PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 513th Meeting of the Society was held at the Lord Rank Research Centre, High Wycombe, Buckinghamshire, on Thursday, 18 March 1971, when the following papers were presented.

COLLOQUIUM ON 'CEREAL AND VEGETABLE STARCHES IN USE: THEIR CHEMISTRY AND BIOCHEMISTRY'

Aspects of the Chemistry of Vegetable Starches

By C. T. GREENWOOD. (*Flour Milling and Baking Research Association, Chorleywood, Herts. WD3 5SH, U.K.*)

The physical and chemical characteristics of a starch depend uniquely on the botanical sources, and consequently a wide diversity of starches is found in vegetables. The fundamental properties of typical vegetable starches will be outlined, including the amylose/amylopectin ratio, the changes that occur during growth of the plant and the susceptibility of the granule to enzymic attack, and the ease with which dispersion of the resultant starch components will be discussed. The features of these typical vegetable starches, which differ from the cereal starches, will be outlined.

The Structures of Amylopectins and Glycogens

By W. J. WHELAN. (*Department of Biochemistry, University of Miami, Miami, Fla. 33152, U.S.A.*)

An enzyme (isoamylase) capable of the total debranching of amylopectin and glycogen has been discovered in a species of *Cytophaga* (Gunja-Smith, Marshall, Smith & Whelan, 1970). An examination of amylopectin and glycogen after sequential treatment with phosphorylase, β -amylase and isoamylase revealed facts at variance with the regularly debranched version of the structure proposed by Meyer & Bernfield (1940). A new arrangement of the unit chains of both macromolecules has therefore been proposed (Gunja-Smith, Marshall, Mercier, Smith & Whelan, 1970). The structure will be discussed in terms of its compatibility with the action patterns of the enzymes of metabolism.

Consideration will also be given to the roles of hydrolytic enzymes in starch and glycogen synthesis. Such enzymes may make important contributions to the polysaccharide structure when there is a lesion involving one of the normal enzymes of synthesis.

A fuller account of this work has been published by Whelan (1971).

Gunja-Smith, Z., Marshall, J. J., Mercier, C., Smith, E. E. & Whelan, W. J. (1970). *FEBS Lett.* **12**, 101.

Gunja-Smith, Z., Marshall, J. J., Smith, E. E. & Whelan, W. J. (1970). *FEBS Lett.* **12**, 96.

Meyer, K. H. & Bernfield, P. (1940). *Helv. chim. Acta*, **23**, 875.

Whelan, W. J. (1971). *Biochem. J.* **122**, 609.

Some Industrial Aspects of the Enzymic Degradation of Starch

By D. J. MANNERS. (*Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1 1HX, U.K.*)

Although the use of amylolytic enzymes in the food and fermentation industries is steadily increasing (see, e.g., Saletan, 1968), the present paper is confined to a discussion of only two developments. These are the production of glucose and glucose syrups from starch, and the replacement of malt by barley and enzyme preparations in the brewing process.

Starch may be quantitatively converted into D-glucose by amyloglucosidase (glucoamylase) preparations (for a review see Fleming, 1968). The substrate is attacked from the non-reducing end and the stepwise hydrolysis of successive glucosidic linkages gives glucose as the primary product. Amyloglucosidases are produced by many fungi, e.g. *Aspergillus* sp., *Rhizopus* sp., *Endomyces* sp., and several examples have been selected for large-scale production, particularly by the Japanese fermentation industry.

Amyloglucosidases have only limited action on starch granules, and a preliminary treatment of these is necessary. In the 'acid-enzyme' conversion process the starch is treated initially with hot mineral acid under pressure, to effect gelatinization and solubilization, and to bring about some degradation of polysaccharide material. This 'thinning' action is then followed by enzymic saccharification of the neutralized starch hydrolysate. More recently, preliminary treatment of the starch with a relatively thermostable bacterial α -amylase has been used in an 'enzyme-enzyme' conversion (Denault &

Underkofler, 1963). The saccharification may take up to 96h at 45–60°C and pH 3.3–4.4, depending on the properties of the amyloglucosidase. The final yield of glucose, or mixture of glucose and maltosaccharides at intermediate stages of hydrolysis, is not related to the botanical source of the starch. The enzymic saccharification is equally effective with wheat, maize and potato starches, although since these starches differ in certain physical properties, e.g. gelatinization temperature, the exact conditions of the preliminary 'thinning' treatment may have to be varied.

Some amyloglucosidase preparations also show transglucosidase activity, i.e. the ability to form oligosaccharides such as isomaltose and panose, by the transfer of a glucose residue to glucose or maltose. This side reaction may significantly decrease the final yield of glucose and must therefore be minimized either by the selection of a strain of mould that is low in transglucosidase, or by preliminary purification of the enzyme preparation.

In addition to the manufacture of D-glucose by the complete saccharification of starch, partial enzyme conversions yield glucose syrups, which are mixtures of glucose, maltose and various amounts of higher maltosaccharides. The exact composition of the glucose syrups, which are widely used in the food confectionery industry (Maiden, 1970), can be controlled by the conditions of the saccharification.

A substantial amount of research effort is being devoted to the preparation of enzymes that are bound to insoluble supports (for a review see Barker & Epton, 1970). A promising development for the future is the binding of amyloglucosidase to DEAE-cellulose (Bachler, Strandberg & Smiley, 1970). This may enable a continuous system for the conversion of starch into glucose to be developed, provided that adequate flow rates through the columns can be maintained, by an effective preliminary α -amylolysis. Columns of this type have been operated for more than 3 weeks without any apparent loss in activity.

The traditional brewing process involves the use of malted barley, in which the cereal carbohydrates have been made more susceptible to enzymic degradation by the dissolution of the endosperm cell walls during germination. During the last few years attempts have been made, with various degrees of success, to replace the malt to a large extent by ungerminated grain. This is treated with a mixture of hydrolytic enzymes, and the resultant worts have then been converted into acceptable beers.

In theory, the action of a thermostable α -amylase on starch will give maltose and small amounts of glucose and α -limit dextrins. In the presence of other carbohydrases (e.g. maltase, limit dextrinase) the conversion of the whole starch into fermentable

sugars should be virtually quantitative. In practice, the starch granules in ungerminated cereals may not be wholly accessible to amylolytic degradation, since the endosperm walls contain substantial amounts of β -glucan and hemicelluloses. The presence of these polysaccharides, which are normally degraded during malting, may result in incomplete degradation of the starch, and give rise to a wort of high viscosity. Both factors may cause filtration problems. In the direct production of wort from barley it is therefore essential to consider the solubilization and enzymic degradation both of starch and of the other cereal polysaccharides.

Favourable results have recently been reported with Brew-n-zyme, a bacterial enzyme preparation having high amylolytic and proteolytic activity (Wieg, Hollo & Varga, 1969; Wieg, 1970). The enzyme preparation, which also shows various endo- β -glucanase and β -glucosidase activities (D. J. Manners & G. Wilson, unpublished work), retains its α -amylolytic activity at temperatures up to 90°C. Experiments have been carried out with mixtures of 80% barley and 20% malt, with Brew-n-zyme added at a concentration of 0.075% based on the weight of barley. The resultant worts have been brewed on both the pilot-plant and industrial-brewery scale to give a lager-type beer with very satisfactory analytical and organoleptic qualities. Although there are certain technological difficulties in the optimum grinding of the barley and in the filtration of the mash, it is clear that the enzymic digestion of ungerminated barley provides an alternative to malt that may offer substantial production and economic advantage to the brewing industry.

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