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Modification of barley starch by α-amylase and pullulanase

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The hydrolysis of the amylopectin of gelatinized waxy barley starch by *Bacillus licheniformis* α-amylase occurred in a non-random way, showing accumulation of smaller-size hydrolysis products. The hydrolysis of barley starch by *Bacillus acidopullulyticus* pullulanase also began with a rapid decrease in the molecular size of amylopectin, indicating that the enzyme was capable of hydrolyzing the α-1,6-linkages in the middle of the amylopectin molecule.

INTRODUCTION

α-Amylases have long been used for starch liquefaction in the production of dextrose syrups. Pullulanases are mainly used in syrup production to improve yields (Norman, 1982). Both α-amylase and pullulanase can, however, also be used to produce starch hydrolysis products with higher molecular weight. Low-DE-maltodextrins and other enzymatically modified starch products with specific functional properties have many existing and potential applications both in the food industry as nonsweetening stabilizing agents, emulsifiers and fat replacers, and in the paper industry as adhesives and in coating and surface sizing of paper. Pullulanase treatment has been suggested for improvement of starch gelling properties in confectionery (Chiu & Zallie, 1989).

The aim of this study was to monitor the early stages of depolymerization of starch for further development of specific high-molecular weight starch hydrolysis products.

MATERIALS AND METHODS

The barley starch used was a commercial product of Raisio Group (Finland). The waxy barley starch was obtained from Alko Ltd (Finland). The enzymes were *Bacillus licheniformis* α-amylase (Sigma, type XII A, A-3403) and a previously purified *Bacillus acidopullulyticus* pullulanase (Lappalainen *et al.*, 1991).

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The changes in the molecular size distributions of pregelatinized starches were studied using gel permeation chromatography (GPC-HPLC) with refractive index detection and by post-column iodine addition and spectrophotometric detection (Suortti & Pessa, 1991). μHydrogel columns 2000, 500 and 250 were used in series and eluent was 50 mM NaOH. The action of enzymes on the microstructure of gelatinized starch was studied using light microscopy with the smear technique and iodine staining (Autio, 1990). The solubility of starches was determined as described previously (Pessa *et al.*, 1992).

RESULTS AND DISCUSSION

Granule residues were still visible in a 5% barley starch paste after gelatinization in boiling water bath for 10 min. The α-amylase treatment caused rapid solubilization of the granule residues, which was well visualized by light microscopy (results not shown). The degradation of amylopectin was also observed by GPC. The hydrolysis mechanism of amylopectin was clearly seen in waxy barley starch (Fig. 1). The hydrolysis proceeded in two stages: *B. licheniformis* α-amylase first hydrolysed amylopectin between the clusters to rather high molecular weight products. Post-column iodine binding at 630 nm showed that at the same time the changes in the amylose fraction were smaller (Fig. 1(b)). As the hydrolysis proceeded, smaller amylopectin fragments were observed (Fig. 1). Acid hydrolysis of barley starch granules has been shown to proceed in an

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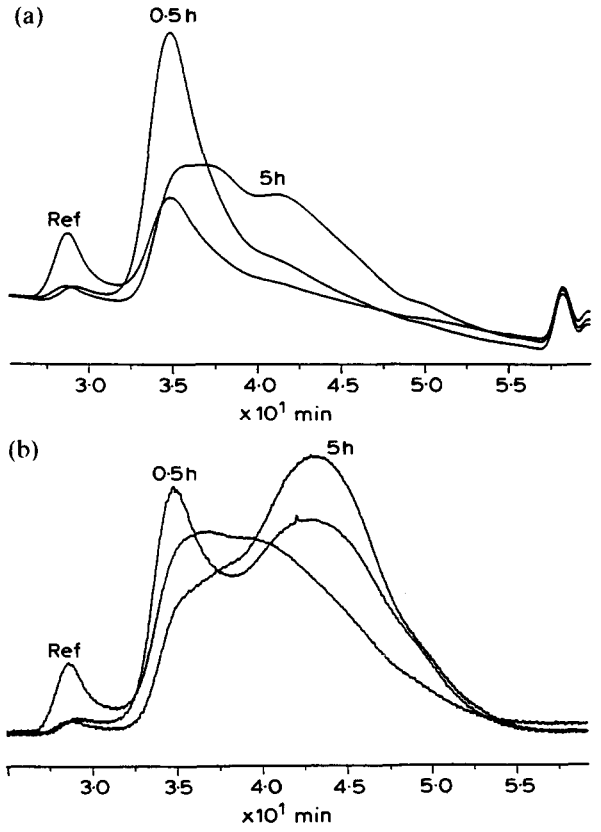

Fig. 1. The effect of α-amylase treatment (5 mU/g, 70°C, pH 6·0) on the molecular size distribution of waxy barley starch: (a) refractive index detection, (b) iodine binding at 630 nm.

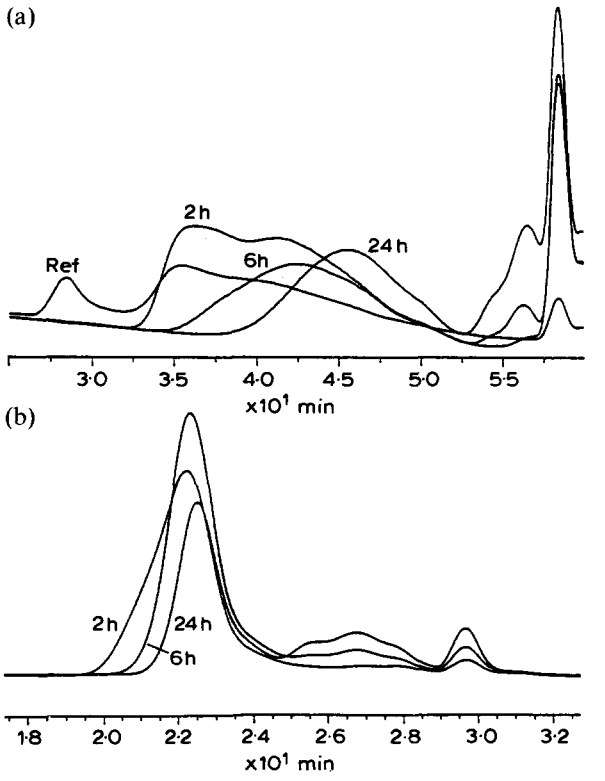

Fig. 2. The effect of pullulanase treatment (100 nkat/g, 50°C, pH 5·0) on the molecular size distribution of barley starch. Refractive index detection: (a) large molecules, (b) small molecules.

analogous way (Autio *et al.*, 1992). Rapid degradation of amylopectin and the non-random action of α-amylase has also been reported by Bertoft (1986) and Chang-Rupp and Schwartz (1988).

During the first changes in molecular size distribution very little formation of reducing sugars was observed (Table 1). Only when all the intermediate hydrolysis products of amylopectin had further degraded, the progress of the hydrolysis was reflected as a substantial increase in reducing sugars. The solubility of barley starches treated with α-amylase increased with the degree of hydrolysis (Table 1). The hydrolysis products of waxy barley starch were more soluble than those of ordinary barley starch (Table 1).

The debranching of starch by pullulanase also started by a rapid decrease in the molecular size of amylopectin, indicating that the enzyme effectively hydrolysed the α-1,6-linkages inside the amylopectin molecule between the clusters (Fig. 2(a)). As the hydrolysis proceeded, the simultaneous gradual release of the external chains of amylopectin was observed (Fig. 2(b)). The trimodal distribution of the external chains of barley starch amylopectin has also been reported earlier (MacGregor & Morgan, 1984).

Table 1. Properties of barley and waxy barley starches treated with α-amylase: 5 mU/g, 70°C, pH 6·0, ethanol precipitation, acetone and air drying

Starch type	Time (h)	Reducing sugars (%)	Solubility at 85°C (%)
Normal	—	0·9	21
Normal	0·5	0·7	33
Normal	2	1·4	31
Normal	5	1·9	41
Waxy	—	0·4	52
Waxy	0·5	0·5	74
Waxy	2	0·9	78
Waxy	5	1·6	70

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