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The Determination of Starch in Composite Foodstuffs by High-Performance Liquid Chromatography

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ABSTRACT

A method for the determination of starch and its degradation products was investigated. High-performance liquid chromatography with a mass (light scattering) detector was used to determine the free mono and disaccharides and the starch and starch degradation products were determined as glucose, following acid hydrolysis of the carbohydrate in the sample.

Model and commercial samples were analysed by both high-performance liquid chromatography and a traditional titrimetric method. The results obtained compared favourably between the two methods. The method was found to be rapid, reproducible and can be easily automated.

INTRODUCTION

The determination of starch has traditionally posed problems for the analytical chemist, as its chemical nature has dictated that it be determined indirectly. Polarimetric methods such as that of Ewers (first published in 1905) and methods based on total saccharification and subsequent titration (Lane & Eynon, 1923) are still extensively used for the determination of starch.

Under the Common Agricultural Policy (CAP) of the European Community, laboratories in member states are required to determine the starch content of foodstuffs, to ensure that the appropriate levies are

collected and refunds paid. A recent Commission Regulation (1987) redefined starch as 'starch and its degradation products (including glucose)'; the previous definition of starch was those polymers of glucose insoluble in 40% aqueous ethanol. In order to determine starch/glucose, therefore, new methodology was required. An enzymatic procedure using amyloglucosidase to hydrolyse soluble starch into glucose, based on that of Blake and Coveney (1979), was initially investigated, using sodium hydroxide to solubilise the starch (Keppler & Decker, 1974), in place of dimethylsulphoxide//hydrochloric acid. This procedure proved unsatisfactory for samples containing maltodextrins, lower molecular weight glucose oligomers and glucose. When treated with dilute sodium hydroxide, such samples were observed to become yellow or orange coloured, and this phenomenon is apparently due to the Lobry de Bruyn-van Ekenstein rearrangement (Nef, 1904), in which reducing sugars react with alkali forming saccharinic acids. These reaction products are not hydrolysed during the conversion of soluble starch to glucose, and a lower glucose content was therefore obtained.

High-performance liquid chromatography (HPLC) has been used successfully for the determination of free sugars in composite food samples using a polar bonded column (Hunt *et al.*, 1977; Macrae & Dick, 1981). Therefore an HPLC procedure was used for such samples for the determination of free mono and disaccharides, and extended for the determination of starch as glucose. In this procedure the starch and starch degradation products were determined as glucose following acid hydrolysis of the carbohydrate in the sample. The procedure has been shown to be accurate and reproducible when using model samples of varying composition, and has undergone successful collaborative testing in European laboratories.

MATERIALS AND METHODS

Materials

Samples of wheat flour, maltodextrin and dried glucose syrup were obtained from commercial sources. Samples of wholemeal bran biscuits, scone mix and sponge mix, declared to contain maltodextrin and/or glucose syrup, were purchased from a local retail outlet.

Fructose, glucose, sucrose, maltose and lactose were obtained from Fisons (Loughborough, UK) and acetonitrile, HPLC grade, was obtained from Rathburn Ltd (Walkerburn, UK).

Methods

Hydrolysis

Samples were homogenised and 1 g sub-samples of these were heated under reflux for 3 h with 100 ml deionised water containing 2 ml concentrated hydrochloric acid (SG 1·16) to hydrolyse the starch. After cooling, the hydrolysate was transferred quantitatively to a 200 ml one-mark volumetric flask, made up to the mark with deionised water and filtered through a Whatman No. 42 filter paper. A 50·0 ml aliquot of the filtrate was transferred using a pipette into a 100 ml volumetric flask and made up to the mark with acetonitrile. Further dilutions were carried out as necessary with acetonitrile/water (50:50 v/v) in order to bring the glucose concentration into the range 0·5 to 2·5 g/litre, prior to analysis by HPLC.

Extraction of free sugars

Sub-samples (10 g) of the previously homogenised sample (qv) were extracted by shaking vigorously with 50 ml deionised water in a 100 ml volumetric flask. This was then diluted with deionised water to the mark. The extracts were then filtered; a 50·0 ml aliquot of the filtrate was taken and diluted to 100 ml with acetonitrile in a one-mark volumetric flask for HPLC analysis of the free sugars.

Chromatographic analysis

An LKB (Bromma, Sweden) series 2150 HPLC pump, a Negretti and Zambra (Eastleigh, UK) M190 injection valve fitted with a 20 μl loop and an ACS 750/14 mass detector (Macclesfield, UK) were used with a Chrompack (Middleburg, Holland) Chromsep™ cartridge system consisting of two 100 × 3 mm^2 glass columns packed with Hypersil-APS and an ODS guard column. The mobile phase used was acetonitrile/water (82:18 v/v), degassed using helium, pumped at a flow rate of 1·0 ml/min. The detector settings employed were as follows:

—Photomultiplier, 3.
—Evaporator, 55.
—Attenuation, 32.

Peak areas were determined using a Spectra-Physics (Hemel Hempstead, UK) SP4290 computing integrator. Quantitation was achieved by chromatography under identical conditions of standard solutions of the sugars fructose, glucose, sucrose, maltose and lactose, 0·1% in acetonitrile/water (50:50 v/v).

Calculation

The calculations used to determine the percentage of starch and starch degradation products (as starch) were as follows:

(a) % starch $= (Z - F - 0{\cdot}53S) \times 0{\cdot}9$
when the glucose content (G) is greater or equal to the fructose content (F); or

(b) % starch $= (Z - G - 0{\cdot}53S) \times 0{\cdot}9$
when the glucose content (G) is less than the fructose content (F)

where

—Z is the glucose content determined by HPLC after hydrolysis with hydrochloric acid
—F is the fructose content determined by HPLC without hydrolysis
—G is the glucose content determined by HPLC without hydrolysis
—S is the sucrose content determined by HPLC without hydrolysis

RESULTS AND DISCUSSION

Results

Samples of wheatflour, maltodextrin and dried glucose syrup were analysed by the above procedure in order to determine the level of starch and starch degradation products. In addition, mixtures of these, to simulate probable samples, were also analysed. Comparative analysis (Lane and Eynon procedure) was also carried out on some of these samples (Table 1).

TABLE 1
Results for the Determination of Starch/Glucose by HPLC following Acid Hydrolysis

Sample	*% Starch and starch degradation products (as starch)*	
	By HPLC	*By Lane and Eynon procedure*
Wheat flour	71·0, 72·2	71·9, 71·9
Maltodextrin	87·9, 88·8	89·2, 89·2
Dried Glucose Syrup	84·5, 82·7	83·2, 84·8
Wheatflour/maltodextrin (70:30)	76·3, 76·6	76·8, 76·8
Wheatflour/maltodextrin/dried glucose syrup (55:21:24)	76·0, 76·2	

The results show very good agreement between the two procedures used. The wheatflour/maltodextrin/ dried glucose syrup result agreed very well with that expected from the results obtained for the individual components (75·0%).

Commercial samples of wholemeal bran biscuits, scone mix and sponge mix were also analysed by both procedures. Results are given in Table 2.

Once more, good agreement between the two procedures was obtained. The slight discrepancy in the results for the wholemeal bran biscuits is probably due to the high bran content; the Lane and Eynon procedure determines total reducing sugars, which may not be 100% glucose following the hydrolysis of bran. Chromatograms obtained for both standard solutions of sugars and samples, before and after acid hydrolysis are shown in Figs 1 to 4.

The procedure described is simple and has been shown to be accurate for typical food components of known composition. It has the advantage of utilising the same HPLC conditions as for the determination of free sugars. In addition, the procedure could be readily automated, which would make it considerably more cost-effective than any gravimetric, titrimetric or enzymatic procedure.

An initial concern in the development of this procedure was the concentration of acid which was being introduced onto the HPLC column, and the possible deleterious effects this could have on column performance and lifetime. In order to prevent this presenting a problem a number of possible clean-up procedures were investigated. These involved neutralisation with dilute alkali and/or the use of ion-exchange resins to remove cations. A number of different cation-exchange and mixed resins were used. When the acid hydrolysate was neutralised, and no ion-exchange clean-up employed, the salt formed gave rise to a peak on the chromatogram which interfered with glucose. The use of ion-exchange resins to remove this

TABLE 2
Results for the Determination of Starch/Glucose by HPLC in Commercial Samples following Acid Hydrolysis

Sample	*% Starch and starch degradation products (as starch)*	
	By HPLC	*By Lane and Eynon procedure*
Wholemeal bran biscuits	44·3, 44·0	47·8
Scone mix	59·7, 59·8	60·2
Sponge mix	47·0, 46·2	46·6

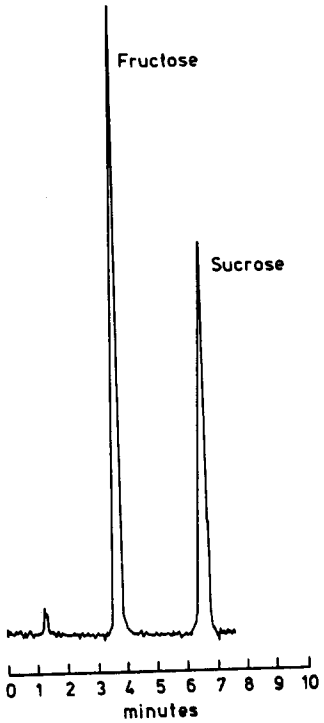

Fig. 1. Mixed sugar standard (0·05%).

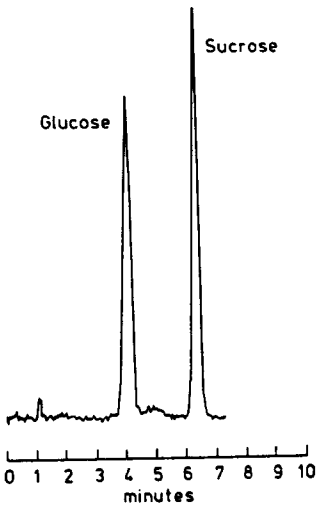

Fig. 2. Scone mix before acid hydrolysis 1·6% sample solution.

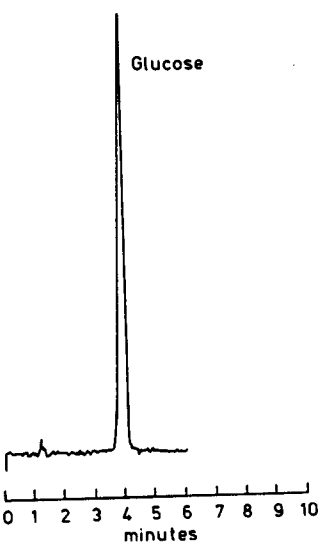

Fig. 3. Glucose standard (0·05%).

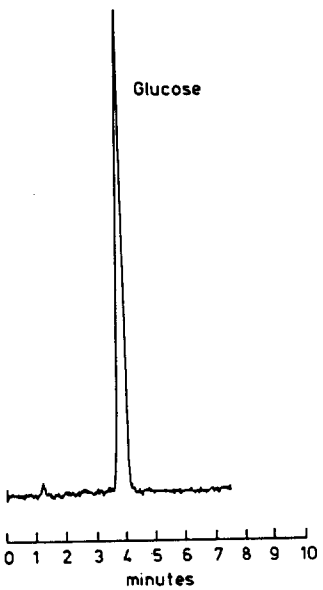

Fig. 4. Scone mix after acid hydrolysis 0·08% sample solution.

interference resulted in significant losses of glucose, giving rise to low and variable recoveries. The use of ion-exchange resins without neutralisation gave rise to similar results. This is probably due to glucose ionising to some extent in aqueous solution, and being removed by the ion-exchange resins. Therefore no clean-up or neutralisation procedures were used prior to HPLC analysis. The maximum level of hydrochloric acid being introduced onto the column was 1·13 μmoles, and no deterioration of column efficiency was observed following a large number of analyses (>30).

CONCLUSIONS

A simple, accurate HPLC procedure for the determination of starch and starch degradation products as glucose in composite foodstuffs has been developed. Results obtained on model and commercial samples compare well with those obtained using traditional procedures. The method is rapid, integrates well with that used for free sugars, can be easily automated if necessary, and requires less operator skill than an enzymatic procedure. The procedure could be validated further if a reference material of certified starch content were available.

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