Physical Characteristics, Enzymatic Digestibility, and Structure of Chemically Modified Smooth Pea and Waxy Maize Starches

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The pasting properties, enzymatic digestibility, and fine structure of smooth pea starch (SP), acetylated smooth pea starch (Ac-SP), an acetylated distarch phosphate derivative of smooth pea starch (AcDP-SP), waxy maize starch (WM), and a hydroxypropyl distarch phosphate derivative of waxy maize starch (HpDP-WM) were investigated. Acetylation decreased syneresis in SP gels. Chemical modification also decreased the susceptibility of the gelatinized AcDP-SP and HpDP-WM to α-amylase action. Structural characterization of the unmodified starches revealed the typical bimodal chain distribution of the amylopectin molecule. Debranching of AcDP-SP and HpDP-WM was incomplete, the debranched chains accounting for only 57–58% of the amylopectin present in the unmodified starches. β-Amylolysis limits of WM, SP, HpDP-WM, and AcDP-SP were 58, 66, 40, and 34%, respectively. Chromatography of the debranched AcDP-SP and HpDP-WM before and after β-amylase treatment suggested that acetylation occurred exclusively in certain parts of the granule, whereas hydroxypropylation was more uniform throughout the starch granule.

The intermediate amylose content (30–40%) legume starches are characterized by a stable hot paste viscosity (functional requirement for canning starches) and a high tendency for phase separation (syneresis) in their gels (undesirable property). Although no actual syneresis data were reported, Comer and Fry (1978) first suggested chemical modification as a means to improve the resistance to syneresis of these starches. In an attempt to minimize syneresis and maintain the heat stability of these starches, we have prepared several substituted (acetate, monophosphate, and hydroxyalkyl), cross-linked-substituted, and oxidized smooth pea starches to investigate the effect of chemical modification on gel properties. This report presents information on the pasting characteristics of some of these derivatives. Of the various derivatives studied, only gels of the acetylated samples combined desirable hot paste characteristics and resistance to syneresis. Acetylated derivatives were also the easiest to prepare. Furthermore, since very little is known about the location of modifying groups on the starch macromolecules (Hood and Mercier, 1978), structural analysis of acetylated smooth pea starch and a hydroxypropyl distarch phosphate derivative of waxy maize starch was carried out to provide further insight into the selectivity of the modification reactions of granular starch.

MATERIALS AND METHODS

Preparation of Starch Derivatives. Smooth pea starch (SP) was prepared from dehulled seeds (*Pisum sativum* L. cv. Trapper) by a wet-milling process (Biliaderis et al., 1979). Unmodified (WM) and hydroxypropyl distarch phosphate (HpDP-WM) waxy maize starches were provided by National Starch and Chemical Corp. (Bridgewater, NJ). The starches were defatted by hot extraction with 85% methanol for 48 h. Acetylation of smooth pea starch was carried out in aqueous suspension (100 g of starch/160 mL of water) in two consecutive treatments by slowly adding dropwise acetic anhydride (0.04 mol/treatment) according to Wurzburg (1964) except that 40% aqueous sodium silicate solution was used to maintain the pH between 8 and 9. The acetylated starch (Ac-SP) was neutralized and washed several times with water and ethanol. Cross-linking in aqueous starch slurries (100 g/160 g of water) was carried out by using 3 mL of carbon tetrachloride containing 0.1 g of phosphorus oxychloride (0.00065 mol) at pH 10.5. Cross-linked pea starch (distarch phosphate) was acetylated (AcDP-SP) as described above. The reaction temperature for both acetylation and cross-linking was 30 °C. The degree of substitution (D.S.) of the acetylated and hydroxypropyl derivatives was determined according to Smith (1967) and Johnson (1969), respectively.

Pasting Properties. Amylograms were prepared on a Brabender Visco-Amylograph with a 700 cm/g sensitivity cartridge at 75 rpm by using 8% (w/w) starch slurries. Syneresis of starch gels was determined by measuring the liquid exuded by the gel (50–60 g) after one freeze–thaw cycle or after storage at 5 °C for 7 days. For both measurements the gels were stored in sealed plastic containers to keep the gel and exudate in equilibrium. The amount of exudate was expressed as the percentage weight loss by the gel.

Digestibility of Starches by Hog Pancreatic α-Amylase. Starch (1 g) dispersed in 5 mL of 0.1 M phosphate buffer (pH 6.9) was mixed with 10 mL of Me_2SO and heated for 30 min in a boiling water bath. The sample was then autoclaved for 30 min at 130 °C. After being cooled, the slurry was mixed with 80 mL of phosphate buffer (0.1 M, pH 6.9) and stirred to eliminate any gellike material. Five milliliters of hog pancreatic α-amylase (Type VI-A, No. A-6880, Sigma Chemical Co., St. Louis, MO) solution [0.1% (w/v) in 0.1 M phosphate buffer] was added and the mixture incubated at 37 °C. At selected intervals (0.33, 1, 2, and 5 h) 2.0-mL aliquots were withdrawn, and after dilution to 10 mL with water they were boiled for 5 min to inactivate the enzyme. Reducing capacities of the hydrolysates were determined by the neocuproine method (Dygert et al., 1965) and total carbohydrates by the phenol–sulfuric acid method (Dubois et al., 1956). The percent hydrolysis was then calculated as

$$\% = \frac{\text{mg of reducing sugar (as maltose)}}{\text{mg of total carbohydrate (as maltose)}} \times 100$$

Structural Characterization of Starches. β-Amylolysis was carried out at 37 °C by adding 0.1 volume of 0.1% (w/v) barley β-amylase (Fluka A.D., Buchs, S.G., Switzerland) in water (8 IU/mL) to 1.0 volume of the buffered (0.05 or 0.1 M acetate, pH 4.7) polysaccharide solution (0.5–2 mg/mL). When the reducing power be-

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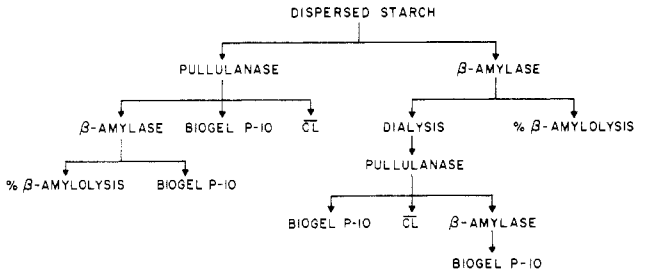

Figure 1. Analytical scheme used to study the structure of smooth pea and waxy maize starches.

came constant, the solutions were boiled to inactivate the enzyme, and the amount of liberated maltose (neocuproine method) was expressed as a percent of the total carbohydrates (phenol–sulfuric acid method).

For preparation of the β-limit dextrins, 330 mg of starch was boiled in 7.5 mL of 80% Me_2SO in 0.1 M acetate buffer (pH 4.7) for 5 min with constant agitation. After dilution with acetate buffer to a final 20% Me_2SO concentration, the dispersed starch samples were autoclaved for 30 min at 130 °C. β-Amylolysis was then carried out for 48 h as described above. The digestion was twice repeated after dialysis through a Spectrapor membrane with a molecular weight cutoff of 6000–8000 (Spectrometrics Co., Los Angeles, CA) to remove maltose. The β-limit dextrins were then dialyzed against water at 4 °C and freeze-dried.

Dispersed starches or β-limit dextrins (30–40 mg in 5 mL of 20% Me_2SO in 0.1 M acetate buffer, pH 5.5) were debranched with 40 IU (0.1 mL) of crystalline pullulanase (Hayashibara Biochemical Laboratories Inc., Okayama, Japan) for 18 h (Harada et al., 1972; Mercier and Kainuma, 1975). Due to the high tendency for retrogradation of unmodified SP dispersions, complete debranching was achieved with 80 IU of pullulanase and in more dilute solutions (20 mg/5 mL) for 4 h. The digest vials were subsequently heated in boiling water for 20 min to inactivate the enzyme and analyzed according to Mercier and Whelan (1970) as follows: (a) total carbohydrate concentration by the phenol–sulfuric acid method, (b) total reducing power by Nelson's reducing sugars method (Nelson, 1944), (c) β-amylolysis as described above, and (d) gel chromatography on Bio-Gel P-10, 100–200-mesh (Bio-Rad Laboratories, Richmond, CA) columns (2.6 × 95 cm) eluted by the ascending method at 22 °C with 0.1 M acetate buffer (pH 4.8) containing 0.02% sodium azide, at a flow rate of 20 mL/h. Recoveries from the columns varied from 92 to 101%. The columns were calibrated by fractionating debranched waxy maize amylopectin (100 mg). The average degree of polymerization ($\overline{DP}$) of each fraction (4 mL) was determined by dividing the total carbohydrate concentration (phenol–sulfuric acid) by its reducing capacity (Nelson, 1944). The average chain length ($\overline{CL}$) of the debranched polysaccharides was determined by (Marshall, 1974)

$\overline{CL}$ =
total carbohydrate of pullulanase digest (as glucose)/
reducing capacity (as glucose)

The overall analytical scheme for the structural characterization of the starches is summarized in Figure 1.

Iodine affinities were measured by potentiometric titration at 30 ± 0.1 °C according to Schoch (1964) except that Me_2SO was used to disperse the starch samples, instead of 1 N KOH, to prevent hydrolysis of the starch acetates by alkali. The wavelength of maximum absorption (λ_{max}) of the iodine–polysaccharide complexes was determined according to Bailey and Whelan (1961).

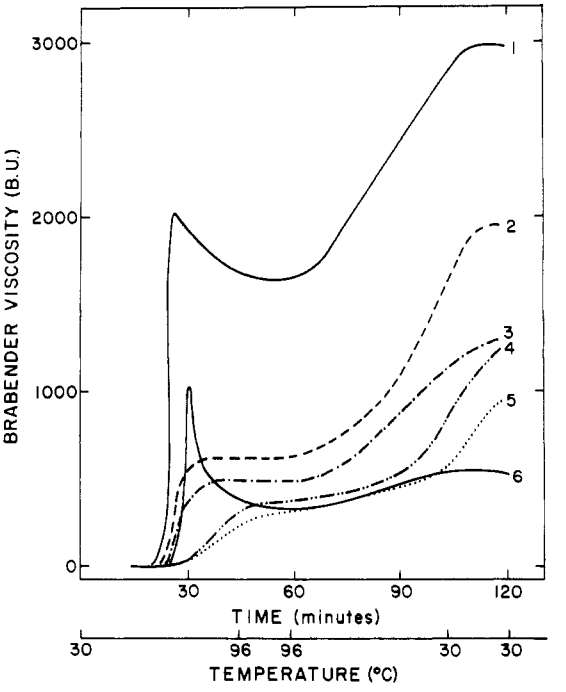

Figure 2. Brabender pasting curves of waxy maize (6), hydroxypropyl (D.S. = 0.09) distarch phosphate waxy maize (1), acetylated (D.S. = 0.06) distarch phosphate smooth pea (2), acetylated (D.S. = 0.06) smooth pea (3), smooth pea (4), and distarch phosphate smooth pea (5) starches.

RESULTS AND DISCUSSION

The pasting curves of the native and substituted WM and SP are shown in Figure 2. Acetylation of SP (D.S. = 0.06) decreased the initial pasting temperature and slightly increased the hot paste viscosity. However, the acetylated samples maintained the shear stability during heating characteristic of the unmodified starch. Crosslinking of SP before acetylation not only resulted in a stable hot paste viscosity but also allowed for better control of granule swelling during the acetylation step. For determination of the efficiency of acetylation in inhibiting retrogradation and, therefore, in improving resistance to syneresis, syneresis data were obtained for gels of acetylated SP starch with different levels of substitution. Figure 3 shows that with increasing substitution of acetyl groups there is a continuous improvement in the stability of both Ac-SP and AcDP-SP gels. At a D.S. of 0.06 the amount of exudate is minimized. It is possible, therefore, to prepare derivatives of legume starches by minor chemical modification that have potential applications in the food industry. For example, the shear stability and low viscosity at high temperatures (i.e., better heat-transfer properties), resistance to syneresis and high viscosity after cooling of the AcDP-SP are desirable features in canning starches.

Previous studies have shown that chemical modification decreases the susceptibility of gelatinized starches to enzymatic hydrolysis (Leegwater and Luten, 1971; Yoshida et al., 1973; Hood and Arneson, 1976; Wootton and Chaudhry, 1979, 1981; Hahn and Hood, 1980). Accordingly, gelatinized native and chemically modified SP and WM were treated with a preparation of pancreatic α-amylase to evaluate the in vitro digestibility of these samples. The data shown in Table I indicate that the digestibilities of the HpDP-WM, Ac-SP, and AcDP-SP were slightly lower than those of the unmodified starches. The degrees of hydrolysis of the 5-h hydrolysates relative to those of the unmodified starches were 87%, 94%, and

Table I. Percent Hydrolysis of Hog Pancreatic α-Amylase Hydrolysates of Native and Chemically Modified Smooth Pea and Waxy Maize Starches

incubation time, h	hydrolysis, %				
	SP[a]	Ac-SP[a]	AcDP-SP[a]	WM[a]	HpDP-WM[a]
0.33	16.8 ± 0.1[b]	23.6 ± 0.3	18.6 ± 0.2	20.2 ± 0.1	16.0 ± 0.2
1	45.5 ± 2.5	35.1 ± 1.3	35.9 ± 3.5	32.3 ± 2.1	29.4 ± 0.4
2	61.4 ± 0.3	48.3 ± 1.4	50.8 ± 1.8	46.0 ± 1.7	43.8 ± 2.4
5	71.4 ± 0.6	67.0 ± 1.3	64.8 ± 1.2	56.0 ± 1.6	48.8 ± 0.8

[a] SP = smooth pea, Ac-SP = acetylated (D.S. = 0.06) smooth pea, AcDP-SP = acetylated (D.S. = 0.06) distarch phosphate smooth pea, WM = waxy maize, and HpDP-WM = hydroxypropyl (D.S. = 0.09) distarch phosphate waxy maize. [b] $n = 2$.

91% for HpDP-WM, Ac-SP, and AcDP-SP, respectively. It has been suggested that hydroxypropyl (Leegwater, 1972) and hydroxyethyl (French et al., 1974) groups restrict α-amylase attack on adjacent α(1→4) glucosidic linkages. Acetyl substituents would be expected to exert a similar effect.

The ability of the modified samples to bind iodine was diminished as evidenced by decreases in λ_{max} and iodine affinity values (Table II). These trends reflect the disrupting effect of the modifying groups on the formation of stable helical inclusion structures between iodine and starch molecules. These results also demonstrate the drawbacks of using iodometric methods to quantitate the amylose in chemically modified starches (Colburn and Schoch, 1964). The β-amylolysis limits of WM and SP were 58.2% and 66.1%, respectively (Table II). The corresponding values for the modified samples were 39.7% (HpDP-WM), 35.6% (Ac-SP), and 33.6% (AcDP-SP), which constitute only 68%, 54%, and 52%, respectively, of the amounts of hydrolysis obtained with the unmodified starches. This implies that either amylose or the external amylopectin chains, or both, contain modifying groups.

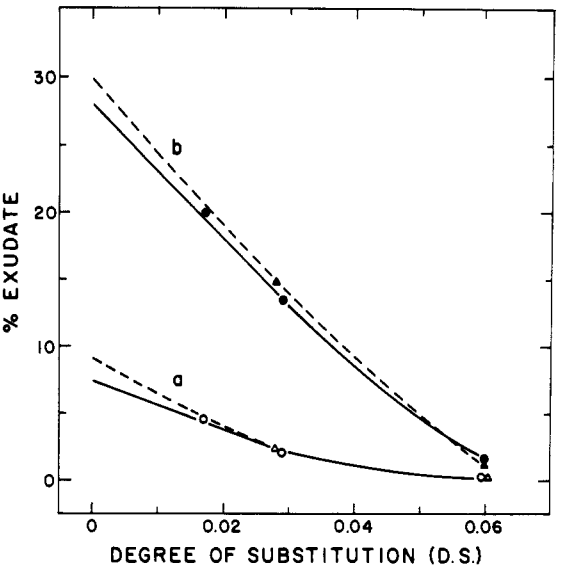

Figure 3. Syneresis of acetylated (—) and acetylated distarch phosphate (---) smooth pea starch gels: (a) one freeze–thaw cycle; (b) storage at 4 °C for 7 days.

Debranching of the modified starches with pullulanase was also incomplete as seen by the much higher values obtained for the apparent $\overline{CL}$, as compared to those of the unmodified samples, and the incomplete β-amylolysis of the debranched polysaccharide digests (Table II, $P\beta_1$ = 46.2–59.4%). These results suggest that some of the modifying groups are located near the branch points of the amylopectin molecules so that effective association between pullulanase and the substrate is inhibited.

The elution profiles of pullulanase-debranched starches and their β-limit detrins on Bio-Gel P-10 are shown in Figures 4 and 5 while the percentage distributions of the eluted carbohydrate materials are presented in Table III. The presence of the chains II ($\overline{DP}$ 45–48) 45–48) and III ($\overline{DP}$ 13–15) in the debranched digests of the unmodified WM (Figure 4a) and SP (Figure 4c) is in agreement with previous structural studies on various starches (Robin et

Table II. Structural Characteristics of Unmodified and Modified Smooth Pea and Waxy Maize Starches

starch	D.S.[b]	iodine affinity	λ_{max},[c] nm	β-amylolysis, %	apparent CL	molar ratio of chains III/ chains II	$P\beta_1$[d]	apparent $\overline{CL}$ of β-limit dextrin
WM[a]		0.01	550	58.2 ± 0.1[e]	20	10.6	104.0 ± 1.0[e]	10.0
HpDP-WM[a]	0.09		520	39.7 ± 1.1[e]	34		59.4 ± 0.9[e]	20.5
SP[a]		6.2	617	66.1 ± 1.2[e]	34	8.9	105.2 ± 1.1[e]	11.1
Ac-SP[a]	0.06	4.8	597	35.6 ± 0.4[e]	68	8.8	50.3 ± 0.8[e]	25.3
AcDP-SP[a]	0.06	4.6	595	33.6 ± 1.0[e]	74	8.7	46.2 ± 1.3[e]	27.0

[a] As in footnote a of Table I. [b] Degree of substitution. [c] λ_{max} of the iodine-polysaccharide complexes. [d] Percent β-amylolysis of the debranched starch. [e] $n = 3$.

Table III. Carbohydrate Distribution (Percent) of Bio-Gel P-10 Eluted Starches and β-Limit Dextrins after Debranching with Pullulanase (P, β_1P) or after Debranching and β-Amylase Treatment ($P\beta_1$, $\beta_1P\beta_2$)

	starch						β-limit dextrin						
	P for chains			$P\beta_1$ for fractions			β_1P for fractions				$\beta_1P\beta_2$ for fractions		
sample	I	II	III	40–56	57–107	108–127	40–56	57–84	85–103	104–127	40–56	57–105	106–127
WM[a,b]	3	22	75	1		99		40	25	35		1	99
HpDP-WM[a]	42	18	40	27	30	43	37	35	18	10	25	55	20
SP[a]	35	17	48	2		98	3	40	21	36	1	2	97
AcDP-SP[a,b]	61	11	28	43	9	48	49	25	15	11	39	27	34

[a] As in footnote a of Table I. [b] Data presented are means of duplicate chromatographic runs. Duplicate values had an average variation of 5.79% (standard error of mean = 1.38).

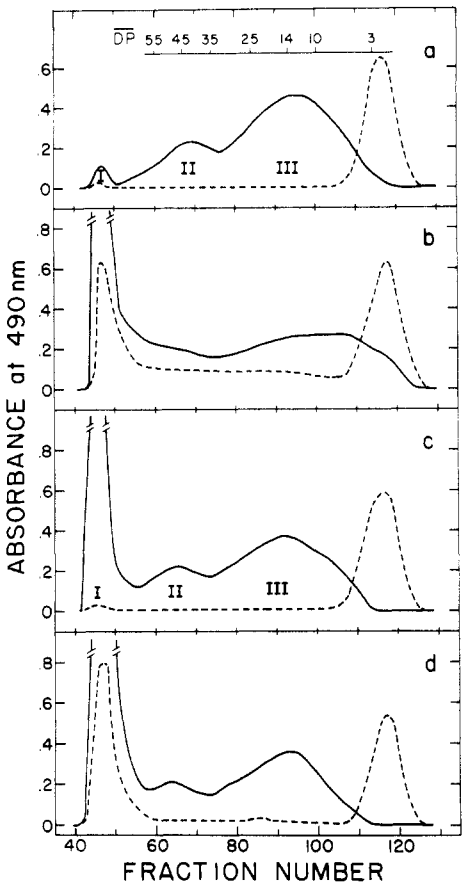

Figure 4. Elution profiles of starches after debranching with pullulanase [P (—)] or after debranching and subsequent β-amylase treatment (---)] on a Bio-Gel P-10 column (2.6 × 95 cm) eluted with acetate buffer (0.1 M, pH 4.8) containing 0.02% sodium azide at a flow rate of 20 mL/h at 22 °C: waxy maize (a), hydroxypropyl (D.S. = 0.09) distarch phosphate waxy maize (b), smooth pea (c), and acetylated (D.S. = 0.06) distarch phosphate smooth pea (d) starches.

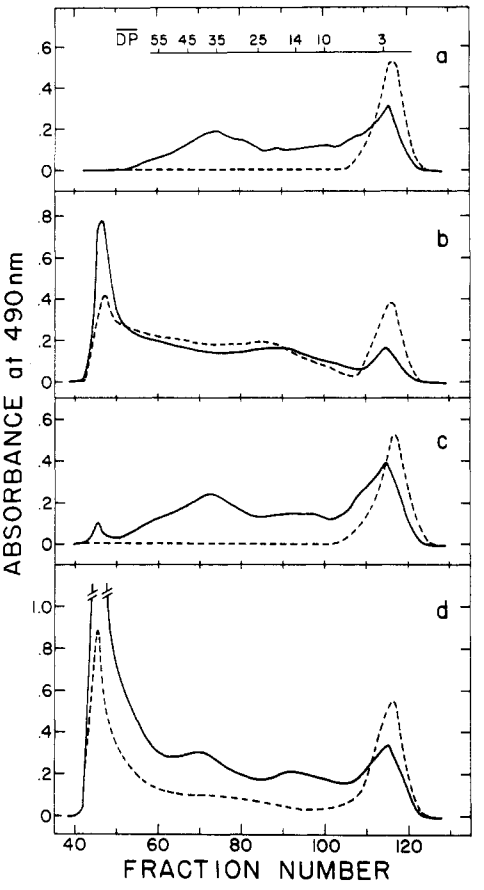

Figure 5. Elution profiles of β-limit dextrins after debranching with pullulanase [β_1P (—)] or after debranching and subsequent β-amylase treatment [$\beta_1P\beta_2$ (---)] on a Bio-Gel P-10 column (2.6 × 95 cm) eluted with acetate buffer (0.1 M, pH 4.8) containing 0.02% sodium azide at a flow rate of 20 mL/h at 22 °C: waxy maize (a), hydroxypropyl (D.S. = 0.09) distarch phosphate waxy maize (b), smooth pea (c), and acetylated (D.S. = 0.06) distarch phosphate smooth pea (d) starches.

al., 1974, 1975; Lii and Lineback, 1977; Ikawa et al., 1978; Biliaderis et al., 1981a) and consistent with current concepts of amylopectin structure and starch granule organization. The calculated molar ratios of chains III/chains II were 10.6 and 8.9 for WM and SP, respectively (Table II). Since β-amylolysis of the debranched digests of WM and SP was complete (Table II, $P\beta_1$ = 104–105%), the gel-excluded carbohydrate material (fraction I) must be linear-type material. The peak at the void volume (V_0) of the SP (Figure 4c) comprised 35% of the total polysaccharide (Table III), which is very close to the amylose content (31%) of this starch as obtained by iodometry.

Chromatography of the debranched HpDP-WM (Figure 4b) revealed peaks at V_0 and at $\overline{DP}$ 10–14 while no distinct peak at $\overline{DP}$ 45 was seen. As compared to WM, approximately half of the chains III were liberated during debranching while 42% (Table III) of the amylopectin had been eluted at the V_0, presumably due to its extensive modification. β-Amylolysis of the debranched HpDP-WM was only 59.4% as compared to 104.0% of the WM (Table II, $P\beta_1$ column). Although most of the debranched chains were completely hydrolyzed to maltose or maltotriose by β-amylase, the chromatogram of the $P\beta_1$ digest clearly demonstrates that a fraction of these chains is resistant to the action of this exoenzyme, implying the presence of some modifying groups near the non-reducing ends.

In contrast to that of HpDP-WM, the profile of the debranched AcDP-SP (Figure 4d) revealed the typical bimodal (chains III and II) chain distribution of the unmodified sample. Furthermore, the molar ratios of chains III/chains II in Ac-SP and AcDP-SP (8.7–8.8, Table II) were similar to that (8.9) of SP. The fact that β-amylase completely hydrolyzed the debranched chains suggests that the chains III and II of AcDP-SP did not contain modifying groups. However, 61% of the debranched AcDP-SP was eluted at V_0 (Table III). Since the amylose content of SP is 31%, approximately 30% of the gel-excluded polysaccharide, therefore, must have been modified amylopectin. This latter value corresponds to 43% of the original amylopectin content of SP and is comparable to that (42%, Table III) obtained for the gel-excluded polysaccharide from the HpDP-WM chromatogram. Furthermore, it is of interest to note that Hood and Mercier (1978) reported chromatograms of debranched hydroxypropyl distarch phosphate of tapioca starch (D.S. = 0.045; amylose content of unmodified starch 17%) and its β-limit dextrin that were similar to those obtained in the present study for HpDP-WM. Thus, the observed differences between the profiles of HpDP-WM and AcDP-SP could not be explained on the basis of a preferential modification of the amylose component of SP or differences in the D.S. The results must rather reflect differences in the distribution of the modifying groups in the branched starch molecules. It appears that acetylation of SP occurred exclusively in certain parts of the granule, presumably the outer lamellae, while hydroxypropylation of WM was more uniform throughout the granule.

The elution profiles of the debranched β-limit dextrin

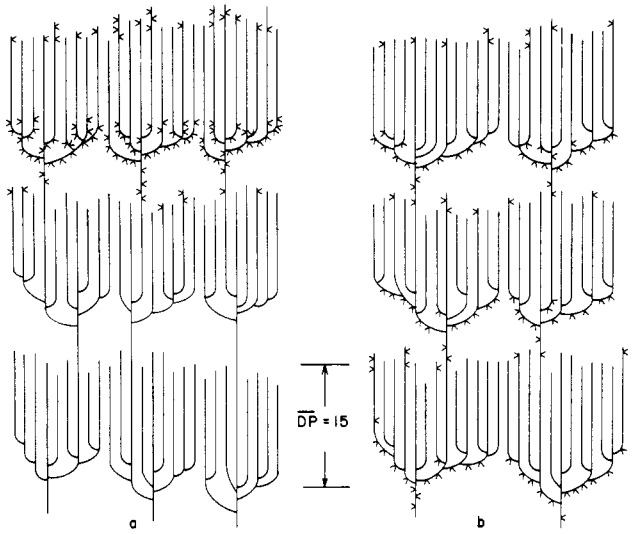

Figure 6. Proposed model structures for modified amylopectin of acetylated distarch phosphate smooth pea (a) and hydroxypropyl distarch phosphate waxy maize (b) starches.

of WM (Figure 5a) and SP (Figure 5c) revealed a peak at $\overline{DP}$ 35, a broad fraction at $\overline{DP}$ 8–20 and the maltose–maltotriose peak at $\overline{DP}$ 2.5 derived from the external stub branches of these dextrins. The gel-excluded material (3%, Table III) in the case of the β_1P of SP (Figure 5c) must correspond to the branched amylose fraction of this starch (Biliaderis et al., 1981a). Debranching of the β-limit dextrins of the modified samples with pullulanase was incomplete as evidenced by the much higher values obtained for the apparent $\overline{CL}$ of these dextrins (Table II) and the chromatograms of Figure 5b,d. After the V_0, the chain distribution of the AcDP-SP debranched digest (Figure 5d) was similar to that of the unmodified sample (Figure 5c). In contrast, there was a more uniform chain distribution over the entire elution volume in the case of HpDP-WM (Figure 5b). Further β-amylolysis and chromatography showed that most of the debranched chains of HpDP-WM β-limit dextrin contained modifying groups near their nonreducing ends as compared to those of AcDP-SP which were, by far, more accessible to β-amylase action. The above trends are similar to those obtained from structural studies on the whole starches (Figure 4a–d) and provide additional evidence that there is in fact a difference between HpDP-WM and AcDP-SP in the distribution of the substituents in the branched starch molecules.

In the following discussion we attempt to envisage the distribution of the modifying groups within the starch molecules based on the results of this study and in view of the current theories on fine structure of amylopectin and molecular organization of the starch granule.

Among the various model structures proposed for amylopectin, the cluster type of structure originally suggested by French (1972) is most in accord with the hydrodynamic behavior of the molecule, the structural features of the amylopectin and its degradation products by acids or α-amylase, and the mode of starch biosynthesis and the semicrystalline nature of the granule. Thus, studies on the granular residues of acid-treated cereal (French, 1972; Robin et al., 1975; Yamaguchi et al., 1979; Watanabe and French, 1980), potato (Robin et al., 1974), and legume starches (Biliaderis et al., 1981b) suggested that closely packed short ($\overline{DP}$ 12–15) chains of amylopectin (chains III, Figure 4) form the starch crystallites while regions of dense branching are less organized and, therefore, more susceptible to acid hydrolysis. Accordingly, it would appear reasonable to expect chemical modification to occur more frequently inside the amorphous regions.

The calculated molar ratios of chains III/chains II (Table II) suggest that there are about 200 and 180 glucose units per each $\overline{DP}$ 15–$\overline{DP}$ 45 cluster of chains for WM and SP, respectively. For lightly cross-linked starches like those of HpDP-WM and AcDP-SP (about 1 phosphate group/950 anhydroglucose units) enzymatic action is mainly influenced by the level of substitution (Hood and Mercier, 1978; Wootton and Chaudhry, 1979). On the basis of the D.S. values, there are 90 hydroxypropyl and 60 acetyl groups per 1000 glucose residues in HpDP-WM and AcDP-SP, respectively. Thus, the average concentration of the modifying groups per amylopectin cluster will be about 18 hydroxypropyl and 11 acetyl groups for HpDP-WM and AcDP-SP, respectively. However, the fact that almost all debranched chains III and II of AcDP-SP (Figure 4d) as well as those of its β-limit dextrin (Figure 5d) were completely hydrolyzed by β-amylase suggests that high-density substitution exists only in certain parts of the amylopectin, which renders these regions exceedingly resistant to debranching enzyme action (i.e., modified polysaccharide elutes at V_0), while the rest of the molecule remains unmodified and thus accessible to both pullulanase and β-amylase. On the other hand, the data obtained for HpDP-WM favor a more uniform distribution of the substituents in the starch macromolecules. The model structures presented in Figure 6 depict the suggested substituent distribution and location of the modifying groups in the branched starch molecules of AcDP-SP and HpDP-WM. Factors that may influence the rate and selectivity of the modification reactions at a macromolecular level and thus account for the above differences in the distributions are the reactivity of the starch hydroxyl groups, specific area and organization of the starch granule, diffusion rate and reactivity of the coreactant, and steric factors due to the bulkiness of the substituent groups. While no firm conclusions can be drawn due to the limited number of reaction products studied, our data and those of Hood and Mercier (1978) suggest that the nature of the coreactant is more important in determining the substituent distribution than the physicochemical characteristics of the starch granule. This is an area of starch research in need of further exploration. Nevertheless, one must point out the possible implications of the distribution pattern to starch granule integrity, solubility, and other physical properties of the modified polysaccharide. An assessment of the substituent distribution may be more meaningful than an average value of D.S. in characterizing chemically modified starches.

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Some Functional Properties of Acylated Wheat Gluten

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Commercial wheat gluten was chemically modified by reaction with either succinic or citraconic anhydride. In the neutral pH range, the acylated glutens showed greater water solubility, emulsification capacity, and water holding capacity than an unmodified control sample. The modified glutens also showed greater water adsorption than the unmodified gluten. Gluten modified with succinic anhydride was similar in functionality to the gluten treated with citraconic anhydride except that after holding at pH 3, the latter showed evidence of substantial reversibility of the acylation.

A number of protein sources such as alfalfa leaf, fish, peanut flour, and soy flour have been investigated for possible incorporation into formulated foods. Chemical derivatization of groups such as the free ϵ-amino group of lysine in proteins with acid anhydrides has resulted in improved functional properties for various food proteins (McElwain et al., 1975; Childs and Park, 1976; Kinsella and Shetty, 1979). A reversible acylation could have advantages in that during digestion, amino groups such as those of lysine would be deacylated and the lysine would become nutritionally available. Brinegar and Kinsella (1979) have studied the deacylation of soy protein after modification with citraconic anhydride and found substantial reversibility under mild acidic conditions.

Wheat gluten is a readily available protein source that has been used extensively in baked products and pet foods (Sarkki, 1979). Gluten proteins are uniquely suited for dough-forming characteristics because of hydrophobicity, low water solubility, and the reactions of sulfhydryl groups. However, some of these same properties can limit other uses of gluten in foods. Gagen and Holmes (1972) and Grant (1973) have studied the acylation of wheat proteins, and certain characteristics were found to improve with the modification. Some functional properties of the acylated glutens have not been explored and the use of citraconic anhydride for forming reversible derivatives of gluten has not been previously investigated. It was the purpose of this research to evaluate the effect of acylation with succinic and citraconic anhydrides on some functional properties of wheat gluten and to determine the degree of reverisbility of the acylation of gluten.

MATERIALS AND METHODS

Acylation of the Gluten. Acylation with both succinic and citraconic anhydrides was performed in a procedure similar to the method of Friedman (1978). Commercially available vital gluten (Pro 80, Henkel Corp., Minneapolis, MN) was used throughout the study. Manufacturer's data indicated this material was 75% protein, which is typical for vital wheat gluten products (Kalin, 1979). The gluten was made into a slurry with a 1:4 gluten:water ratio, and the pH was adjusted to 7.5–8.5 with 1 N NaOH. To the slurry 0.5-g increments of either succinic anhydride or citraconic anhydride (Eastman Organic Chemicals) were added. The pH was readjusted to 7.5–8.5 with 2 N NaOH after each addition of the anhydride. The amount of anhydride added was varied in a preliminary experiment where the weight of anhydride was either half, the same as, or twice the weight of dry gluten. Because there was no significant change in nitrogen solubility of the modified protein samples with increasing levels of anhydride, a ratio of 1 part of anhydride to 2 parts of gluten was used throughout the remainder of the study. After the addition

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