A NOTE ON SUGAR DETERMINATION BY THE ANTHRONE METHOD

JUTTA CERNING-BEROARD, Station de Biochimie et Physico-Chimie des Céréales de l'I.N.R.A. au C.E.R.D.I.A., 91305 Massy, France

The anthrone method for determining carbohydrates has been known for many years. The method has been applied with various modifications in concentration of anthrone and acid, heating time, and temperature (1–3). It is known that the rate and extent of color development with anthrone vary for many sugars having similar structures (2,3), just as encountered in the phenol-sulfuric acid (3,4) or orcinol (4) methods. However, these observations are usually based on experiments with pure sugars, and there is little information concerning the consequences of these results on sugar determination in cereals, cereal products, and other plant materials. Such differences do not necessarily preclude the use of anthrone in determining total sugars. Anthrone has an advantage over the phenol and orcinol procedures (5) by being applicable in 80% ethanol extracts, and it also reacts specifically with fructose at 50°C. We have studied the possible application and limitation of the anthrone method in ethanol extracts of corn, potatoes, and horsebeans.

MATERIALS AND METHODS

Ethanol extracts (6) of potato, corn, and horsebean samples were used. Total sugars were determined by anthrone (1); fructose was estimated by the anthrone method at 50°C (7,8). Glucose (6), sucrose (6), and raffinose (8) were determined enzymatically. In addition, quantitative paper chromatography was used to determine α-galactosides (9).

RESULTS AND DISCUSSION

Glucose is normally used to establish a calibration curve for the anthrone method and total sugars are expressed in terms of glucose. Results expressed in this way are arbitrary. Nevertheless, when glucose, fructose, sucrose, and small amounts of raffinose are present (8), the use of glucose as a standard does not introduce a significant error. In fact, the sum of sugars determined individually by the enzymatic and anthrone procedure at 50°C, using the same sugar as a standard, is in very close agreement with total sugars estimated by the anthrone method and expressed in terms of glucose (Table I).

However, when the anthrone method is used for determination of total sugars in legume seeds, such as horsebeans, that are predominantly composed of galactosides (9), the choice of glucose as a standard yields a value which is considerably lower than the sum of the individual sugars (Table II). Under identical experimental conditions, equal quantities of raffinose or stachyose produce a lower absorbance than does glucose (Fig. 1). Reactions of α-galactosides, such as stachyose, with anthrone have not been reported so far. One may assume that verbascose, because of its additional galactose moiety, would yield lower absorbance than an equal amount of stachyose, since galactose

produces a much lower absorbance than does glucose (3).

It is difficult to propose a standard mixture of sugars which imitates that existing in legume seeds. Our knowledge of the proportions of different sugars in legume seeds remains fragmentary, and proportions vary with variety and growing conditions (9).

Furthermore, pure anhydrous stachyose is expensive, while verbascose is not available commercially.

When total sugars, directly determined by anthrone (Table II), are expressed in terms of raffinose instead of glucose, the results for horsebeans and horsebean flour are 5.58 and 6.63%, respectively, the latter number much closer to the sum of individually determined sugars.

Further systematic sugar analyses are needed to decide whether raffinose would be preferable for establishing the calibration curve. Expressing total sugars in legume seeds (horsebeans) in terms of raffinose could possibly lead to results that are in better agreement with absolute values.

TABLE I
Sugar Content (% Dry Matter)[a] of Freeze-Dried Potatoes and Corn

Sample	Glucose[b]	Fructose[b]	Sucrose[b]	Raffinose[b]	Total Sugars	
					Sum of determined	Directly by anthrone
Potato	1.60	1.42	0.70	...	3.72	3.69
Potato	2.63	2.74	1.60	...	7.10	7.00
Corn	0.04	0.14	0.90	0.23	1.31	1.38
Corn	0.06	0.25	1.20	0.21	1.72	1.80

[a]Mean value of three determinations.
[b]Glucose, sucrose, and raffinose were determined enzymatically; fructose was estimated by the anthrone method.

TABLE II
Sugar Content (% Dry Matter)[a] of Horsebeans and Horsebean Flour

Sample	Verbascose[b,c]	Stachyose[c]	Raffinose[c]	Sucrose[c]	Total Sugars	
					Sum of determined	Directly by anthrone
Horsebean	2.57	0.83	0.45	1.60	5.45	4.50
Horsebean flour	2.61	1.06	0.67	2.36	6.70	5.50

[a]Mean value of three determinations.
[b]Includes higher molecular weight α-galactosides.
[c]Determined by paper chromatography.

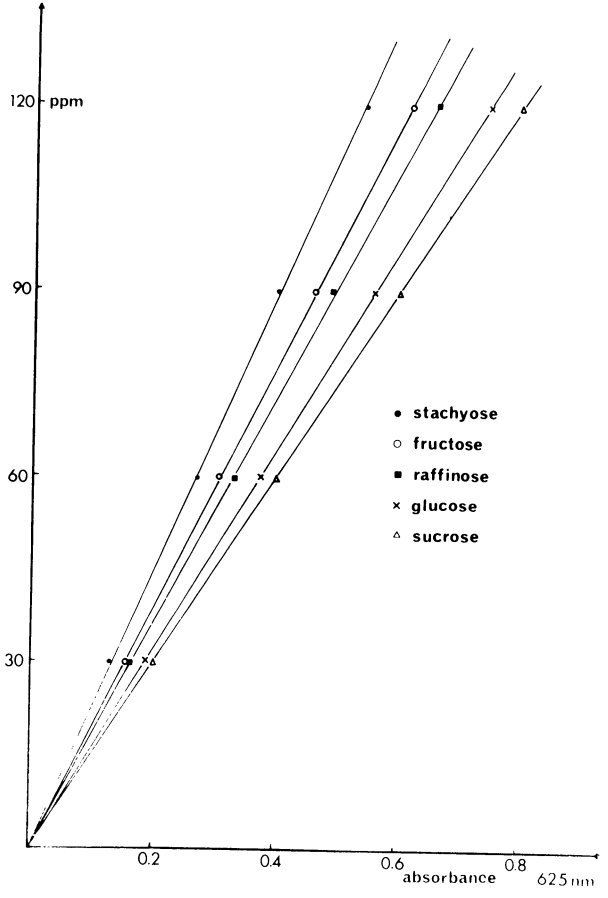

Fig. 1. Reaction of various sugars with the anthrone reagent (2% anthrone dissolved in ethyl acetate); heating time 12 min in a boiling-water bath.

Literature Cited

1. LOEWUS, F. A. Improvement in anthrone method for determination of carbohydrates. Anal. Chem. 24: 219 (1952).
2. HODGE, J. E., and HOFREITER, B. T. Determination of reducing sugars and carbohydrates. I. Analysis and preparation of sugars. In: Methods in carbohydrate chemistry, ed. by R. L. Whistler and J. N. BeMiller. Academic Press: New York (1962).
3. MONTREUIL, J., and SPIK, G. Microdosage des glucides. Fascicule. 1. Méthodes colorimétriques de dosage des glucides totaux. Faculté des Sciences de Lille, France (1963).
4. 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 (1956).
5. TOLLIER, M. T., and ROBIN, J. P. Adaptation du dosage automatique des oses et oligosides par l'orcinol sulfurique : conditions d'application aux extraits végétaux. Ann. Technol. Vég. (in press).

6. CERNING-BEROARD, J. The use of invertase for determination of sucrose. Application to cereals, cereal products, and other plant materials. Cereal Chem. 52: 431 (1975).
7. JOHNSON, G., LAMBERT, C., JOHNSON, D. K., and SUNDERWIRTH, S. G. Colorimetric determination of glucose, fructose and sucrose in plant materials using a combination of enzymatic and chemical methods. J. Agr. Food Chem. 12: 216 (1964).
8. CERNING, J. Contribution à l'étude de l'évolution de la composition glucidique des grains de céréales au cours de leur maturation: maïs, blé, orge. Thèse de Doctorat d'Université, mention Sciences, Lille, France (1970).
9. CERNING, J., SAPOSNIK, A., and GUILBOT, A. Carbohydrate composition of horse beans *(Vicia faba)* of different origins. Cereal Chem. 52: 125 (1975).

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