The Nature of Insoluble Starch Particles in Liquefied Corn-Starch Hydrolysates[1]

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ABSTRACT

Hydrolysates prepared from corn starch by conventional industrial thinning procedures invariably contain small amounts of insoluble starch particles (ISP). The ISP have been isolated and characterized by microscopic appearance, lipid and starch content, X-ray diffraction, solubility, and iodine absorbancy. Results indicate that the ISP in acid-thinned hydrolysates are primarily amylose in a degraded and associated form. In contrast, the ISP in enzyme-thinned hydrolysates are complexes of degraded amylose and free fatty acids. After clarification and storage in the refrigerator, additional ISP form in both types of hydrolysates. The second crops of ISP are different from each other and from their corresponding initial crops.

Conventional industrial processes for producing dextrose from starch involve the partial hydrolysis of starch with either acid or enzyme followed by further hydrolysis to dextrose using glucoamylase. Corn starch thinned by either process invariably contains small amounts of insoluble starch particles (ISP). During subsequent saccharification to dextrose with glucoamylase, the ISP in acid-thinned hydrolysates are solubilized almost completely; whereas the ISP in enzyme-thinned hydrolysates are solubilized only partially. The minute insoluble particles not only reduce dextrose yield but, more importantly, reduce production rate by impeding filtration.

The composition and nature of ISP have not been adequately determined. Komaki (1-6) in a series of publications discussed the preparation of ISP by enzymatic hydrolysis of various starch species and pointed out the negative influence of the particles on filterability. Komaki also showed that, of the common starches, corn starch yields hydrolysates containing the highest concentration of ISP. Based on reaction with iodine, Komaki assumed that the starch particles were mainly linear fragments. He further concluded that the ISP were highly associated as indicated by insolubility in boiling water and resistance to glucoamylase or α-amylase attack. From his studies Komaki concluded that a substantial portion of the starch particles pre-exists as micelles in the original starch granules. The studies also defined thinning conditions which reduce, but do not eliminate, the formation of ISP in corn-starch hydrolysates.

Maezawa et al. (7) demonstrated that a fatty acid-amylose complex is formed when incompletely liquefied corn starch is cooled below 30°C. and that the complex is resistant to enzymatic attack. Fukumoto et al. (8) also recognized the role that fatty acids play in forming ISP. They reported that the relatively high fat content of corn starch is responsible for significant levels of starch particles in enzymatic hydrolysates.

The objective of the present work was to more fully characterize ISP present in low-D.E. hydrolysates of corn starch prepared by acid- and enzyme-thinning.

MATERIALS AND METHODS

Preparation of Hydrolysates

Corn-starch hydrolysates of 11 to 12 D.E. were prepared at a total solids level of

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about 32% using single-stage acid- and enzyme-thinning processes. Acid-thinning was conducted at a pressure of 18 p.s.i.g. and a hydrochloric acid normality of 0.015. Enzyme-thinning was conducted with *Bacillus subtilis* bacterial α-amylase at 90°C. One portion of the enzyme-thinned hydrolysate was heat-treated at 121°C. for 15 min. and the other portion left untreated.

Analysis of Hydrolysates

Each hydrolysate was cooled to 60°C. and total insolubles determined by immediately slurrying a weighed portion (50 to 70 g.) with 2.000 g. filter aid. Each sample was then filtered immediately through a tared circle of filter paper (5.5 cm., S & S 576) and washed with about 200 ml. of 60°C. distilled water. Each residue containing ISP, fat, and protein was quantitatively recovered, dried at 80°C. for 4 hr. in a vacuum oven, weighed, and insolubles expressed on a total dry substance basis. Protein was determined by the Kjeldahl method (9) and fat by an acid hydrolysis technique using carbon tetrachloride as the extractant (10). Insoluble starch (first crop ISP) was calculated by difference, i.e., total insolubles minus fat plus protein.

Isolation of Insoluble Starch Particles

The process developed for isolating ISP in corn-starch hydrolysates is schematically diagrammed in Fig.1. A portion (4 to 12 liters) of each hot, freshly prepared hydrolysate was adjusted to pH 4.3 and passed through an 8-in.-diameter basket centrifuge rotating at 1,500 r.p.m. The basket was lined with filter paper

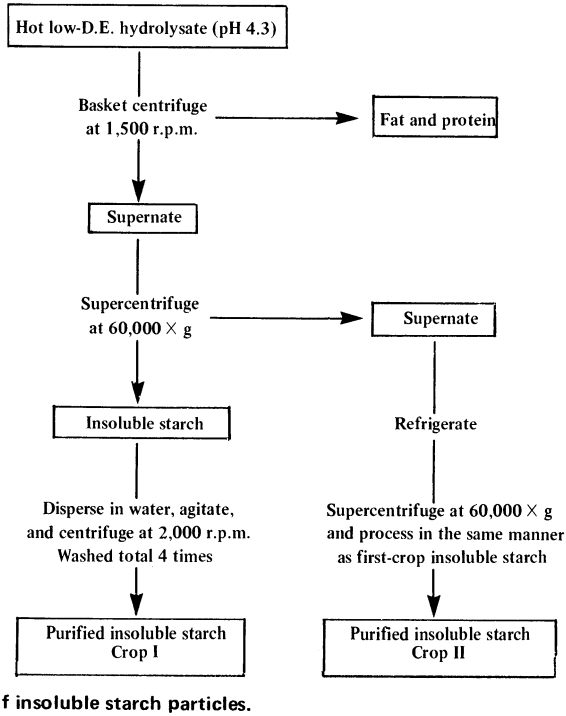

Fig. 1. Isolation of insoluble starch particles.

TABLE I. INSOLUBLES IN LOW-D. E. HYDROLYSATES

Insolubles	Acid Hydrolysate % d.b.	Enzyme Hydrolysate	
		Untreated % d.b.	Heat-treated % d.b.
Total	2.2	2.1	2.1
Fat	0.5	0.4	0.5
Protein	0.3	0.2	0.4
Starch (by diff.)	1.4	1.5	1.2

backed by cloth twill. The minute starch particles passed through the paper and cloth while the fat and protein were retained in the basket. The starch particles were then separated from the effluent by passage through a Sharples solid bowl supercentrifuge operated at 60,000 X g. The particles, deposited in the rotor as a heavy paste, were removed, dispersed in water, agitated, and spun in an International centrifuge at 2,000 r.p.m. The aqueous dispersion and centrifuging steps were repeated four times to wash the starch particles free of solubles. The supernates from each washing were discarded. The effluent from the supercentrifugation step was collected, stored in the refrigerator for 4 to 8 days at 4°C., and a second crop of starch particles isolated as described above. Each crop of ISP was dispersed in water to give a stock suspension containing 26 to 120 mg. dry substance per ml. Aliquots of each stock suspension were used for characterization studies.

Analysis of Insoluble Starch Particles

Fat was determined by an acid hydrolysis technique using carbon tetrachloride

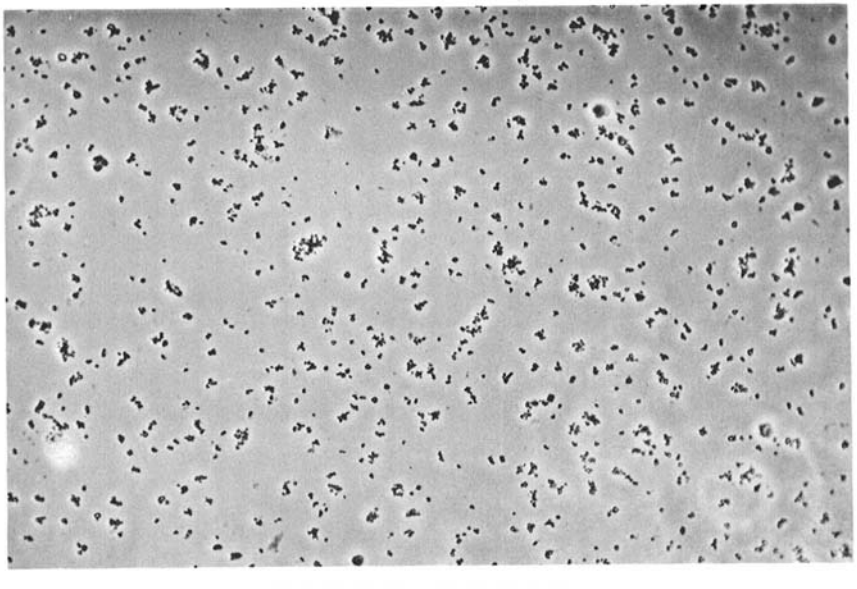

Fig. 2. First-crop ISP. Phase contrast, magnified 250X.

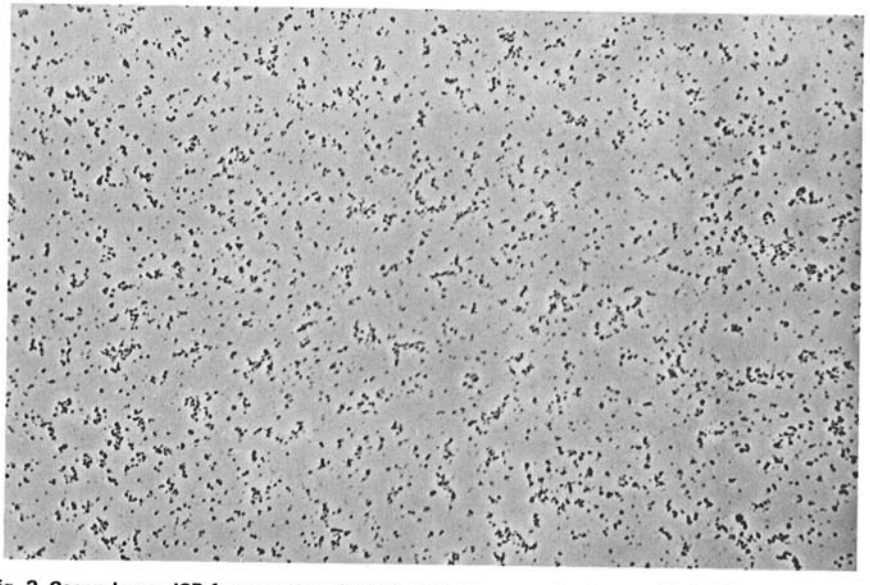

Fig. 3. Second-crop ISP from enzyme hydrolysate. Phase contrast, magnified 250×.

as the extractant (10). Total carbohydrate in each crop of ISP was determined by analyzing the acid hydrolysate from each fat determination using the α-naphthol procedure (11) and expressing the results as starch.

X-ray diffraction analyses were conducted as described by Zobel (12).

Carbohydrate composition of each crop of ISP was determined by paper chromatography (13). A solvent system of 2:3:1:3 butanol:propanol:ethanol:water was used for saccharide separation and α-naphthol for color development. For analysis, first crop samples were dissolved in 90% dimethyl sulfoxide (DMSO) and second crop samples in 1N sodium hydroxide followed by neutralization with HCl. In one case (ISP from acid hydrolysate), the salt formed on neutralization was removed by ion-exchange refining prior to analysis.

To determine iodine absorption spectra, a stock solution of each ISP fraction was prepared containing 1.0 mg. of d.s. per ml. of 90% DMSO. A 3-ml. aliquot of each stock solution was then reacted with iodine reagent (14) for 30 min. at 25°C. in a total volume of 500 ml. Iodine absorption spectra were then determined over the wavelength range of 370 to 700 nm. using an automatic recording DK-2 spectrophotometer.

Dextrose equivalent values were determined by the Schoorl's reducing sugar method (15).

Samples were examined and photomicrographed using a polarizing Carl Zeiss photomicroscope.

RESULTS

Composition of the insoluble fraction in each hydrolysate is shown in Table I. Total insolubles, fat, and protein were each determined by direct analysis. Insoluble starch, i.e. first-crop ISP, was calculated by difference.

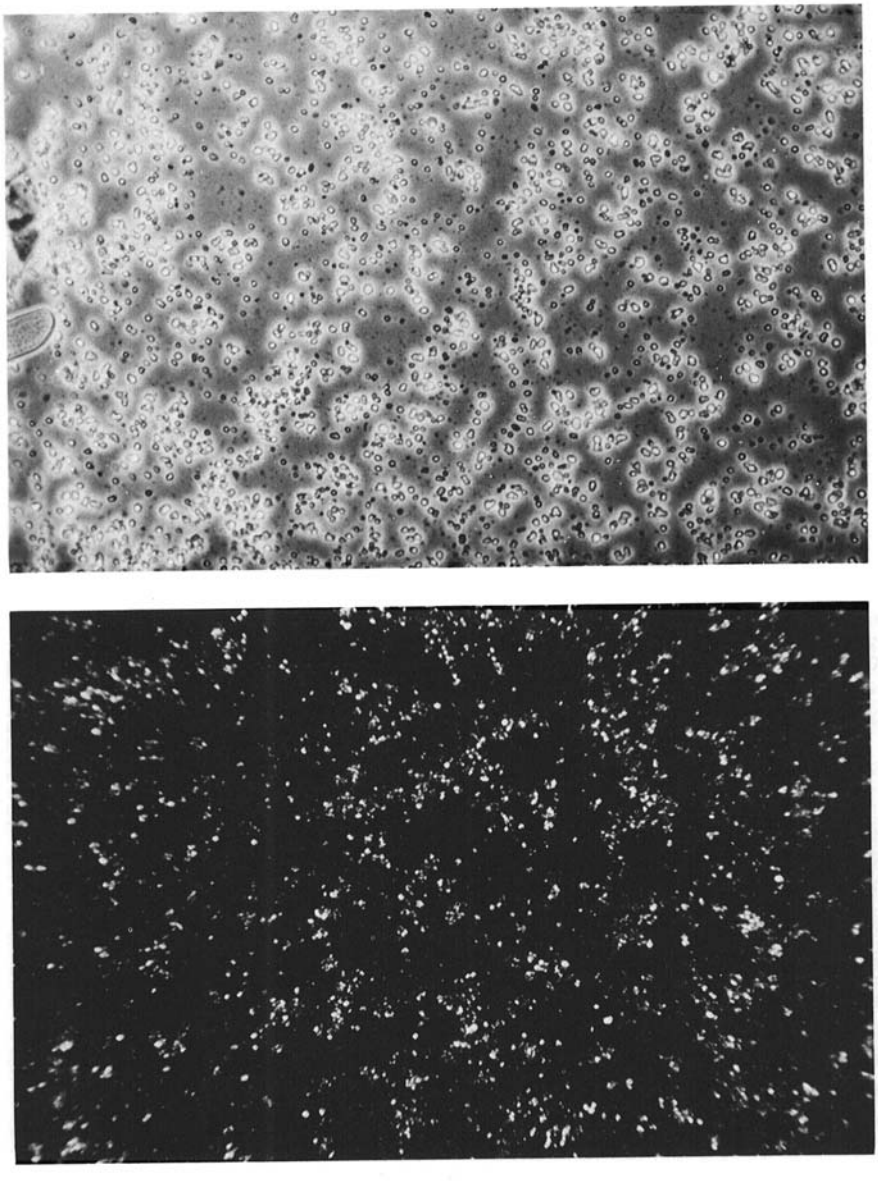

Fig. 4. Second-crop ISP from acid hydrolysate. a) (top) Phase contrast; b) (bottom) polarized light showing birefringence. Both magnified 250×.

ISP from acid-thinned hydrolysate and from heat-treated and untreated enzyme-thinned hydrolysate were characterized. However, since the ISP from the latter two hydrolysates showed identical characteristics, results are only shown for the ISP isolated from the untreated hydrolysate.

Microscopic Appearance

Microscopic examination did not reveal any differences in the first crop of ISP from hydrolysates produced by acid and enzyme liquefaction. The typical appearance of first-crop particles is illustrated in Fig. 2, a photomicrograph of the particles isolated from the acid hydrolysate magnified 250 times. The particles are 1 to 2 μ in size, nonbirefringent, and stain blue with iodine.

The second crop of ISP from each hydrolysate differed from the first crop. The second crop of particles from the enzyme hydrolysate are less than 0.5 μ in size and nonbirefringent (Figure 3). Because of a strong tendency for the particles to aggregate and since the particle size is at the limit of resolution of the optical microscope, it was impossible to obtain a meaningful photomicrograph depicting the actual size of the particles. In contrast, the second crop of particles from the acid hydrolysate are spherocrystals, 2 to 4 μ in size, and weakly birefringent (Figs. 4a and 4b). The latter particles are similar to the synthetic amylose spherulites crystallized by Sandstedt (16) and to hydrolyzed dextran spherulites (17).

TABLE II. SOLUBILITY CHARACTERISTICS OF ISP

	Enzyme Hydrolysate		Acid Hydrolysate	
	Crop I	Crop II	Crop I	Crop II
Boiling water	Ins.	Ins.	Ins.	Sol.
90% DMSO	Sol.	Sol.	Sol.	Sol.
28% $CaCl_2$	Sol.	Sol.	Sol.	Sol.
0.25N NaOH	...	Sol.	...	Sol.

Solubility Characteristics

The solubility characteristics of each crop of ISP are shown in Table II. With one exception, each crop of ISP is either insoluble or only slightly soluble in boiling water. The exception is the spherocrystal particles isolated as the second crop from acid hydrolysate. Each crop of ISP is soluble in 90% DMSO or 28% calcium chloride solution–solvents commonly used to dissolve starch. Both second crops of ISP dissolve in 0.20 to 0.25N sodium hydroxide to give clear solutions. In contrast, dilute aqueous dispersions of each first crop show very unusual behavior on the addition of sodium hydroxide. As alkali is added to the particles shown in Fig. 3 there is a noticeable reduction in opacity at about 0.15N but, on further alkali addition, filamentous structures begin to form, as shown in Fig. 5. On further alkali addition, the filamentous structures become larger and better defined (Fig. 6). The structures persist even when the alkali concentration is increased to 9N. Identical structures are obtained when potassium hydroxide is used in place of sodium hydroxide. Further experimentation is necessary to explain this unusual phenomenon.

Composition

The type of X-ray diffraction pattern as well as the starch and lipid content of each crop of ISP is shown in Table III. Lipids in corn starch are primarily in the form of fatty acids (18), and it assumed in this paper that the lipids in ISP are also fatty acids. The ISP exhibit two distinctly different types of X-ray diffraction patterns. The first crop of ISP from enzyme hydrolysate give a definite V-type

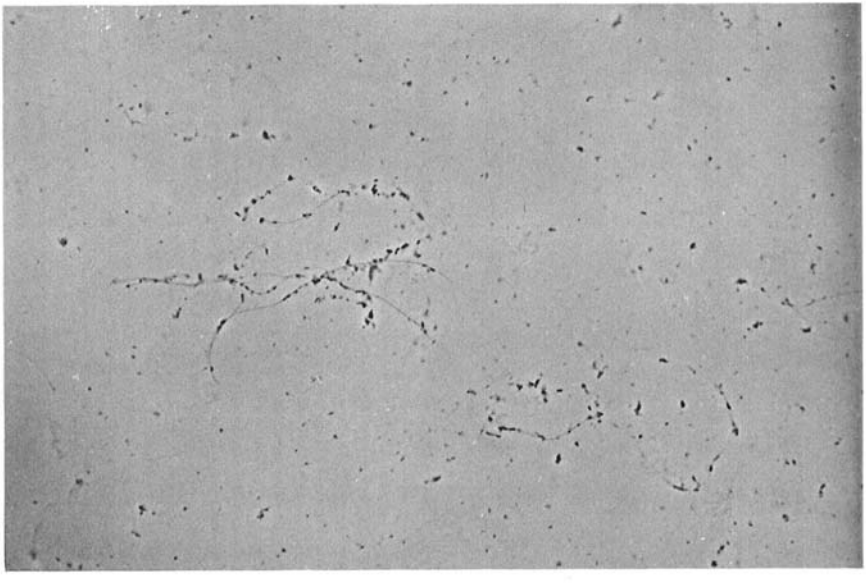

Fig. 5. Initial formation of filamentous structures (0.3N alkali). Phase contrast, magnified 250X.

pattern which is characteristic for amylose-fatty acid complexes. Fat and carbohydrate analyses support this finding since the ISP contain 5.9% fat and 94.1% starch. In contrast, each of the other crops of ISP exhibit an X-ray pattern that is predominantly of the B-type, characteristic of retrograded starch. These ISP contain 0.2 to 2.3% fat, indicating the likely presence of some fatty acid-starch complex–a diagnosis supported by X-ray detection of a trace of V-pattern.

Each crop of ISP is relatively free of simple sugars and oligosaccharides, as shown in Table IV.

Average Degree of Polymerization

The average degree of polymerization of each fraction of ISP was determined using iodine absorbancy and dextrose equivalent values. Data are shown in Table V. Bailey and Whelan (19) have defined the relationship between λ_{max} (wavelength of maximum absorption) and chain length for synthetic amyloses prepared from maltohexaose by phosphorylase catalysis. Using this relationship, the degraded amyloses in the first crops of ISP were estimated to have chain lengths of 32 and 40

TABLE III. COMPOSITION OF ISP

	Enzyme Hydrolysate		Acid Hydrolysate	
	Crop I	Crop II	Crop I	Crop II
X-Ray pattern[a]	V	B, Tr V	B, Tr V	B, Sl Tr V
Starch, % d.b.	94.1	99.0	97.7	99.8
Fat, % d.b.	5.9	1.0	2.3	0.2

[a]Tr = trace; Sl = slight.

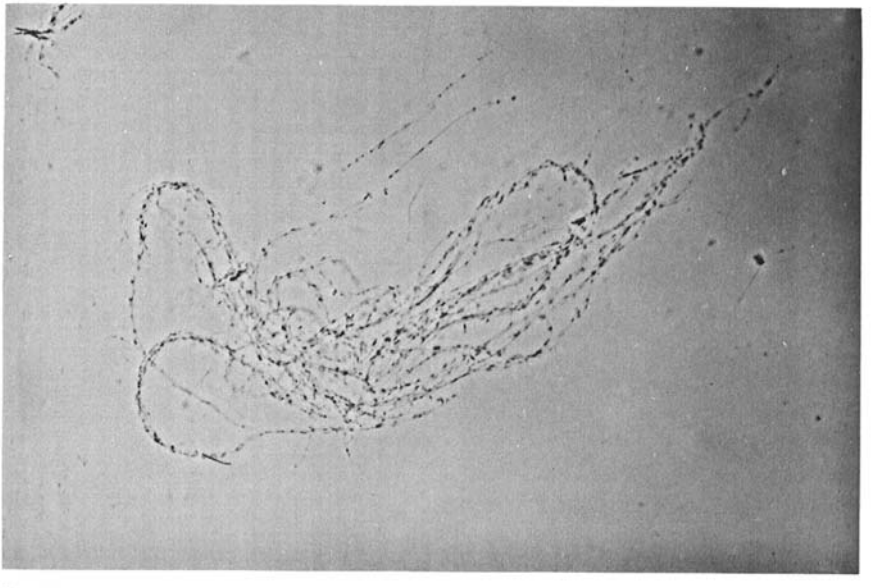

Fig. 6. Completely formed filamentous structures (0.8N alkali). Phase contrast, magnified 250X.

anhydro-glucose units, and the second crops 18 and 33. For comparison, chain length was calculated from dextrose equivalent values using the formula

$$DP\,(\text{chain length}) = [(20{,}000/D.E.) - 18]/162$$

The values obtained are in fair agreement with those obtained from absorbancy

TABLE IV. CARBOHYDRATE COMPOSITION OF ISP

Carbohydrate Distribution	Enzyme Hydrolysate		Acid Hydrolysate	
	Crop I % d.b.	Crop II % d.b.	Crop I % d.b.	Crop II % d.b.
DP-1	0.0	0.0	0.0	0.0
2	0.0	0.2	0.5	0.1
3	0.0	0.1	0.5	0.1
4	0.0	0.6	0.5	0.0
5	0.0	1.4	0.5	0.2
6	0.0	1.4	0.6	0.3
7	0.0	1.4	0.9	0.6
8+	100.0	94.9	96.5	98.7

TABLE V. ISP DEGREE OF POLYMERIZATION

	Enzyme Hydrolysate		Acid Hydrolysate	
	Crop I	Crop II	Crop I	Crop II
λ_{max} NM	558	544	541	508
Average, DP	40	33	32	18
D. E.	1.3	3.9	4.8	5.6
Average, DP	...	32	26	22

measurements. Chain lengths obtained by either method should be regarded as approximations only.

CONCLUSION

The results of this study indicate that insoluble starch particles may form in corn-starch hydrolysates by either of two entirely different mechanisms, depending upon whether acid or enzyme is used in liquefaction. During the initial stage of hydrolysis, amylose, the linear component of corn starch, is partially degraded. Evidently, the linear fragments that are produced by the partial hydrolysis of amylose are the primary precursors of ISP regardless of method of hydrolysis. During acid-thinning, the amylose fragments associate with each other through hydrogen-bonding, a phenomenon termed retrogradation when applied to starch. In contrast, the linear fragments produced during enzyme hydrolysis are apparently insolubilized by complexing with fatty acids. Based on X-ray and compositional data, it is speculated that the fragments coil around the fatty acid molecules to form a typical helical complex.

The ISP in acid-thinned corn starch are solubilized almost completely on subsequent saccharification with glucoamylase. In contrast, the ISP in enzyme-thinned corn starch are highly resistant to enzymatic attack during saccharification. In the latter case, the linear starch fragments not only are tightly complexed with free fatty acids but are in a helical form which apparently prevents the enzyme from forming the alignment required for hydrolytic scission.

Acknowledgments

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