Problems in the Analysis of Polysaccharides in Foodstuffs

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This article is a summary of a lecture given by Dr. Southgate at the first symposium of the newly formed Food Chemistry Group of the Chemical Society, held on 14th May, 1974 at 23, Savile Row, London.

Many foods contain a mixture of polysaccharides, and this is responsible for many of the problems in their analysis. The polysaccharides which concern the analyst are often classified according to their role in plants but the distinction is arbitrary. Starch, dextrins and inulin are spoken of as "reserve polysaccharides". The algal polysaccharides could enter that classification, but plant gums might deserve another classification. Lignin is not a polysaccharide, but it is intimately associated with the polysaccharides in the cell wall, so methods of analysis must take lignin into account.

The analysis of foods for polysaccharides involves four main stages.

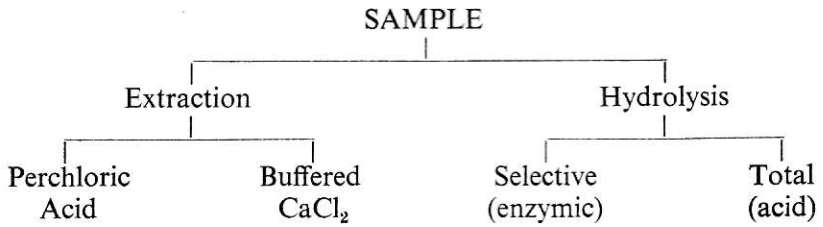
1. The preparation of the sample. The particulate nature of the plant cell wall constituents makes preparation of a homogenous mixture difficult in many instances, and grinding forage materials has been known to make some of the polysaccharide constituents more susceptible to enzymic hydrolysis.

2. Extraction with alcohol. Free sugar interferes with determinations of polysaccharide so it is removed by means of hot alcohol. Methyl, ethyl or isopropyl alcohol have been used in concentrations of about 70 per cent. or 80 per cent. by volume. These extractions are usually done at boiling point and repeated extractions are necessary. Fructosans readily hydrolyse, so foods containing organic acids should be neutralised before being extracted. Lipids are conveniently removed at the same time. This is not essential but lipid-free materials are usually easier to handle.

3. Isolation of individual polysaccharides. Separation represents the most difficult stage in polysaccharide analysis. Only two examples are discussed here.

4. Measurement. Again only two examples are discussed namely starch and plant cell-wall polysaccharide—often designated 'fibre'.

Starch is the most important polysaccharide for the food analyst. The approaches to its measurement might be summarised as follows:—



In many foods starch is the major polysaccharide present and the amounts of other readily hydrolysable polymers are quite small. In such cases, or where one can accept a lower degree of accuracy, one may use acid hydrolysis followed by measurement of reducing sugars.

One of the recommended AOAC methods for cereals is a method involving hydrolysis with HCl. The hydrolysates tend to be discoloured and one suspects that destruction or modification of other carbohydrates takes place. In the presence of appreciable amounts of protein, humin is formed accompanied by losses in carbohydrate. It is possible to get theoretical yields of glucose with a food starch if one adopts the principles used in the preparation of amino acid hydrolysates. About 4 hours under reflux with one per cent. sulphuric acid gives a good conversion when the amount of starch is limited to between 200 and 400 mgm per litre. When other polysaccharides are present however, acid hydrolysis often gives high values for starch. To some extent the use of glucose oxidase procedures and the measurement of glucose in the hydrolysate minimises such interference but beta-glucans yield glucose on hydrolysis. When interfering polysaccharides are present therefore, the method for starch must either include a selective hydrolysis stage or some form of selective extraction.

The use of an enzyme for selective hydrolysis has much to commend it and many taka-diastase preparations with low activity toward other polysaccharides, or highly specific amylo-glucosidases have become available. Poor yields of glucose and high yields of maltose sometimes occur, so qualitative examination of the hydrolysate is advisable before one makes any assumptions about the ratio of glucose to maltose. Some dextrins obtained in baking are incompletely hydrolysed by enzymes.

Selective extraction of starch is difficult to achieve quantitatively with any one extracting medium. The difficulties are even greater if one wishes to examine other polysaccharides in the starch-free residue. Extraction with $CaCl_2$ followed by polarimetric measurement is a well established procedure for cereals. The method depends on a knowledge of the specific rotation of the particular starch under examination, and in mixtures or where dextrins are present the results can be misleading. If other optically active polymers are in the extract one has another problem.

Extraction with perchloric acid has been used as a basis for several methods for starch, and reproducible results have been obtained when the extracted starch has been measured colorimetrically by the anthrone or a related colorimetric procedure. The precipitation of a starch-iodine complex has also given good results. If one defines starch as a mixture of alpha 1-4 and 1-6 polyglucosides then enzymatic procedures are best and chemical methods should be regarded as attempts to approximate the measurement of these polymers.

PLANT CELL-WALL POLYSACCHARIDES

This material is usually called 'fibre' by the clinicians. The initial problem therefore is one of terminology. In the classic view there were four major

components in plant cell walls, namely pectic substances, hemicelluloses, cellulose and lignin. Modern work has shown that this arbitrary classification is unreal and alkali fractionation in particular really produces progressive degradation of the hemicellulose fraction.

TABLE I

CELL-WALL POLYSACCHARIDES

Constituent	Property used in fractionation	Polysaccharide type
Pectic substances	Sol. in hot water or NH_4 oxalate	Polygalacturonan
Hemicellulose	Sol. in dil. NH_4OH	
	Precipitated by acid	Heteroglycans
	Precipitated by adding alcohol	Xylans,
	Sol. in strong alkali	Arabinoxylans and Uronans
Cellulose	Insol. in 17·5% NaOH	Glucosans and contaminants
Lignin	Insol. in 72% H_2SO_4	

It is probably better at the present time to divide cell-wall polysaccharides into cellulosic and non-cellulosic, the latter ranging from those rich in uronic acid (pectic substances under the old nomenclature) to those low in uronic acid. The practising analyst may say at this point that he is not concerned with these niceties. One appreciates his problem, but he should be working toward the measurement of defined substances.

The analysis of substances in the plant cell wall has been dominated by the crude fibre procedure. It has an ancient heritage, having been devised in the mid-19th century in an attempt to determine non-digestible carbohydrate in animal foods. It has considerable disadvantages as a measure of cellulose and lignin since the fraction weighed has variable composition from food to food.

The method of the Fertilisers and Feeding Stuffs Regulations is usually adopted, giving "crude fibre". Modified methods have been sought to simplify the procedure and to reduce the nitrogen content of the residue of fibre. There are two such major modifications.

1. *Normal Acid Fibre.* This is obtained by simply extracting with boiling normal sulphuric acid. The residue still contains some nitrogen.

2. *Acid Detergent Fibre.* The sample is extracted with boiling normal sulphuric acid containing detergent, or as described in Technical Bulletin 27, Ministry of Agriculture, Fisheries and Food. In addition to providing a better measure of cellulose and lignin, the fibre obtained by this method is suitable for further measurements of its components.

The acid detergent method has recently been accepted as "first action" official method of the A.O.A.C.

The non-cellulosic polysaccharides are usually lost in the acid hydrolysis stages of fibre determination, so only a part of the cell wall is measured. A neutral detergent method has been reported to give a good measure of total cell wall material but it gives high and unreliable values when starch is present.

In the absence of starch (or after starch has been removed with enzymes), the difference between neutral detergent and acid detergent fibre results can be used to give an estimate of the non-cellulose fraction. Such methods have been developed with the routine analyst in mind.

There is an alternative approach which sets out to fractionate the plant cell wall. Fractionation is time-consuming and does not commend itself to the routine analytical laboratory, but the development of complete analytical schemes forms a necessary forerunner and a reference method for subsequent abbreviated schemes. One scheme first delignifies with chlorine or hypochlorite and the chlorinated residues are extracted. Although the stage is essential with woody tissue it can often be omitted in the analysis of foodstuffs. Then the scheme divides the cell-wall polysaccharides into arbitrary fractions—each containing a number of polysaccharides. The estimation of the materials dissolved at each stage is not straightforward. Polysaccharides tend to co-precipitate upon addition of alcohol or acetone as fine gelatinous precipitates difficult of recovery, and they are usually contaminated. Total carbohydrate in the fractions can be estimated colorimetrically without prior hydrolysis, but one should try to divide the fractions into their component sugars and then determine the different monosaccharides.

TABLE II

FRACTIONATION OF CELL-WALL POLYSACCHARIDES

Sample treatment	Fraction of cell wall obtained
Delignification	Matrix polysaccharides
Extraction with hot water and EDTA	Pectic substances
Extraction with 4% NaOH	Hemicelluloses
Extraction with 17·5% w/v NaOH	Residue of cellulose and insoluble ash

The most difficult stage in measuring the component sugars of the non-cellulosic polysaccharides is the hydrolysis of the polysaccharide, especially when uronic acids are present. They tend to undergo decarboxylation and the hydrolysis products include disaccharides which resist further hydrolysis. The decarboxylation reaction forms the basis of classical assay procedures for uronic acid but colorimetric procedures based on a reaction with carbazole are more sensitive and may be carried out on intact polysaccharide. The colour-yields with different uronic acids are not identical however. Some chemical modification of uronic acids (reduction or methylation) is essential if one hopes to get quantitative hydrolysis, but enzymic methods are being sought.

Cellulose is usually estimated gravimetrically, either as the difference between acid detergent fibre and lignin, or as the residue remaining after removal of lignin with alkaline permanganate. These procedures depend on the specificity of the extracting medium but other methods measure directly the glucosans extracted into 72% H_2SO_4.

TABLE III

EXAMINATION OF CELL-WALL FRACTIONS

Fraction	Method of Assay	
Pectic substances	Gravimetric:	Precipitation of Ca salt
	Colorimetric:	Carbazole either direct or after hydrolysis
Hemicelluloses	Gravimetric:	
	Measurement of sugars after hydrolysis:	Colour GLC Enzymic
	Distillation of furfural:	
Cellulose	Gravimetric:	After hydrolysis
	Colour:	

Only a few of the problems have been mentioned, but they suffice to indicate which aspects of the problems need to be resolved. They are:

1. The terminology. The terms used by analysts, biochemists and structural organic chemists do not coincide. There is unnecessary confusion and some rationalisation is desirable, but the ideal will only be reached when specific methods for the different polysaccharides become available.

2. Analytical research Investigations need to be carried out to find specific extraction and hydrolytic procedures, particularly in connection with the specific polysaccharides in cell walls.

3. Final assay of sugar Rationalisation and improvement of existing procedures are needed, with the introduction of automatic procedures.

The Royal Institute of Chemistry

Mastership in Chemical Analysis

The Regulations for the Post-Graduate Examination in the Analysis of Food, Drugs and Water, leading to a Mastership in Chemical Analysis (M.Chem.A.), have been revised and are to be implemented on 1st January, 1975.

The revision has been undertaken to bring the examination into line with the contemporary needs of law enforcement agencies especially since the reorganisation of Local Government on the 1st April, 1974.

The Regulations are a guide to the comprehensive nature of the duties carried out by the Public Analyst the discharge of which demands a unique expertise unlikely to be obtained from a course of training leading to any other qualification.

Copies of the revised Regulations may be obtained from the Examinations Officer, The Royal Institute of Chemistry, 30 Russell Square, London.