A NOTE ON THE ENZYMATIC REDUCTION OF PROTEIN IN HIGH-AMYLOSE CORN STARCH[1]

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Recent articles published by the Northern Regional Research Laboratory on experimental wet-milling of high-amylose corns containing starch with 49 to 57% amylose indicate that recovery of low-protein starch presents some difficulty (2,3). In the experimental milling where starch-gluten separation was carried out on starch tables, double

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tablings were necessary to obtain a fair separation. While it would be expected that better results would be obtained by using centrifuges and other machinery commonly found in the starch industry, it seemed of interest to investigate another method of reducing the protein content of this starch. The treatment of corn starch with a proteolytic enzyme to reduce the protein content has been described in the patent literature (4). In this procedure, the proteins in the starch were made soluble by the enzyme pepsin, and were removed by decantation and filtration.

This paper reports results of a short study on the effect of certain variables in the reduction of proteins by the enzymatic action of trypsin and papain on high-amylose corn starch (57% amylose content).

The starting material was prepared from corn with starch containing 57% amylose by the small-scale wet-milling procedure described by Anderson *et al.* (3).[2] The starches used in this work were from the first tabling of the mill starch, and their protein contents varied from 0.81 to 1.27%. In all experiments 50 ml. of starch slurry (18.7 – 19.6% dry solids) were subjected to the enzyme treatments. One-percent stock solutions of trypsin and papain[3] were prepared by dissolving the enzyme in distilled water and filtering it to remove any sediment. The bases of selection for these enzymes were their commercial availability, relatively high purity, and freedom from amylases, etc. Because of the limited scope of the study, other enzymes were not investigated.

The experiments were carried out in the following manner: The pH of the starch slurry was adjusted to the desired level with either 1*N* acetic acid or 1*N* sodium hydroxide, the enzyme solution was then added, and the mixture was held at the specified temperature for the required period of time, with occasional shaking. Continuous agitation had negligible effect on these small samples. The starch slurry was then filtered, washed, and dried. A control sample was included in each test.

The moisture in the starch was determined by drying a sample for 4 hours at 100° – 110°C., under a vacuum of 28 in. of mercury. Protein (N × 6.25) was determined by the improved Kjeldahl method for nitrate-free samples (1).

Like most proteolytic enzymes, trypsin and papain have an opti-

[2] Corn was provided by the American Maize-Products Co., Roby, Indiana, and the National Starch and Chemical Corp., Plainfield, N. J. The corn was grown from strains developed by Bear Hybrid Corn Co., Decatur, Ill.

[3] Difco trypsin (Ref. No. 359773) and purified papain (Meer Standard Lot 8-5050XXP). Use of these enzymes does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over others not mentioned.

mum pH range in which their hydrolytic activity is at its greatest. Experiments conducted to establish the proper pH for carrying out each protein hydrolysis showed that in the pH range 4.3 – 6.1 papain reduced the protein content of the starch from 0.81 to 0.60%. A higher pH range, 6.1 – 8.8, was found suitable for trypsin, with the protein content reduced from 0.81 to 0.52 – 0.56%.

As little as 0.2% of trypsin was found to reduce the protein content of the starch from 1.27 to 0.42%. When the enzyme concentration was increased to as high as 2.0%, no further protein reduction was attained. An addition of 0.5% of papain resulted in a reduction of protein from 1.27 to 0.56%. As noted with trypsin, increasing the enzyme concentration had no effect on protein reduction.

It was noted that both papain and trypsin could be used effectively at a wide range of temperatures to reduce protein content. The greatest enzyme activity occurred with both enzymes at a temperature of 40°C. The best protein reduction was obtained with trypsin, where the protein in the high-amylose starch was reduced from 1.27 to 0.42%.

Proteolytic activity started almost immediately upon addition of the enzymes to the starch slurry and hydrolysis of the protein was completed in about 2 hours. When papain was used, a reduction in protein content from 0.81 to 0.69% was realized in 2 hours of hydrolysis; in 16 hours, 0.66% of protein remained in the starch. Similarly, in 2 hours trypsin reduced the protein content of the starch to 0.52%; the same reduction was attained after 16 hours of proteolysis.

Literature Cited

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