Food Chemistry **35** (1990) 109–116

Enzymatic Determinations of Glucose, Sucrose and Maltose in Food Samples by Flow Injection Analysis

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(Received 3 October 1988; revised version received and accepted 17 February 1989)

ABSTRACT

Several sugars were determined in food samples with a flow injection system that used a combination of immobilized and soluble enzymes. The food samples were selected to have a broad range of sugar concentrations (0·1–30%). The proposed method is more rapid than official methods, yet comparable results are obtained with the particular food samples studied. Because the method uses enzymatic reactions, it is more selective than standard, non-enzymatic procedures. The method has been applied to standard solutions of glucose, sucrose and maltose and to honey, wheat flour, white wine and a soft drink. The precision of the enzymatic method is 2% RSD.

INTRODUCTION

Analytical procedures employing immobilized enzymes have become increasingly common in recent years. Since invertase was first immobilized by adsorption (Nelson & Griffin, 1916), many new applications (Messing, 1975; Chibata, 1978; Carr & Bowers, 1980; Trevin, 1980) and several new methods of immobilization have been proposed (Glass & Rand, 1982; Ralis *et al.*, 1983; Riffer, 1983). For quantitative methods, immobilized enzymes combine the selectivity and sensitivity of aqueous enzymes with the

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Food Chemistry 0308-8146/89/$03·50

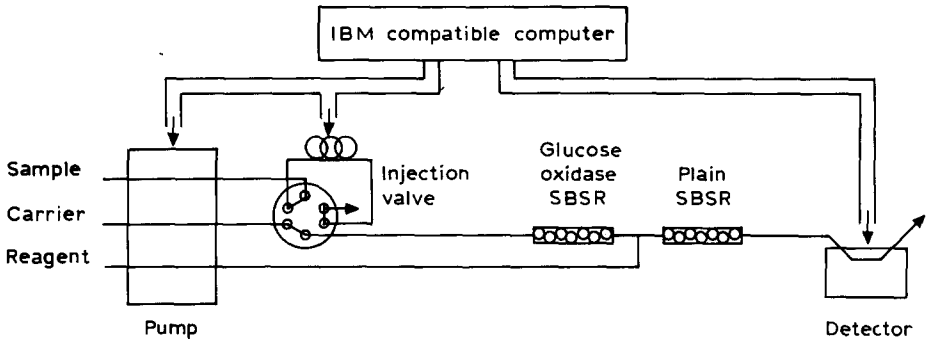

Fig. 1. FIA manifold for glucose determinations.

convenience and low cost of reusable reagents. Recently, immobilized enzyme reactors have been used in conjunction with flow injection analysis (FIA) (Johanson *et al.*, 1983; Mottola, 1983; Olson *et al.*, 1985). FIA is an unsegmented continuous flow analysis technique that is now widely employed for automated colorimetric determinations (Ruzicka & Hansen, 1981; Betteridge *et al.*, 1983; Patton & Crouch, 1986; Toei, 1987; Valcarcel and Luque de Castro, 1987). FIA systems provide automated sample transport and reagent mixing, reproducible timing and controlled dispersion. For the determination of sugars in food samples, several reports have appeared in which immobilized enzyme reactors are used in conjunction with FIA systems (Glass & Rand, 1982; Aldcorn *et al.*, 1985; Maquieira *et al.*, 1987).

In the work reported here, glucose oxidase (Miller *et al.*, 1977; Masoom & Townshend, 1984) was immobilized on non-porous glass beads which were packed into a single bead string reactor (SBSR) (Reijn *et al.*, 1981) and used in a FIA manifold to determine glucose (see Fig. 1). In order to determine sucrose and maltose in food samples, soluble invertase and maltase were used to convert the two disaccharides to glucose prior to their measurement with the immobilized enzyme/FIA system.

For the spectrophotometric determination of glucose, the following reactions were utilized (Trinder, 1969; Thompson, 1982).

$$H_2O + O_2 + \beta\text{-D-glucose} \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{AAP} + \text{DCPS} \xrightarrow{\text{peroxidase}} \text{red dye} + H_2SO_4$$

where AAP = 4-aminoantipyrine and DCPS = 3,5-dichloro-2-hydroxyphenyl sulfonic acid.

The absorbance of the red dye generated in the second reaction was monitored at 510 nm, the wavelength of maximum absorption. The flow

injection determination of glucose utilized the optimum conditions of flow rate, temperature, peroxidase concentration, AAP and DCPS concentrations and pH described previously (Stults *et al.*, 1987*a*).

The combination of soluble enzymes for converting sugars to glucose with an automated, immobilized enzyme/FIA system for glucose makes the determination of glucose, sucrose and maltose highly specific and quite rapid in comparison with the official methods.

MATERIALS AND METHODS

Apparatus

Glucose was determined with a flow injection apparatus (Fig. 1), which consisted of a 12-channel peristaltic pump (Ismatec, Glattbrugg, Switzerland) with flow-rated pump tubing (Technicon Instruments, Tarrytown, NY, USA), a pneumatically activated injection valve with a 30 μl sample loop (Rheodyne Inc., Cotati, CA, USA), and a miniaturized flow-through filter colorimeter designed and constructed in this laboratory (Patton & Crouch, 1986). An IBM PC compatible microcomputer, equipped with an RTI-815 (Analog Devices, Norwood, MA, USA) interface board, controlled the pump speed, sample injection and data acquisition. Software was written in QuickBASIC (Microsoft Corp., Rendmond, WA, USA). Additional details are given in the thesis of Stults (1987).

The sample containing glucose passed first through a SBSR which had glucose oxidase covalently bonded to 0·6-mm diameter non-porous glass beads (Proper Mfg., LI City, NY, USA). The beads were packed into 10 cm of 0·86 mm i.d. poly(tetrafluoroethylene) tubing (Benton-Dickinson, Parsipanny, NJ, USA) as reported by Stults *et al.* (1987*a*). The sample was then mixed with a reagent stream that contained horseradish peroxidase, AAP and DCPS (see below). The colored product was formed in a plain SBSR, about 40 cm in length.

By using the appropriate pump tubing, the flow rate of the carrier stream was made ten times that of the reagent stream in order to minimize both the consumption of peroxidase and dilution of the sample.

Reagents

All chemicals (reagent grade) were used without further purification. The stock solutions were prepared in distilled water and diluted with 0·05 M phosphate buffer (3·39 g KH_2PO_4 + 3·53 g Na_2HPO_4/liter). Anhydrous α-D(+) glucose grade III, sucrose grade II and maltose grade II (all from Sigma

Chemical Co., St Louis, MO, USA) were used to prepare the standard solutions. The reagent for the Trinder reaction, prepared immediately before use, contained 0·8 mg ml^{-1} peroxidase, 1 mM 4-aminoantipyrine (AAP), and 1 mM 3,5-dichloro-2-hydroxyphenyl sulfonic acid (DCPS), dissolved in 0·05 M phosphate buffer.

The enzymes used were horseradish peroxidase (Sigma, Type II, activity 150–200 units mg^{-1}), glucose oxidase (Sigma, Type II, from *Aspergillus niger*, activity approx. 17 800 units g^{-1}), invertase (Sigma, grade VII, from Baker's Yeast, activity approx. 400 units mg^{-1}), and maltase (Sigma, Type IV, from Brewer's Yeast, activity approx. 4 units mg^{-1}).

Glucose oxidase was immobilized on non-porous glass beads by the procedure described by Stults *et al.* (1987*a*). Attempts to immobilize invertase and maltase, using the same experimental conditions, failed.

Preparation of the samples

The samples tested were a wheat flour, a light honey, a soft drink and a dry white wine. The soft drink sample was degassed, while the honey sample was diluted 1:10 prior to use. The wheat flour sample was treated to obtain a clear extract. One gram of flour was wetted with 1 ml of ethyl alcohol. After addition of distilled water to make a final volume of 10 ml, the mixture was shaken for 1–2 min. The resulting suspension was then centrifuged at 10 000 rpm for 20 min. The other samples were used as received without treatment.

Procedure

For the determination of glucose, each sample was transferred into a separate 10-ml volumetric flask; the amounts taken were 100 μl of the degassed soft drink, 300 μl of the wine, 100 μl of the diluted honey, and 1·0 ml of the flour extract. The four flasks were filled to volume with 0·05 M phosphate buffer to make the working solutions. The FIA determinations were begun after a delay of 15 min to allow the α and β forms of D-glucose to reach equilibrium in the phosphate buffer (Stults *et al.*, 1987*b*). After injection, approximately two min elapsed under the conditions used before the FIA signal was acquired with the computer data acquisition system. Peak absorbance values were used in all cases.

For determination of sucrose, the same procedure was used except that the samples were treated with invertase prior to the glucose determination. Similarly maltose was determined after conversion to glucose with maltase. For these sugars, after the samples were transferred into 10-ml volumetric flasks (as above), solid invertase or maltase was added. After allowing two

min for the conversion reaction to proceed, the volumetric flasks containing the treated samples were filled to volume with the buffer solution. The FIA determinations of glucose were again begun 15 min after the dilution. The amounts of invertase and maltase needed for rapid conversion were determined empirically.

Six replicate measurements were done for each sample. The conditions for all measurements were: flow rate 0·5 ml min^{-1}, temperature 22°C, peroxidase concentration 0·8 mg ml^{-1}, AAP and DCPS concentration 1 mM, and pH of the carrier and reagent 6·86.

RESULTS AND DISCUSSION

Results of glucose, sucrose and maltose determinations are presented in Table 1 and compared to results obtained for reducing (glucose and maltose) and non-reducing (sucrose) sugars by the official AOAC method (AOAC, 1980). Although the mutarotation of D-glucose reaches equilibrium within 15 mins in 0·05 M phosphate buffer (Stults *et al.*, 1987*b*), more time was required for the equilibration of the α and β forms of D-glucose obtained from converted maltose (with maltase). At the end of 15 mins, the glucose values from converted maltose were 80% of the final values obtained when equilibrium was reached. The reason for this slower equilibration from converted maltose is not clear at this time. However, the percentage conversion after 15 mins was found to be quite reproducible. Hence to save time in the maltose determinations, the initial glucose values obtained after 15 min were multiplied by a factor of 1·25 to obtain the final values.

For constructing the calibration curves for sucrose and maltose, solid invertase and maltase were added to aliquots taken from stock standard solutions, which were 2% in sucrose and maltose. This procedure was found

TABLE 1
Sugar Determinations

Sample	*FIA*			*Reducing sugars glucose + maltose* (%)	*Non-reducing sugars sucrose* (%)	*Error (%)**	
	Glucose (%)	*Sucrose* (%)	*Maltose* (%)			*Reducing sugars*	*Non-reducing sugars*
Wheat flour	0·74	0·16	1·36	2·13	0·15	−2	6
Light honey	33·12	3·49	5·12	36·65	3·36	4	4
Soft drink	4·03	0·37	0·28	4·12	0·34	4	7
White dry wine	0·41	0·10	0·10	0·47	0·09	7	6

* The relative difference between the values found by FIA and by the AOAC method.

to be necessary in order to obtain a high enzyme concentration while using small amounts. The desired conversions were then obtained within two min. After this delay, the stock solutions were diluted with 0·05 M phosphate buffer to make the working standards for the measurements.

The calibration curves for glucose and converted maltose were linear over the concentration range 0·010–0·080%. The calibration curve for converted sucrose was linear over the concentration range 0·010–0·120%. The slopes of the calibration graphs were 0·0432, 0·0416 and 0·0429 absorbance mM^{-1} for glucose, sucrose and maltose respectively.

Glucose oxidase immobilized on non-porous glass loses activity slowly with time. This makes it necessary to construct new calibration curves daily. Figure 2 shows the FIA absorbance maxima for a 2·2 mM glucose standard over a 33 day time period. The reactor has lost over half its initial activity in this period. The enzyme reactor used for this study was stored during the nights at 4°C to minimize this loss.

The dilution of the original samples was adjusted according to their expected glucose concentrations; 1:100 for the soft drink, 3:100 for white wine, 1:1000 for the light honey, and 1:100 for wheat flour.

From Table 1 it can be seen that the results of the enzymatic method compare very favorably with results obtained by the AOAC method. The relative errors between the enzymatic method and the AOAC method were

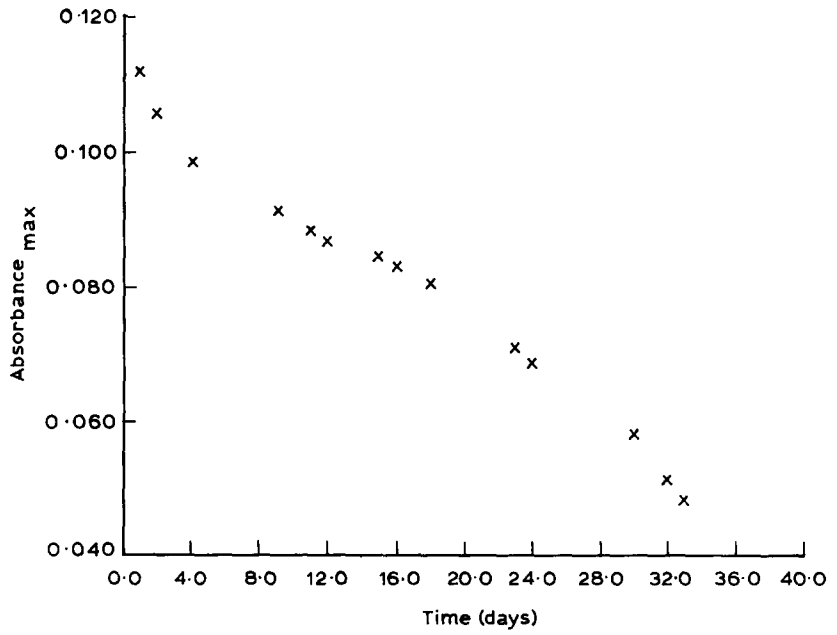

Fig. 2. Glucose oxidase activity loss with time.

always less than 10%. Standard additions applied on three samples (honey, wine and soft drink) gave a recovery of 99·7–103·4%. In the case of the flour sample the additions were made on the 1:10 extract, and the recovery was found to be 98·2%. The standard deviation of the measurements was SD = ±0·02 or 2% RSD (for $n = 6$).

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Dr Cheryl Stults who developed the computer-controlled FIA system used here. Dr Peter Wentzell and Mark Victor provided assistance with some of the software. The authors appreciate the help of Kris Kurtz in preparing some of the immobilized enzyme reactors.

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