dextrose*.—Purified anhydrous dextrose (National Bureau of Standards, Standard Sample 41).

(f) *Tris buffer, pH 7.6*.—Add 384 ml 0.8M HCl to 500 ml 0.8M Tris solution, adjust to pH 7.6 if necessary, and make to 1 L.

(g) *Glucose oxidase reagent*.—Filter a solution of 800 mg glucose oxidase and 16 mg peroxidase in 200 ml Tris buffer into an amber glass bottle. Filter a solution of 180 mg *o*-tolidine dihydrochloride in 260 ml water into the same bottle. Mix, and store in refrigerator overnight before use. Reagent is stable under refrigeration for at least 6 weeks.

#### Procedure

Weigh (to 0.1 mg) 0.98-1.02 g honey. (The following treatment must be carried through the heating and cooling procedure with one sample at a time immediately after it is first dissolved.) Dissolve the sample in water, transfer into a 100 ml volumetric flask, and make to volume. Dilute a 5.00 ml aliquot to 100 ml in a volumetric flask and place three 18 mm test tubes containing 2.00 ml aliquots of the diluted solution into a boiling water bath for 2 minutes. Cool in a water bath, wipe dry, and allow to come to room temperature.

Dissolve 30-35 mg (weighed to 0.1 mg) standard dextrose in water and make to 200 ml in a volumetric flask. Use 2.00 ml aliquots for standards, either heating them as above or letting the solution stand for at least 2 hours before use. Intersperse 2 test tubes of standard between each 3 tubes containing aliquots of a honey sample. When not more than 60 tubes (12 samples plus standards) have been processed, add 5.00 ml glucose oxidase reagent (brought to room temperature) to the tubes at 1 minute intervals, to allow time later to acidify and read them. After the first tube has stood at room temperature for 60 minutes, add 0.1 ml 4N HCl to the first 2 tubes,

<sup>2</sup> Mention of company and trade names does not imply endorsement by the Department over others of a similar nature not named.

immediately mixing each. Determine the absorbance of the first tube at 500  $m\mu$  by the displacement bar technique (11). Next add acid to the third tube, mix, replace in the rack, and read the second tube. Then acidify the fourth, mix and replace, and read the third tube, and so on. About 1 minute is required for acidification and reading. The color should be a clear violet with no brown cast.

To determine absorbance in 1 cm cuvettes, allow sufficient time when adding reagent to permit later transfer and measurement. Alternatively, read the samples and standards 1 hour after acidification, after which time the color is stable.

Within the ordinary range of dextrose content of honey, dextrose in the 2 ml aliquot may be calculated by direct reference to the average absorbance of the adjacent standard tubes.

#### Calculation

Absorbance of sample  $\times$  (mg dextrose weighed)/(absorbance of dextrose)  $\times$  (1000)/(mg sample weighed) = % dextrose in sample.

#### Use of Commercially Available Reagent

*Glucostat reagent.*—Prepare the reagent as directed by the manufacturer (Worthington Biochemical Corp., Freehold, N.J.) by their method 1A.

#### Procedure

Prepare honey solutions and standards as described above. To 2 ml samples in test tubes, with tubes of standard dextrose solution interspersed, add 8 ml Glucostat reagent at 15-second intervals. After the first tube has incubated at room temperature for exactly 10 minutes, add, with the same timing, 1 drop 4*N* HCl to each tube, with immediate mixing. After 5 minutes, determine the absorbance of samples and standards in 1 cm cuvettes at 450  $m\mu$ .

#### Results and Discussion

The analyses of 12 honey samples for dextrose by the selective adsorption method and by the two glucose oxidase procedures are shown in Table 1. Variance due to samples was found to be highly significant ( $p = .005$ ), while that due to methods was just significant at the .05 level of probability. Duncan's Multiple Range Test (12) showed that the means for the two glucose oxidase methods differ significantly ( $p = .05$ ) but

neither differs significantly from the mean of the selective adsorption method, which falls between them.

**Table 1. Dextrose content of honey by three procedures**

Sample	Glucose Oxidase Tris %	Selective Adsorption %	Glucostat %
Eastern fall flower	35.26	34.62	34.30
Eastern fall flower	34.98	34.25	34.72
Clover	34.56	34.29	34.10
Rape	36.57	35.85	35.46
Polish rape	37.83	37.64	37.95
Gallberry	28.88	28.40	29.18
Tulip tree	26.60	27.47	27.15
Basswood	31.20	30.72	29.83
Sweet clover-alfalfa	34.92	35.10	34.98
Alfalfa	33.61	33.04	32.20
Sweet clover	33.71	33.66	33.67
Safflower	34.75	34.56	34.16
Mean value	33.57	33.30	33.14

Since the two glucose oxidase procedures do give results differing from each other, one higher and one lower than the SA method, it is of interest to determine which of the two enzyme procedures may be more accurate. In a study of the accuracy of the SA method (5), it was found that unanalyzed material in the monosaccharide fraction obtained by the charcoal column separations averaged 0.40% of the entire sample of honey, and polarimetric measurement indicated that this was largely dextrose. The glucose oxidase-Tris method, here shown to give results averaging 0.27% higher than the SA method, would thus appear to be more accurate than the Glucostat procedure; indeed this reasoning implies that it is more accurate than the analytical method used for dextrose determination (hypiodite oxidation of glucose corrected for fructose oxidation) in the SA method. The Glucostat method, however, does appear to be sufficiently accurate for routine control applications.

Five samples of a honey were weighed and analyzed with the glucose oxidase-Tris reagent by displacement photometry. Results were 35.86, 36.17, 35.58, 35.32, and 36.25%,

giving a standard deviation of 0.39. This may be compared with a value of 0.38 previously reported (4) for dextrose by the SA method, with 4 samples analyzed by 3 analysts in one laboratory.

**Table 2. Effect of pretreatment on absorbance values of freshly-dissolved dextrose standards**

Treatment	Absorbance
21.1 mg/100 ml Concentration	
Analyzed at once	0.294
Boiled 2 min. before analyzed	0.334
Unboiled, analyzed after 2 hr	0.337
Boiled, analyzed after 2 hr	0.336
20.0 mg/100 ml Concentration	
Analyzed at once	0.317
Boiled 2 min.	0.338
Boiled 5 min.	0.340
Boiled 10 min.	0.337

Frequent erratically high dextrose values were traced to the use of dextrose standard solutions immediately after solution. Heating the standard tubes as described, or allowing them to stand long enough, yielded concordant results. Heating the honey sample tubes was originally intended to inactivate enzymes, but it probably also accelerates mutarotation. Table 2 shows the effect of pretreating the dextrose solution on the absorbance values obtained. The last four

values were obtained by heating dextrose solutions before the final dilution, to eliminate the effect of solvent evaporation. The difference in color yield seen for the two weighings in the table (done on different days and probably due to different room temperatures) is compensated for in the analytical procedure by including standards in every set of analyses.

#### Acknowledgment

Statistical counsel by J. N. Boyd is acknowledged with appreciation.

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