Carbohydrate Polymers **15** (1991) 265–269

STARCH: Legislation on Purity Measurement

J. F. Kennedy,[a] V. M. Cabalda[a] & K. Jumel[b]

[a]Research Laboratory for the Chemistry of Bioactive Carbohydrates and Proteins, School of Chemistry, University of Birmingham, Birmingham B15 2TT, UK
[b]Chembiotech Ltd, Institute of Research and Development, University of Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, UK

(Received 15 April 1990; accepted 10 May 1990)

ABSTRACT

Substantial rebates are given to end-users of food grade starches, having minimum purity of 97%, in non-food applications. This paper presents the various methods for starch purity determination being considered by the EC Starch Expert Group for legislation, with discussions on their respective disadvantages. Discussions on possible improvement and optimization of these existing non-enzymic and enzymic procedures are also given.

Legislation regarding the measurements of starch purity in starches has become an increasingly important matter for the European Community (EC). This paper investigates the reason for the problems and past and present proposals for its solution.

Starch is present in all staple foods such as rice, potato, wheat, maize, oats, sago, etc. It is made up of a mixture of linear and branched homopolymers (amylose and amylopectin respectively) of D-glucose. Amylose is a predominantly linear polymer, consisting of α-D-glucopyranose units linked by $(1 \rightarrow 4)$ bonds, whereas amylopectin is made up of short chains of α-D-glucopyranose units linked by $(1 \rightarrow 4)$ bonds, which are joined by $(1 \rightarrow 6)$ linkages to form a highly branched structure. It is due to this structure that starch is such a food reserve — the breakdown of the starch molecule to glucose, within the metabolism is relatively slow, thus providing a sustained source of energy. However, starch is also of major use as a raw material for the production of hydrolysates such as sweeteners in drinks and confectionery and as thickeners of sauces, pies, etc., leading to a large demand for food grade starches. Since these starches are highly profitable the demand was met by more and more

Carbohydrate Polymers 0144-8617/91/$03.50

starch manufacturers switching to food grade (i.e. high purity) starches, and ultimately creating a large surplus.

In order to reduce this surplus within the EC, substantial rebates are now given to end-users who utilize food grade starches (minimum purity 97%) in non-food applications for example as a feedstock in the chemical industry. However, such a rebate scheme requires close monitoring of starch purity and it is the lack of a method officially adopted by the EC for this purpose which is the cause of the problem.

There is no shortage of methods for measuring starch being put forward for official adoption but the arguments within the EC starch working party seem to be never-ending, partially due to the intransigence of some sectors who are unwilling to compromise on their particular method and partly due to scientific based arguments on the acccuracy and reproducibility of the methods. Basically, the proposed methods can be classified into two categories: non-enzymic methods and enzymic methods.

NON-ENZYMIC METHODS

These are the methods still most widely used today, since they are more convenient and produce higher more favourable values (from the viewpoint of rebate) of starch purity values than do the enzymic methods. However, most of these methods have serious drawbacks concerning accuracy, reproducibility and reliability and may not be applicable to all types of unmodified starch. The methods which fall into this category are: the Difference Method, the Ewer's Polarimetric Method (OJEC, 1972), the Calcium Chloride Polarimetric Method (AOAC, 1984) and the Saccharification Method (OJEC, 1969).

The Difference Method is based on the assumption that the difference between the sum of the percentage contents of protein, ash, lipid and moisture content of the starch and 100%, is equal to pure starch. This leads almost certainly to an overestimation of the starch purity since non-starch components such as soluble dextrins, hemicelluloses, nucleosides, etc., would not be detected if present. However, this method has been and is still used as the reference method by the EC and starch manufacturers.

The other non-enzymic methods rely on the dispersion of starch by various reagents and measurement of the optical rotation (polarimetric methods) or the amount of reducing sugar produced (Saccharification Method). The problem with these methods is that their repeatability and reproducibility values vary by far too much to be permanently adopted

as official methods for measuring starch purity in starch. Polarimetric methods are instrument operator dependent to some extent and saccharification methods may be easily interfered with by contamination, and both these procedures are dependent upon the conversion of the starch to glucose — a conversion which may or may not be complete, no checks being made in the assay procedure. However, the modified Ewer's method has temporarily been adopted as an official method, despite its sometimes reported lack of reliability, until a better method is found. Apart from the above mentioned drawbacks, non-enzymic methods also suffer from a lack of specificity and are, therefore, open to abuse.

However, at present, starch manufacturers and customs are reluctant to change to enzymic methods, due to the lower purity data obtained and due to the convenience and ease with which non-enzymic methods can be performed.

ENZYMIC METHODS

Due to the problems regarding accuracy, reliability and specificity experienced with the non-enzymic methods, the measurement of starch purity in starch has been approached from a different angle. Enzymes would break the starch molecule down into single glucose units which could then be easily determined. However, the development of these methods was not trouble-free either, with problems mainly arising from incomplete hydrolysis of the starch and, therefore, an underestimation of its purity content.

The development of enzymic methods was led by the Swiss enzyme method (OJEC, 1986). This method suffered from incomplete hydrolysis due to the amount of enzyme used — amyloglucosidase, which slowly hydrolyses the $(1\rightarrow6)$ branch points in starch. However, the amylose/amylopectin ratio in starches differs depending on the type of starch and its origin and the amount of enzyme used was insufficient to give repeatedly complete hydrolysis of all unmodified starches.

Recognition of the importance of the specificity of the method, however, led to further developments and a method by the Association Francaise de Normalisation (Afnor) has recently been ring-tested within the EC. This method also uses amyloglucosidase as the starch degrading enzyme but in higher concentrations and has shown considerable improvement on the Swiss method. However, recent gel permeation chromatography (gpc) studies by our research groups at Birmingham University have shown that some starches are still not

completely hydrolysed with up to 25% of the starches still existing as malto-oligosaccharides (Kennedy *et al.*, in press).

In order to improve hydrolysis still further, the Birmingham Research Group introduced a multi-enzyme regime consisting of α-amylase, amyloglucosidase and pullulanase which is a debranching enzyme. The use of this method has been shown by gpc to hydrolyse a wide range of starches virtually completely (99·5%) (Kennedy *et al.*, 1989) to glucose and it is hoped that this method will undergo ring-testing in the near future.

The above shows that the use of highly specific enzyme systems will become increasingly desirable due to their specificity towards starch. However, reproducibility and repeatability of the methods still have to be proven before a decision regarding their adoption for official EC use can be made. Previous concern by present users and advocates of non-enzymic methods regarding enzyme preparations and possible errors in glucose determination are easily answered. Liquid enzyme preparations are commercially available and relatively cheap and stable, and the accuracy of glucose determinations can be increased by the use of automatic glucose analysers or HPLC methods. HPLC equipment is present in most analytical laboratories nowadays and for the cost of an extra column, reliable glucose determinations could be made.

It is obvious that the EC urgently needs a reliable method for the determination of starch purity in starch. Most non-enzymic methods have been shown to suffer from a lack of reproducibility and a definite lack of specificity, whilst it remains to be proven unequivocably that enzymic methods can totally hydrolyse all types of unmodified starches although the most recent studies indicate that this is the case. Speedy ring-testing of the most recent enzymic methods should, therefore, be carried out, so that a decision on an official method can be made before the next harvest.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Agriculture, Fisheries and Food, UK, and is Crown Copyright.

REFERENCES

AOAC (1984). *Methods of Analysis Manual.* Association of Official Agricultural Chemists, Washington, DC, p. 254.

Kennedy, J. F., Cabalda, V. M., Stevenson, D. L. & White, C. A. (1989). In *Biomedical and Biotechnological Advances in Industrial Polysaccharides*, ed. V. Crescenzi, I. C. M. Lea, S. A. Paoletti, S. S. Stiwala & I. W. Sutherland. OPA, Amsterdam, pp. 313–33.
Kennedy, J. F., Cabalda, V. M. & White, C. A. (in press). *Starke*, (RP 372).
Official Journal of the European Community (1969). **L141**, 261–2.
Official Journal of the European Community (1972). **L123**, 75–6.
Official Journal of the European Community (1986). **L158**, 3–5.