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METHODS OF CARBOHYDRATE ANALYSIS

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ABSTRACT

Modern methods of carbohydrate analysis are utilised to solve many of the same chemical problems as those of the past three or four decades. The difference is that the diversification of carbohydrates and sweetners has complicated the analytical problem and the development of instrumental technology has allowed an entire battery of analytical methods to be employed.

Methods of analysis may be selected for sensitivity, specificity, speed, facility and precision, and may broadly be divided into rapid routine control procedures (often involving combination of physical and traditional 'wet' chemical methods) and instrumental methods. The former include reducing power determination, modified aldehyde colorimetric reactions, refractometry and osmometry whereas the latter cover a range of sophisticated spectrometric, enzymic, immunological and chromatographic procedures.

Of singular interest in carbohydrate analysis is polarimetry. Although the determination of sugar by polarimetry is often by no means as sensitive as other methods (eg.g. gas liquid chromatography) it is highly specific. Moreover, the optical rotation of a sugar reflects its stereostructure and the polarimetric method therefore allows accurate assignment of ring size, conformation and configuration as well as changes in these caused by interactions with food components.

Modern food science and technology has resulted in a plethora of novel derivatised carbohydrates including sugar glycerides, benzoylated sugars (for intense bittering), chlorinated sugars (for intense sweetening), oxidised sugars (including lactones, as acidulants) and hydrogenated sugars (for bulk sweetening). Analysis of these is more difficult than simple sugars or, with combinations of the different types, almost impossible. However, for traditional food carbohydrates, modern analytical methods are already contributing substantially to food standardisation, quality and the prediction of sensory and nutritional character.

INTRODUCTION

Although modern food carbohydrate anlaysis covers much of the same area of food standardisation, quality control, nutrition and safety as it has for many decades, the emphasis is now on sophisticated instrumental and labour-saving devices to improve precision, increase speed and refine the sensitivity of determination.

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Carbohydrate analysis may be aimed at determining mono-, oligo- or polysaccharides in foods or frequently mixtures of all three types. The results may have implications for the sensory or metabolic effects of food products and may, in conjunction with other determinations, e.g. protein estimation, allow the computation of legal requirements for food composition. As in many other areas of modern food science and technology, carbohydrate analysis must be developed, not only to cope with existing food standardisation and composition, but also to anticipate the demands of a rapidly developing industry in which novel products, novel components and newly-reported interactions provide a constant challenge to the food chemist.

Food carbohydrate analysis in the 1980s therefore covers a range of instrumental, physical, chemical, enzymic, immunological and sensory procedures each of which is a science in itself. A recent volume summarises the field in both theoretical and practical detail (Birch, 1985) and it is clear that a prodigious analytical effort is engendered at the development stages of new food products and components. Perhaps it is true that food anlaysts have previously been preoccupied with maintaining standards but are now more concerned with nutritional implications. Certainly the advent of the dietary fibre theory, for example, has catalysed many analytical efforts in one complex corner of food carbohydrate chemistry (James and Oleander, 1981; Southgate, 1976; Birch and Parker, 1983), and this has helped in understanding, not only the composition of fibre, but its role in nutrition and gut metabolism.

The two main roles of carbohydrates in foods are as (1) energy sources, fillers and thickeners (e.g. starches), texturisers (e.g. cellulose, hemicellulose and pectin fibres) and (2) as sweeteners (sugars) Birch and Parker, 1982). Analytical techniques for food carbohydrate have therefore been developed to determine their concentrations and to monitor their ability to exercise these functions. The latter implies the study of structure/activity relationships of food carbohydrate and involves linkage analysis, conformational investigation and definitive synthesis. On the other hand, modification of food carbohydrate leads to products which no longer fall within the legal definition of carbohydrate $[C_x\ (H_2O)_y]$. Nevertheless the chemistry of such modified products is often so similar to that of the parent components that the appropriate analytical procedures are similar or identical.

TRADITIONAL PHYSICAL, CHEMICAL, RAPID AND CONTROL METHODS OF ANALYSIS

Traditional rapidly physical methods for analysis of carbohydrate-rich foods are hydrometry, refractometry and polarimetry. Whereas hydrometry and refractometry measure the total density and refracting power of the solution of carbohydrate and other substances, polarimetry measures the optical activity (usually at a fixed wavelength) and reflects the identity and concentration of the carbohydrate itself. Polarimetry therefore represents a specific and sensitive technique for measuring a particular food carbohydrate, not only in the presence of other food components, but also in the presence of other carbohydrates. Sucrose, for example, may be determined by determining the change in optical rotation which accompanies hydrolysis (inversion) of the sample.

Another physical technique for rapid determination of dissolved carbohydrate is osmometry, utilising for example the cryogenic principle of depression of freezing point. However, since osmotic pressure is one of the colligative properties of a solution, the tecniqueis only applicable to solutions of carbohydrate devoid of protein, minerals and other water-soluble food components.

Traditional wet chemical procedures for carbohydrate analysis include reducing power determination (usually with Fehlings solution or modifications of this) and total carbohydrate methods which involve the dehydration of all common types of carbohydrate to furfural derivatives and the coupling of these to amines or phenols to give coloured complexes. Although the use of Fehling's solution procedures only measures reducing sugars, all non-reducing sugars may be converted to reducing sugars by hydrolysis with dilute acid. Much information about common mixtures of food sugars can therefore be obtained by reducing sugar determination before and after hydrolysis and the classical method for doing this is the volumetric procedure of Lane and Eynon (1923). Such methods may be combined with the traditional rapid physical method to give the compositions of major food carbohydrates in mixture, which in turn shows us how such mixtures were formulated. It must be emphasised that, although these combinations of traditional methods lack the precision and sensitivity of modern instrumental procedures, there is no way that the modern methods alone can give the same information. Some of the traditional methods are therefore still routinely used in the modern food industry.

APPLICATIONS OF TRADITIONAL METHODS OF ANALYSIS TO FOOD PRODUCTS

The common types of food sugar, utilised in mixtures and determinable by combined methods are sucrose, invert sugar (i.e. equimolar D-glucose and D-fructose) and glucose syrup (i.e. partial starch hydrolysate). Such mixtures occur in many syrups, soft drinks, preserves and confectionery and can be analysed by a combination of the Lane and Eynon method with iodimetric oxidation and refractometry (Smith and Norton, 1966) or otherwise by a combination of the Lane and Eynon method with polarimetry and refractometry (Snyder and Diehl, 1959). Dairy products, such as sweetened condensed milk normally only contain two sugars, sucrose and lactose. After the sucrose is determined by the normal double polarization method (before and after inversion) the lactose may be directly computed (arithmetically) from the polarimeter readings. An alternative approach (Birch and Mwangelwa, 1974) is to destroy the lactose (reducing sugar) by treating with alkali and to determine residual carbohydrate (sucrose) by a modified aldehyde reaction, e.g. the phenol-sulphuric method in which absorbance is measured at λ_{max} 490 nm. Lactose can be determined by repeating the phenol-sulphuric acid method without the alkali step. Lactose itself, in food products which do not contain additional sugars (e.g. milk, cheese, whey), can be most conveniently determined by the phenol-sulphuric acid method because proteins and minerals do not interfere (Barnett and Tawab, 1957). After setting up a standard curve the determination only takes a few minutes and clarification is unnecessary.

One of the most common ways of characterising glucose syrups is the so-called "dextrose equivalent" or D.E., which is defined as the reducing power of the sample, calculated as dextrose (D-glucose) and expressed on a dry weight basis. In practice this is done by the Lane and Eynon method and refractometry (to determine solids content). Each sample takes several minutes to complete and a novel difficulty has emerged in the last few years with the production and use of hydrogenated glucose syrups. By definitions those syrups have no reducing power, yet they contain distributions of oligomers analogous to that of the parent gyrup and it would clearly be convenient to characterise them by this distribution. Kearsley (1978) has elegantly demonstrated that the osmotic pressure of glucose syrups is directly proportional to D.E. at constant refractive index. Hence determination of osmotic pressure and refractive index are sufficient for a rapid physical determination of D.E. without reference to chemical procedures. Obviously the

same technique is applicable for determining the "hypothetical D.E." of hydrogenated glucose syrups.

CHROMATOGRAPHY OF CARBOHYDRATES

Chromatography of sugars is the only available method for determining one sugar separately from others. Resolution of isomers may be achieved by paper, thin-layer, gas-liquid and high performance liquid chromatography but for resolution of anomeric forms of the same sugar probably only gas liquid chromatography (GLC) has shown any promise.

Whereas paper and thin-layer chromatography are attractive in terms of simplicity and cheapness most carbohydrate analysis laboratories now employ GLC and high performance liquid (HPLC) systems. GLC is a convenient method for determining food sugars after the molecules are first derivatised by coverting them to trimethylsilyl ethers or other suitable volatile forms (Birch, 1973) and it remains the most sensitive method available for determining trace amounts of sugars in mixtures. Lian-Loh et al (1982) used GLC for the determination of traces of sorbitol and maltitol in biological fluids and excreta during the study of the metabolic fate of maltitol. Such studies are the only means available for ascertaining the safety of novel carbohydrate foods by elucidating the metabolic absorption and excretion patterns (Lian-Loh, 1982). They have contributed to the clearance of many hydrogenated sugars as permitted sweeteners in the U.K. (1983). GLC is also useful for separating mixtures of oilomers such as in glucose syrups. Oligomers up to DP 9 may be directly determined (Birch, 1985).

The great sensitivity of GLC distinguishes it as the best method for estimating trace constituents in the presence of a vast excess of different sugars. Kheiri and Birch (1969) for example determined levels of levoglucosan between 0.01 and 0.20% in glucose syrups by GLC (Table 1) and used this information to identify their method of manufacture. Levoglucosan is a simple, stable anhydro product of glucose and an index of heat/acid treatment.

TABLE 1 : LEVOGLUCOSAN IN GLUCOSE SYRUPS AND THEIR METHOD OF MANUFACTURE

Syrup No.	Levoglucosan (%)	D-Glucose (%)	Maltose (%)	DE	Method of Manufacture
1	0.026	3.8	8.2	21.3	Acid to 10-12 DE then enzyme
2	0.110	18.2	12.7	39.2	Acid only
3	0.103	34.3	21.0	55.8	Acid to 40 DE then enzyme
4	0.084	32.5	26.4	62.6	Acid to 36-40 DE then enzyme

High performance liquid chromatography (HPLC) is the most modern method for resolution of sugar mixtures. It employs a pressurized column and allows the separation of sugars about as rapidly as GLC. The great advantage of HPLC is that sugars dissolved in water may be applied directly to the column and eluted with water or acetonitrile or mixtures of these solvents, i.e. no derivatisation is needed. Some typical column procedures are listed in Table 2 and Macrae et al (1982) and Pinthong et al (1980) have demonstrated the value of HPLC for anlaysis of sugar mixtures in soy products and malted products; mixtures of oligosaccharides as in glucose syrups can be conveniently resolved (Macrae, 1985). A range of different types of detector have been utilised in this method (Macrae, 1985) (Table 3).

TABLE 2 : TYPICAL TYPES OF HPLC COLUMN

1. Ion-exchange methods (e.g. Aminex A-6)
2. Borate complexing procedures (distinguishes sugars by configuration of hydroxyl substituents).
3. Partition (e.g. Spherisorb-NH_2).
4. Absorption with amine-modifier.
5. Size exclusion (e.g. molecular sieves).

TABLE 3 : TYPICAL DETECTORS IN HPLC

1. Refractive Index (RI)
2. Ultraviolet (UV)
3. Derivatisation and Spectrophotometry
4. Moving Wire Detector (FID principle)
5. Mass Detector

INSTRUMENTAL AND MODERN METHODS OF ANALYSIS

One instrumental technique known for about 20 years is Attenuated Infrared Reflectance spectrometry (AIR), also known as Multiple Internal Reflection spectrometry (MIR). In this method a beam of IR radiation is passed through an crystal surrounded by the sample and it undergoes a series of relfections from the walls (in contact with the sample), each reflected causing some absorbance. The total loss by absorbance of the multiply reflected beam at particular wavelengths is proportional to the carbohydrate content and the advantage of the technique is that carbohydrate may be determined as an extract in the presence of other food components (Kennedy et al, 1985) at a wide range of concentrations in aqueous solution.

Other modern techniques include nuclear magnetic resonance (NMR) and mass spectrometry (MS). The former measures properties and functions of total resonating protons and is therefore of use in food extracts which have been purified to contain only carbohydrates. High resolution NMR (in D_2O solutions) allows signals to be assigned to

particular ring protons and anomeric ring protons in particular may be distinguished from other ring protons with facility. Birch and Kheiri (1971) therefore utilised this technique for measuring the total anomeric ring protons of a series of glucose syrups. This offers an alternative (physical rather than chemical) method for measuring degree of hydrolysis and hence DE (Table 4). High resolution NMR studies of carbohydrates in D_2O or other polar solvents is limited by overlapping of signals due to the different ring protons. For this reason most structural studies have been carried out on carbohydrate acetates, benzoates or other suitable derivatives. Singlet, doublet and multiplet signals of the ring protons enable coupling constants to be calculated which in turn allow conformations to be determined.

TABLE 4 : DEXTROSE EQUIVALENTS (DE) AND DEGREE OF HYDROLYSIS BY NMR OF SOME LABORATORY ACID-CONVERTED GLUCOSE SYRUPS.

DE	Degree of hydrolysis (by NMR)
11.0	9.0
15.0	15.0
28.0	25.0
33.0	31.2
68.0	71.5

After receiving pulses of radiation, the protons interact to exchange their spin energies and this allows spin-spin relaxation times to be determined. The latter give clues to the state of carbohydrate molecules in solution and in particular to the ways in which carbohydrates may disturb water structure. These properties, in conjunction with many solution-thermodynamical functions of sugars, are important for predicting their behaviour in food products. The entire subject of NMR analysis of carbohydrates has recently been reviewed by Rathbone (1985).

Mass spectrometry is chiefly of use in the structural elucidation of new compounds. There is a no special way in which its use for carbohydrate analysis differs from other compounds.

A possible future instrumental method for carbohydrate determination is stopped-flow analysis. The method has already been widely applied to studies of enzyme kinetics (e.g. Morpeth et al, 1982) and can be used to study the rates at which molecules are changing. Typical modern instruments allow sweep times of between 5.0 and 0.01 seconds to be studied and rate constants to be automatically calculated. Their value for investigations of very rapid interactions of carbohydrates in food is therefore obvious.

An exciting new moder method of food analysis is immunoassay. Clearly this is most applicable to protein determination and most food carbohydrates are too low in molecular weight to elicit an immune response. But as "haptens" they may be made immunogenic by attaching them chemically to an immunogenically inert carrier macromolecule

such as serum albumin and an antibody can be raised against them (Hitchcock, 1984). Antibodies therefore represent highly specific reagents for food analysis. A very recent development of this is the use of enzyme labels immobilised on an inert support (mmuno-sorbent) or alternatively the antibody may be absorbed onto the inert support. This technique is known as enzyme-labelled immunosorbent assay (ELISA) and has, for example, been used for the determination of collagen (Daussant and Bureau, 1984).

ENZYMIC ANALYSIS OF CARBOHYDRATES

There are now many specific enzymic methods available for the determination of individual sugars and polysaccharides (Bergmeyer, 1984) and these may involve the measurement of absorbance changes at visible or UV wavelengths. Several such methods centre on the coupling system NADP/NADP-H and offer a highly convenient route for analysis of a large number of samples in one batch such as is common in the clinical field. Commercial enzyme "kits" are available for many of the common food sugars and simple colorimetric instruments are available to facilitate the process even further. One such device is the Reflomat (Irmscher et al, 1974), for rapid determination of glucose in blood and plasma. The drawback of such facilities is that they are not precise but rapid and they are only designed for a single purpose, e.g. clinical anlaysis. However, Birch et al (1978) have demonstrated that the Reflomat has a limited applicability for the determination of D-glucose in food samples.

Enzymic methods may be convenient for the determination of small amounts of insoluble polysaccharides even though hydrolysis is not complete. Cellulose, for example, may be partially hydrolysed (solubilised); then total dissolved carbohydrate can be determined by a modified aldehyde procedure as described above (Bergmeyer, 1984). Starch is possibly best determined by disintegrating the sample with dimethyl sulphoxide/HCl mixture then hydrolysis with amyloglucosidase. The resulting glucose is then estimated by hexokinase and glucose 6-phosphate dehydrogenase (G6P-DH). The final measurement is the change in absorbance at 339 nm due to formation of NADP-H. The entire sequence of reactions is therefore as follows (Bergmeyer, 1984):–

(a) $\text{Starch} \;\; 3 \;\; (n\text{-}1)\,H_2O \xrightarrow[\text{hydrolysis}]{\text{Amyloglucosidase}} n\ \text{Glucose}$

(b) $\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate} + \text{ATP}$

(c) $\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow{\text{G6P-DH}} \text{D-gluconolactone-6-phosphate} + \text{NADPH} + H^+$

NOVEL CARBOHYDRATE DERIVATIVES AND SWEETENERS AND THEIR ANALYSIS

Although deoxy sugars and monosubstituted sugars have properties which placed them clearly within the chemical definition of "carbohydrate" they are not legally accepted as carbohydrates in the UK. On the other hand, certain classes of sweet water-soluble substances such as the inositols, would fall within the UK classification of "carbohydrate" because they contain only carbon, hydrogen and oxygen with the latter two elements present in the same proportion as in water. The inositols are cyclic polyols and do not fit within the chemical definition of sugars. They represent only a small proportion of the

large number of derivatives now available for study and use. The new UK Sweeteners in Foods Regulations (HMSO, 1983) listed a number of sugar alcohols permissible in foods as sweeteners (not carbohydrates) and this has led to an almost impossible analytical problem of determining them in foods. The determination of all the individual sugar alcohols in hydrogenated glucose syrup could not yet, for example, be contemplated. Likewise the derivatisation of sugars to form acetates, benzoates (Sheridan et al, 1980) for bittering purposes, produces esters and partial esters akin to the complex mixtures in use as emulsifiers, stabilisers and thickeners in food. Unless a single component is sought for identification purposes the analytical task becomes practically impossible. Probably GLC is the best available method for determining a single molecular species in such circumstances.

Hydrogenation of sugars produces sugar alcohols which are already being widely used in food science and technology. A newer set of derivatives are the oxidised aldoses, i.e. aldonolactones. The lactones themselves are interesting structural analogues of the sugars but, probably because they hydrolyse to aldonic acids once in contact with water, they tend to be sour in taste. D-glucono-δ-lactone is a permitted UK acidulant. An indication of how oxidised glucose syrups may be prepared has already been reported (Gallali and Birch, 1985). Yet another set of derivatives are the chlorosugars. Some of these are already reported as intensely sweet (Hough and Phadnis, 1976); others are reported as bitter (Khan, 1979). Their analytical chemistry has not yet been reported but there is little doubt that chromatography will be the means by which their presence in food could be determined.

SENSORY ANALYSIS OF CARBOHYDRATES

Sensory anlaysis is of practical importance in food manufacture and centres on their quality of sweetness (acceptability) and their sweetening power. Trained taste panels provide the only means to achieve a reliable sensory appraisal and a battery of threshold, ranking, scaling, difference testing and magnitude estimation techniques are available. One recent problem of practical significance with all sweetening agents is their "persistence" of response (Birch, 1985) and intensity/time analyses of sweetness have elaborated the study of structure-sweetness relationship in many novel molecules (Birch and Munton, 1981).

CONCLUSION

Analysis of food carbohydrates covers physical, chemical, simple and complex methods and combinations of these for rapid control and standardisation of food composition. These techniques may need correlation with psychophysical analyses in many food applications. The changing pattern of food carbohydrate chemistry has created an enormous range of problems, the important aspects of which are already being solved by modern analytical procedures.

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