IN VITRO DIGESTIBILITY OF HYDROXYPROPYL DISTARCH PHOSPHATE AND UNMODIFIED TAPIOCA STARCH[1]

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ABSTRACT

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Unmodified and modified (hydroxypropyl distarch phosphate) tapioca starches were treated with fungal or pancreatic amylase under experimental conditions designed to simulate *in vivo* digestion conditions. The effects of gelatinization and retrogradation on enzyme susceptibility were investigated. Hydrolysis and alterations in granule structure were evaluated by quantitative reduction of ferricyanide and scanning electron microscopy, respectively. Ungelatinized starches were hydrolyzed to a greater extent by pancreatic amylase than by fungal amylase. The reverse was true for gelatinized starches. Modification of the starch increased the enzyme susceptibility of the ungelatinized, but decreased the susceptibility of the gelatinized starch. Acid pretreatment and retrogradation had little effect on enzyme susceptibility. The relative degree of hydrolysis of the gelatinized modified to the unmodified starch was similar for the two enzyme preparations, suggesting that either enzyme preparation would be suitable for the estimation of the digestibility of modified starches *in vitro*. Most of the gelatinized granules were destroyed by pancreatic amylase. Pores were evident in the few granules that remained.

Modified food starches vary in their susceptibility to enzymatic hydrolysis. This variation depends on the botanical origin of the starch, the modifying agent(s) used and the subsequent chemical bonds and derivatives formed, the extent of granule gelatinization, and the choice of enzyme (1,2).

Hydroxypropyl distarch phosphate is one of the chemically modified starches

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used extensively in the food industry. Modification is carried out with phosphorous oxychloride and propylene oxide. In spite of its wide usage, there is relatively little known about its digestibility or the products of digestion. In one study, a slight decrease in caloric utilization with increasing degree of modification was demonstrated (1).

More research has been carried out on two modified starches related to hydroxypropyl distarch phosphate: distarch phosphate and hydroxypropyl starch. Some reports have indicated that phosphate cross-linking slightly reduces enzymatic hydrolysis while others have stated that cross-linking has no effect on hydrolysis when compared to the unmodified starch (1). The level of phosphorous oxychloride used has been shown to affect the *in vitro* digestibility of the starch (3). Differences between unmodified and modified were reduced or nullified by boiling the starch. The available data suggest that food-grade phosphate cross-linked starches are almost completely digestible.

The *in vitro* digestibility of gelatinized hydroxypropyl starches by pancreatin was decreased with increasing substitution of hydroxypropyl groups (4). Banks *et al.* (5) demonstrated that the degree of substitution, rather than the molar substitution (MS), determined the rate and extent of amylolytic attack on hydroxyethyl amylose. French *et al.* (6) found that the position of the hydroxyethyl group on the anhydroglucose unit affected enzymatic hydrolysis by α-amylase.

The nutritional, physiological, and biochemical effects of short- and long-term consumption of hydroxypropyl distarch phosphate from tapioca have been under investigation in our laboratory. Data on changes in the intestinal microflora of rats on hydroxypropyl distarch phosphate, hydroxypropyl starch, and distarch phosphate suggest that starches containing ether linkages are more difficult to digest than those containing only phosphate linkages (7). The purpose of this study was to evaluate the digestibility of hydroxypropyl distarch phosphate *in vitro* using fungal and pancreatic amylases. The effects of various processing and storage conditions were studied.

MATERIALS AND METHODS

Starch

Unmodified tapioca starch and hydroxypropyl distarch phosphate from tapioca (MS = 0.045) were furnished by Stein, Hall and Co., Inc., New York, N.Y. The percentage hydroxypropyl groups was determined by method No. 1 of Johnson (8) and the MS was calculated from the percentage hydroxypropyl groups (9).

Processing

Starch suspensions (1 to 1.5%) were cooked in 125-ml flasks in a water bath. Cooking times and product temperatures for the different processing treatments (trt) are shown in Fig. 1. Starch was dispersed in either distilled water (pH 7.0) or 0.01*M* citric acid-phosphate buffer (pH 3.5). After being cooked, some samples were stored at 4° to 6°C for 3 days to effect retrogradation.

Hydrolysis

The objective was to select acid and enzyme treatment conditions which would

give meaningful estimates of the *in vivo* digestibility of starch. In the acid treatment, starch slurries were subjected to 0.1*N* HCl for 1 hr at 37° C. At the end of the hr, the slurries were neutralized with 1*N* NaOH. Controls were held in distilled water for 1 hr at 37° C without being acidified.

The criteria applied in selecting enzymes to meet the above objectives were similar to those applied by Friedemann *et al.* (10): 1) commercially available, soluble, standardized preparation; 2) low reducing sugar content; and 3) high potency. Table I shows the reducing sugar contents of several commercially

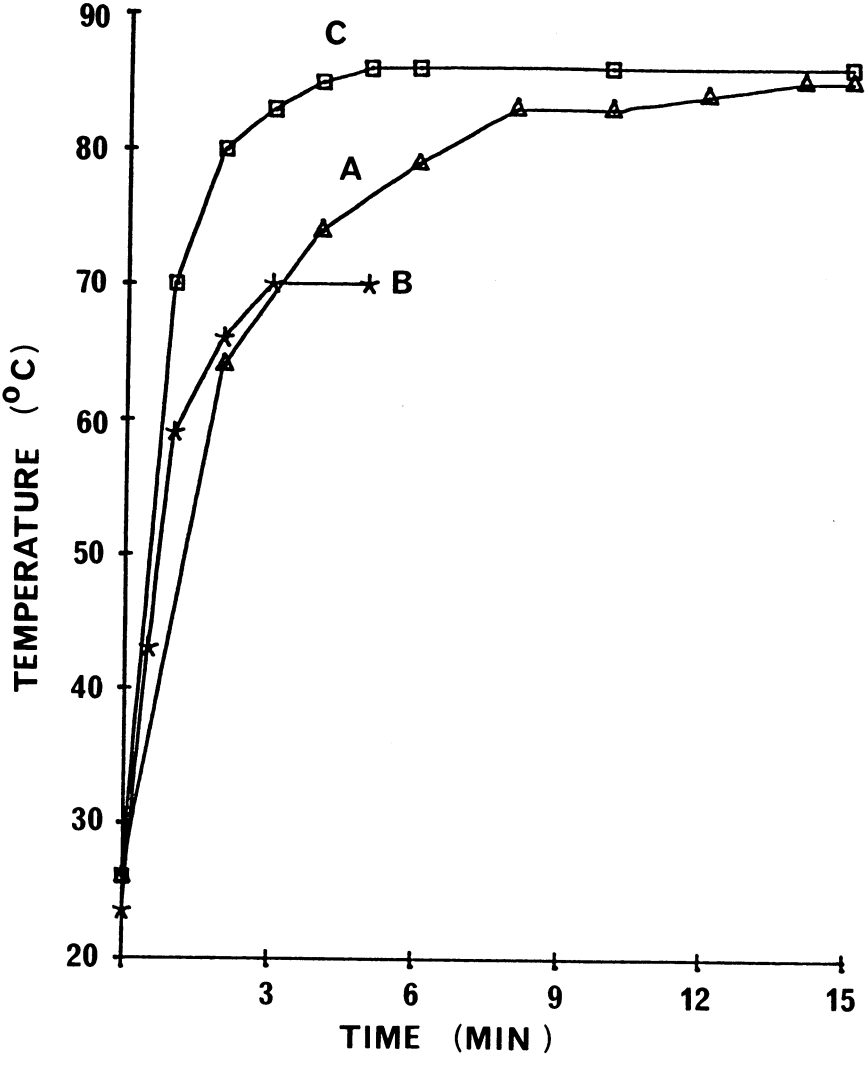

Fig. 1. Temperatures of starch suspensions during gelatinization. A, B, and C were the three treatments applied.

available enzyme preparations that were screened. Based on these considerations, two preparations were selected: Rhozyme S, a fungal amylase derived from *Aspergillus oryzae,* and α-amylase from hog pancreas.

The optimum enzyme concentration was selected to give high but not maximum levels of hydrolysis on the most easily hydrolyzed starch preparation, so that quantitative differences among starches could be determined. Table II shows the amount of hydrolysis with various enzymatic concentrations. With the fungal amylase, 0.04% was used because it met this criterion. Percentage enzyme was calculated as:

$$\frac{\text{wt enzyme}}{\text{vol reaction mixture}} \times 100$$

Although 100% hydrolysis was not attained by pancreatic amylase, 0.0056% was utilized in subsequent experiments. Both enzyme preparations were incubated at 37°C for 1.5 hr at their optimum pH: fungal amylase in 0.04*M* acetate buffer, pH 4.7, and pancreatic amylase in 0.1*M* phosphate buffer, pH 7.0.

TABLE I
Reducing Sugar Content of Enzyme Preparations

	Reducing Sugar %
Pancreatin[a]	50
Amylopsin[a]	50
Viokase[b]	7.5
α-Amylase-hog pancreas[a]	0
Rhozyme S[c]	0

[a]Nutritional Biochemical Corp., Cleveland, Ohio.
[b]Viobin Corp., Monticello, Ill.
[c]Rohm and Haas Co., Philadelphia, Pa.

TABLE II
Effect of Enzyme Concentration on Hydrolysis of Unmodified Starch[a,b]

Fungal Amylase %	Hydrolysis %	Pancreatic Amylase %	Hydrolysis %
0.025	80	0.0003	44
0.050	91	0.0007	50
0.100	99	0.0014	53
0.150	100	0.0028	57
0.200	100	0.0056	60
		0.0112	61
		0.0168	63

[a]% Enzyme = (wt enzyme/vol reaction mixture) × 100.
[b]% Hydrolysis = (mg glucose from standard curve/mg starch) × 100.

Degree of Hydrolysis

Reducing sugar was determined by the ferricyanide reduction method (10). A glucose standard curve was used to convert the absorbance of the ferricyanide-treated hydrolysate to the weight of reducing sugars. Per cent hydrolysis was calculated as:

$$\frac{\text{mg glucose from standard curve}}{\text{mg original starch}} \times 100$$

Microscopy

Gelatinized starches were prepared for examination in a MINI-SEM scanning electron microscope (11).

RESULTS AND DISCUSSION

The ungelatinized starches were hydrolyzed very little by the fungal amylase (Table III). The unmodified tapioca was hydrolyzed 2%. Acid pretreatment increased the amount of hydrolysis to almost 5%. However, this slight increase was not due to the acid. Samples held for 1 hr at 37° C at pH 7.0 were hydrolyzed to the same extent as those treated with acid for 1 hr. Therefore, it is possible that acid has no effect on the enzyme susceptibility of ungelatinized starches *in vivo*. The modified starch was hydrolyzed to a slightly greater extent by fungal amylase than was the unmodified starch. This was probably due to the effect of the modification treatment on the granule structure. Modification, or the reaction conditions associated with modification, may have opened up the granule structure slightly, permitting better penetration of the amylolytic enzyme. These structural changes must have been very slight because no differences have been observed between the granule surfaces of ungelatinized unmodified and modified starches (11). Acid pretreatment had no effect on the fungal amylase susceptibility of the ungelatinized modified starch. However, holding the modified starch for 1 hr in water slightly increased the enzyme susceptibility.

Friedemann *et al.* (10) obtained 25 to 30% hydrolysis with fungal amylase after treatment with 0.1*N* HCl for 1 hr at 95° C. Their values were considerably higher than those we obtained. The difference was undoubtedly due to the temperature during acid pretreatment. We chose 37° rather than 95° C because our objective was to estimate hydrolysis under simulated physiological conditions. Strong acid

TABLE III
Effect of Amylases on Ungelatinized Starches[a]

	Fungal		Pancreatic	
	Unmodified	Modified	Unmodified	Modified
No acid, no hold	2.0 ± 1.2	6.5 ± 0.0	28.2 ± 3.5	32.5 ± 5.4
No acid, hold[b]	4.9 ± 2.5	14.8 ± 5.8	29.3 ± 1.7	33.8 ± 3.2
Acid, hold[b]	4.8 ± 0.9	7.1 ± 2.5	31.3 ± 3.3	37.3 ± 2.3

[a]Mean % hydrolysis ± SD, n ≥ 4.
[b]Held for 1 hr at 37° C.

treatment of starch has been shown to alter granule structure (12,13) and may explain why the conditions of Friedemann *et al.* (10) increased hydrolysis.

Pancreatic amylase hydrolyzed 28 to 37% of the ungelatinized starches. This was a significantly greater hydrolysis than that attained with the fungal amylase on the ungelatinized starches. These results are difficult to explain, particularly in light of those for the two enzymes on gelatinized starches which are discussed later. It may be related to differences in size and three-dimensional structure of the α-amylases from the two sources, although their molecular weights are similar. Like fungal amylase, pancreatic amylase hydrolyzed slightly more of the modified than the unmodified starch. Apparently hydroxypropyl and/or phosphate modification favors both fungal and pancreatic amylase activity in the ungelatinized starches.

The degree of hydrolysis by fungal amylase on the gelatinized starches is shown in Table IV. Gelatinization markedly increased the enzyme susceptibility of the starches. Others have reported similar results (14). Modified starch was hydrolyzed about 20% less than the unmodified starch. Modification, particularly when ether linkages are introduced into the starch molecule, was shown to reduce the enzyme susceptibility of starch (1,2,4,7). The pH during gelatinization apparently had a slight effect on the enzymatic degradation of the unmodified starch molecule. Unmodified starch gelatinized at pH 3.5 was hydrolyzed about 4% more than that gelatinized at pH 7.0. This pH effect was not evident for the gelatinized modified starch.

Cooking temperatures at pH 7.0 had little effect on the fungal enzyme susceptibility of the starches (Table IV). The higher temperature (trt A) destroyed almost all of the unmodified granules whereas the lower temperature (trt B) did not. There were very few modified starch granules broken at the higher temperature. The modified granules were gelatinized less at the lower temperature. Retrogradation had a slight effect on the unmodified starch gelatinized at the lower temperature but had no effect at the higher temperatures. Presumably, this was due to retention of more intact granules at the lower cooking temperature and, therefore, a greater potential for reassociation of the amylose chains during refrigerated storage. This same effect was not evident with the modified starch, probably because the hydroxypropyl groups inhibit retrogradation. Acid pretreatment had no effect on the fungal amylase susceptibility of the gelatinized starches.

TABLE IV
Effect of Fungal Amylase on Gelatinized Starches[a]

	Unmodified		Modified	
	Gelatinized only	Gelatinized and retrograded	Gelatinized only	Gelatinized and retrograded
pH 3.5,[b] trt A[c]	86.3 ± 2.3	87.0 ± 3.5	65.8 ± 3.6	65.0 ± 2.6
pH 7.0, trt A	82.3 ± 4.7	83.0 ± 2.2	65.5 ± 2.3	62.0 ± 4.0
pH 7.0, trt B	83.8 ± 1.5	79.8 ± 1.0	63.8 ± 3.0	61.5 ± 1.0

[a]Mean % hydrolysis ± SD, n ⩾ 4.
[b]Gelatinization pH.
[c]See Fig. 1 for trt conditions.

The effects of pancreatic amylase on gelatinized unmodified and modified starches are shown in Table V. Gelatinization of the unmodified starch increased the amount of hydrolysis by only 30%. This was considerably less than the increase noted in fungal amylase susceptibility after gelatinization. The difference was probably because of the composition of the two enzyme preparations. The fungal amylase contained α- and glucoamylase whereas the pancreatic amylase contained only α-amylase. Thus, because of the different reaction mechanisms of α- and glucoamylase, the degree of hydrolysis and the hydrolysis products was expected to be different.

Gelatinized modified tapioca starch was hydrolyzed about 12% less than the unmodified. Starch gelatinized at pH 3.5 was hydrolyzed 4 to 5% more than that gelatinized at pH 7.0. Retrogradation had a slight effect on the enzyme susceptibility of unmodified starch gelatinized at pH 7.0. This effect was independent of the gelatinization temperature (trt C and B) and did not exist for the modified starch. Retrogradation had no effect on the susceptibility of the modified starch. Lower cooking temperatures did not affect the enzyme susceptibility of the unmodified starch. Acid treatment prior to pancreatic amylase treatment had no effect on the enzyme susceptibility of any of the processed starches.

TABLE V
Effect of Pancreatic Amylase on Gelatinized Starches[a]

	Unmodified		Modified	
	Gelatinized only	Gelatinized and retrograded	Gelatinized only	Gelatinized and retrograded
pH 3.5,[b] trt C[c]	61.5 ± 2.9	59.3 ± 2.2	48.5 ± 3.1	46.3 ± 3.1
pH 7.0, trt C	56.3 ± 4.3	52.5 ± 4.7	44.5 ± 1.2	45.8 ± 5.4
pH 7.0, trt B	56.9 ± 1.2	52.2 ± 1.8	42.5 ± 2.5	41.0 ± 1.3

[a]Mean % hydrolysis ± SD; $n \geq 4$.
[b]Gelatinization pH.
[c]See Fig. 1 for trt conditions.

TABLE VI
Relative Hydrolysis of Gelatinized Unmodified and Modified Starches by Amylases from Different Sources[a]

	Fungal[b]		Pancreatic[c]	
	pH 3.5[d]	pH 7.0	pH 3.5	pH 7.0
Unmodified	100	100	100	100
Modified	76	80	79	79

[a]Based on an index of 100 for unmodified starch gelatinized at the same pH.
[b]trt A.
[c]trt C.
[d]Gelatinization pH.

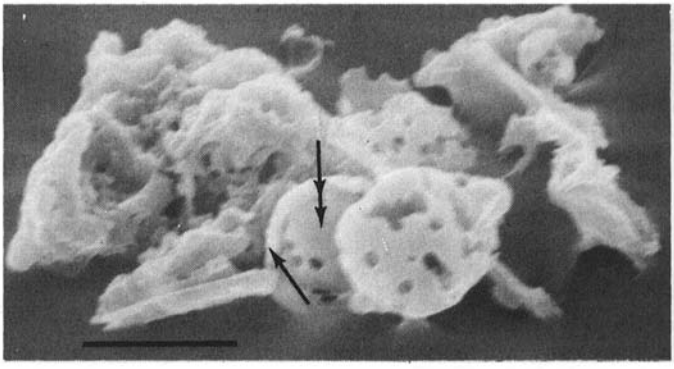

Fig. 2. Scanning electron micrograph of gelatinized hydroxypropyl distarch phosphate treated with pancreatic amylase. Coat—single arrow, core—double arrow. Marker: 10 μm.

The relative enzyme susceptibility of the gelatinized modified to unmodified starches is shown in Table VI. Hydroxypropyl distarch phosphate was 76 to 80% as enzyme-susceptible as the unmodified starch independent of the enzyme utilized or the pH of gelatinization. These values agree with those calculated from the data of Leegwater and Luten (4) for hydroxypropyl potato starch with an equivalent level of substitution. Apparently the phosphate cross-links had no effect on the enzyme susceptibility of hydroxypropyl distarch phosphate. These results suggest that either enzyme preparation (*i.e.*, fungal or pancreatic) could be used to provide an indicator of the relative digestibility of modified to unmodified starches.

The significance of our *in vitro* observations is in the relation between processing and digestion conditions and not in the absolute degree of hydrolysis. It is unrealistic to interpret *in vitro* hydrolysis data as being quantitatively equivalent to the extent of hydrolysis *in vivo*. Rather *in vitro* results should be interpreted to provide indications of the relative digestibility *in vivo*.

Enzymatic treatment affects granule structure. Gallant *et al.* (15) showed that pancreatin attacks unmodified ungelatinized tapioca granules in specific regions of the granule. Others have studied the effect of bacterial amylase on granule structure (16,17,18,19). Electron microscopic observations on amylase-treated starch contribute to the understanding of granule structure and the effects of chemical modification on structure.

Gelatinized modified granules treated with pancreatic amylase are shown in Fig. 2. Most of the granules were destroyed by α-amylase. Previously, we showed (11) that most modified granules are not broken by gelatinization conditions. The granules evident in this micrograph are representative of the few granule ghosts remaining. Some of the granules appear to have been affected to a greater extent than others. This suggests that individual granules vary in their enzyme susceptibility. This variation may be due in part to variations in degree of modification among granules. Pores were evident in the granules. In some locations, it appeared that the pores were so large that they were interconnected with other pores. We have postulated on the basis of transmission electron

micrographs that gelatinized modified granules are comprised of a dense coat and a dispersed core (11). These two areas are evident in Fig. 2. The pores seemed to be in the core and not in the outer coat. It is a tenable hypothesis that the enzyme penetrated the core of the granule through amorphous regions and that enzymatic action resulted in enlargement of these regions. We are currently investigating the effects of different modification treatments on the granule structure and enzyme susceptibility of various modified starches.

Acknowledgments

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