NOTE

# A Modified Method for Total Carbohydrate Analysis of Glucose Syrups, Maltodextrins, and Other Starch Hydrolysis Products

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Use of an anthrone reaction for the analysis of carbohydrates has found widespread acceptance since its first qualitative usage was reported by Dreywood (1946). The basis for the reaction lies in the ability of a carbohydrate, in the presence of acid and heat, to form a furfural derivative followed by reaction with anthrone to yield a characteristic blue-green color (Sattler and Zerban 1948). Sensitivity and simplicity without the need for prior hydrolysis are listed among the major advantages (Koehler 1952). Further, Buckee and Hargitt (1978) reported the anthrone reaction to be superior to other total carbohydrate assays in determining the residual carbohydrate in beers.

The early quantitative methods involved the solubilization of anthrone in concentrated sulfuric acid, followed by its addition to an appropriately diluted sample (Morris 1948, Sattler and Zerban 1948). Because the aqueous sample volume is generally equivalent to one-half the anthrone reagent volume, heat-of-mixing was considered adequate for color formation, but later authors reported improved efficiency by adding a heating step in a boiling water bath (McCready et al 1950, Koehler 1952). A major difficulty with these approaches is the inability to control the amount of heat initially generated by the addition of anthrone reagent and its subsequent effect on the overall results. In an attempt to improve consistency by more evenly distributing this heat, Morris (1948), McCready et al (1950), and Koehler (1952) included specific recommendations for controlled addition of anthrone reagent.

In this laboratory, repeated analyses of maltodextrin preparations and glucose syrups by methods utilizing the anthrone reagent in concentrated sulfuric acid (McCready et al 1950, Koehler 1952) yielded results that were not consistent. Despite close adherence to the recommendations and suggestions put forth by the previous authors (Morris 1948, McCready et al 1950, Koehler 1952) inconsistencies in apparent carbohydrate amounts for identical samples, attributed to uneven sample heating during anthrone reagent addition, continued to be a problem. Approaches based on an anthrone reagent prepared in a more dilute sulfuric acid (Fairbairn 1953) combined with the use of smaller sample volumes (Van Handel 1965) seemed most promising, but neither report proved entirely satisfactory for the analysis of maltodextrin preparations or glucose syrups.

The purpose of the present study was to describe a modified method of total carbohydrate analysis, based on the proposals of Fairbairn (1953) and Van Handel (1965), and to provide experimental evidence supporting the optimum conditions reported for this procedure. This method was designed to be suitable for the analysis of maltodextrins, glucose syrups, and other soluble starch products.

## MATERIALS AND METHODS

Lintner soluble starch (starch sample) and α-D(+)-glucose (glucose sample) were obtained from the Sigma Chemical Co. (St. Louis, MO). The maltodextrin sample was derived from the partial enzymatic hydrolysis of rice flour by a procedure developed in our laboratory (Brooks and Griffin, *unpublished*). Anthrone and concentrated sulfuric acid were purchased from the J. T. Baker Chemical Co. (Phillipsburg, NJ) and Fisher Scientific (Atlanta, GA), respectively.

Samples of glucose, maltodextrin, and starch were solubilized to yield approximately 0.8–1.0 mg of glucose per milliliter. Sulfuric acid and water were mixed in a 2.3:1.0 (v/v) ratio and allowed to cool. The modified anthrone reagent was prepared fresh daily by dissolving 0.1% (w/v) anthrone in the diluted sulfuric acid. All samples were heated in 16× 150 mm screw-cap test tubes to prevent evaporation. Six replicates were used for determination of color stability after heating, whereas all other analyses were performed in triplicate.

To determine the heating time required for maximum color formation, 25-μl aliquots of appropriately diluted glucose, maltodextrin, and starch were mixed with 3 ml of the modified anthrone reagent and placed in a boiling water bath. Samples were removed at fixed time intervals, cooled to room temperature in cold water, and the absorbance at 630 nm was recorded. Water blanks of 25 μl were treated similarly.

Stability of color formation after heating was evaluated by mixing glucose samples and blanks with anthrone reagent and heating for 5 min. After cooling in cold water for 5 min, absorbance measurements were recorded over time.

## RESULTS AND DISCUSSION

Table I shows the results of heating time on color formation in samples of glucose, maltodextrin, and starch. In all cases maximum color formation was achieved within 5 min, followed by significant absorbance losses with subsequent heating. These findings are in disagreement with those of Fairbairn (1953), who reported that maximum color intensity in glucose solutions is not obtained until after 12 min of heating, but these differences may be largely caused by the smaller sample quantities and volumes used in the current study. Citing less rapid color degradation after prolonged heating, Van Handel (1965) advocated treatment at 90° C for 20 min. Yadav et al (1969) also recommended heating in a constant temperature bath at 95° C for 20 min, but we favor a boiling water bath because it is more conveniently regulated, maximum color formation

TABLE I
**Anthrone Color Intensity Readings Based on Heating Time in a Boiling Water Bath**

| Heating Time (min) | Average Absorbance (AU) | | |
|---|---|---|---|
| | Glucose | Maltodextrin | Starch |
| 2 | 0.308 | 0.238 | 0.401 |
| 3 | 0.484 | 0.357 | 0.467 |
| 4 | 0.531 | 0.398 | 0.520 |
| 5 | 0.539 | 0.407 | 0.529 |
| 6 | 0.525 | 0.396 | 0.510 |
| 7 | 0.508 | 0.383 | 0.506 |
| 8 | 0.493 | 0.344 | 0.485 |
| LSD 0.05 | 0.012 | 0.005 | 0.006 |

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TABLE II
Anthrone Color Intensity Readings of Glucose Samples Based on Time after Heating in a Boiling Water Bath

| Time (min) | Average Absorbance (AU) |
|---|---|
| 5 | 0.496 |
| 10 | 0.509 |
| 15 | 0.514 |
| 25 | 0.515 |
| 35 | 0.514 |
| 45 | 0.511 |
| 65 | 0.506 |
| 85 | 0.501 |
| LSD 0.05 | 0.003 |

occurs more rapidly, and samples can easily be removed at the appropriate time before color degradation occurs.

Data on stability of color formation after heating are presented in Table II. Yadav et al (1969) reported that samples should be read within 15 min after heating but supplied no supporting evidence. In this study absorbance values were consistently found to increase 5 to 15 min after removal from the boiling water bath, but those values remained stable for at least the next 25 min. Clegg (1956) reported color stability up to 3 hr after heating, but our results show significant decreases in absorbance within 45 min.

Koehler (1952) reported the differences in heating time necessary for maximum color formation among different carbohydrates and speculated that the ease of formation of a furfural derivative is the regulating factor in the rate of color development. In the same report Koehler (1952) proposes that relative color intensity, with respect to a given quantity of carbohydrate, is dependent on the nature of that derivative. Because glucose syrups, maltodextrin preparations, and other starch products consist almost entirely of glucose and its oligomers, the optimum conditions listed in this study should be appropriate for these products.

Based on the results reported here, the following procedure is recommended. Three milliliters of freshly prepared anthrone reagent is added to 25 $\mu$l of sample diluted to yield approximately 0.5–2.0 mg of glucose per milliliter. After heating in a boiling water bath for 5 min, the samples are removed and cooled to room temperature in cold water. Absorbance values at 630 nm are recorded 15–35 min after heating and compared to a standard curve, ranging from 0 to 50 $\mu$g of glucose, run simultaneously. This procedure eliminates sample heating during anthrone reagent addition and requires less sample volume, reagent, and heating time than previously reported procedures (Fairbairn 1953, Van Handel 1965 Yadav et al 1969).

## LITERATURE CITED


BUCKEE, G. K., and HARGITT, R. 1978. Measurement of carbohydrates in wort and beer—A review. J. Inst. Brew. 84:13.

CLEGG, K. M. 1956. The application of the anthrone reagent to the estimation of starch in cereals. J. Sci. Food Agric. 7:40.

DREYWOOD, R. 1946. Qualitative test for carbohydrate material. Ind. Eng. Chem. Anal. Ed. 18:499.

FAIRBAIRN, N. J. 1953. A modified anthrone reagent. Chem. Ind. 31:86.

KOEHLER, L. H. 1952. Differentiation of carbohydrates by anthrone reaction rate and color intensity. Anal. Chem. 24:1576.

MCCREADY, R. M., GUGGOLZ, J., SILVIERA, V., and OWENS, H. S. 1950. Determination of starch and amylose in vegetables. Anal. Chem. 22:1156.

MORRIS, D. L. 1948. Quantitative determination of carbohydrate with Dreywood's anthrone reagent. Science 107:254.

SATTLER, L., and ZERBAN, F. W. 1948. The Dreywood anthrone reaction as affected by carbohydrate structure. Science 108:207.

VAN HANDEL, E. 1965. Estimation of glycogen in small amounts of tissue. Anal. Biochem. 11:256.

YADAV, K., WEISSLER, H., GARZA, A., and GURLEY, J. 1969. The determination of total carbohydrates in wort and beer. Am. Soc. Brew. Chem. Proc. 1969:59.


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