

Lipid Binding of Modified Corn Starches Studied by Electron Spin Resonance¹

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ABSTRACT

Cereal Chem. 67(3):236-240

Lipid binding capabilities of modified corn starches were studied by electron spin resonance in starch-water-fatty acid systems. Spin labeled stearic acid was used as a probe to report interactions. The corn starches studied included regular, waxy, nonionic hydrophilic, hydrophobic, cationic, and anionic starches. Electron spin resonance spectra were taken at room temperature before and after heating at temperatures up to 95°C.

The spectral response showed components ranging from a dilute spin, broad-line, powder pattern, to a three-line, rapid, isotropic motion, to various degrees of spin broadening even at relatively low probe concentration. Differences in degree of binding as well as degrees of granular probe penetration were found between types of modified starch. Binding was also altered by the presence of salts.

Pearce et al (1985) first used electron spin resonance (ESR) techniques to show that a nitroxide labeled fatty acid is greatly immobilized by granular starch. Strong binding was found for starch with amylose contents ranging from that of waxy corn starch to high-amylose corn starch. Binding was facilitated by the polar solvent, water, and once bound, the spin labeled fatty acid could not be removed by repeated washing with water. Binding did not occur in a nonpolar solvent. Nolan et al (1986) showed that initial binding of a spin labeled fatty acid and of a fatty acid ester is induced by the presence of water and that once binding had occurred, spectral line shape is largely independent of water content at equal starch-probe ratios. Pearce et al (1987) later demonstrated that after heating followed by cooling, binding is not quite as strong, that 5-DOXYL-stearic acid shows greater probe immobilization than 16-DOXYL-stearic acid, and that waxy corn starch shows less probe immobilization than starches of higher amylose content. Biliaderis and Vaughan (1987) used differential scanning calorimetry (DSC) to show that nitroxide labeled fatty acid probes display the amylose-lipid complex melting peak for amylose-containing starches, whereas this peak is absent in waxy corn starch. In the amylose-containing starches, a small fraction of the probe showed a melt transition at about 70–80°C, indicating nonbound probe; this melt transition peak was larger in the waxy corn starches. Since native waxy corn starch also showed the ESR powder pattern, binding must occur with amylopectin as well as with amylose.

Pearce et al (1985) calculated that probe binding does not occur exclusively on the granule exterior, but some binding must occur

in the granule interior in order to obtain the observed dilute spin powder pattern at the 1:0.002 ratio of starch to 16-DOXYL-stearic acid. Thus, it was implied that information concerning probe distribution with respect to the granular surface could be obtained. The objective of the present study was to use chemically modified granular corn starches, in order to further investigate which factors influence fatty acid probe binding to starch chains and where this binding takes place with respect to the granule surface.

MATERIALS AND METHODS

Starch Samples

Waxy corn starch was obtained from American Maize-Products Co. (Hammond, IN). Regular, waxy, commercially modified hydroxypropyl waxy (HPW) and commercially modified acetylated waxy (ACW) corn starches were obtained from National Starch and Chemical Corporation (Bridgewater, NJ). A series of laboratory prepared modifications at known levels of degree of substitution (DS) were also supplied by National Starch and Chemical Corp. These were: hydroxypropyl regular corn starch at DS 0.055, 0.098, and 0.148; hydroxypropyl waxy corn starch at DS 0.055, 0.085, and 0.133; waxy corn starch phosphate monoester at DS 0.0087 and 0.026; quaternary ammonium derivatives of waxy corn starch at DS of 0.036 and 0.052; and a waxy corn starch octenylsuccinate of DS 0.015. The waxy corn starch phosphate monoester was washed three times with 5% ethylenediaminetetraacetic acid (EDTA) at pH 7, then two times with distilled water. All other starch samples were washed five times with distilled water unless otherwise indicated.

Spin Probes

The probes 16-DOXYL-stearic acid and 5-DOXYL-stearic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received. Probes were dispersed in water and slurried 24 hr prior to use with starch as described by Pearce et al (1985, 1987) at probe-water weight ratios of 1:1,000 or 2:1,000 as indicated.

¹ Presented at the AACC 73rd Annual Meeting, San Diego, CA, October 1988. Published as paper no. 16,751 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Projects 18-027 and 18-063.

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Preparation of Starch-Water-Probe Samples

The water-probe two-phase suspension was added to starch at a weight ratio of 1:2 of starch to water-probe suspension. The mixtures were then slurried for 24 hr at room temperature before transfer to 2-mm glass capillary tubes. ESR spectra were run on samples at room temperature. Other samples were heat treated for 4 min at 40, 45, 50, 55, 75, or 95°C with subsequent cooling to room temperature prior to obtaining ESR spectra.

ESR Spectra

Two ESR spectrometers were used in the study. Preliminary work was done using a Varian E-3 with an operating frequency of about 9.33 GHz with center field at 3.2 kG. The remainder of the study was done using an IBM ERO23M with an operating frequency of about 9.77 GHz with center field at 3.5 kG. Spectra were analyzed as described by Pearce et al (1985, 1987).

Mineral Analysis

Sodium and potassium were analyzed on an Applied Research Laboratories spectrometer (model QA137) by inductively coupled plasma (ICP). Samples (1–2 g) of dry starch were ashed overnight at 485°C, then dissolved in concentrated HCl and HNO₃ prior to analysis.

RESULTS

Probe Concentration and Spin Broadened Spectra

Figure 1a and b show the effect on the ESR spectra of doubling the concentration of 5-DOXYL-stearic acid from the 1:0.002 starch to probe ratio used by Pearce et al (1985, 1987) to 1:0.004 for waxy corn starch. Doubling the probe concentration of waxy corn starch resulted in a slightly spin broadened spectrum due to interaction broadening between paramagnetic electrons of adjacent probes. For regular corn starch (Fig. 1c), doubling the probe concentration did not change the spectra and it retained the dilute spin, broad-line, powder pattern observed at the lower probe concentration. The same differences in degree of spin broadening were not seen at this concentration ratio for 16-DOXYL-stearic acid. For comparison, the remaining spectra shown will be for the starch to 5-DOXYL-stearic acid ratio equal to 1:0.004.

Nonionic Hydrophilic Modification of Waxy Corn Starch

Figure 2 shows the room temperature spectra of the nonionic hydrophilic substitutions of the commercially modified ACW and HPW corn starch. The two spectra are very similar, with both

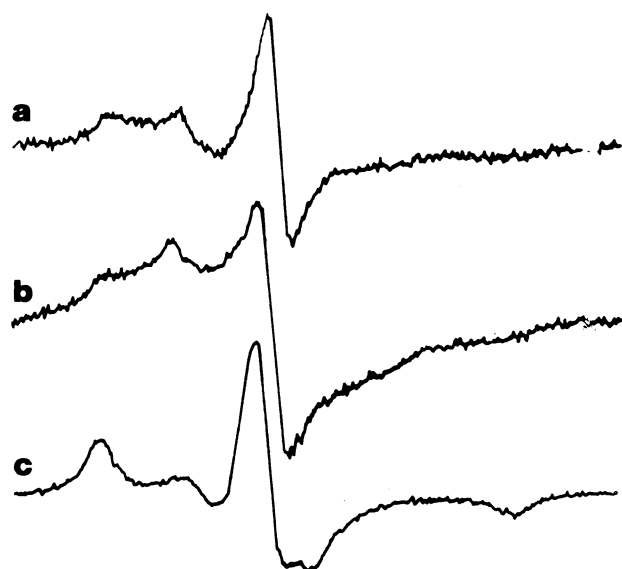


Fig. 1. Electronic spin resonance spectra (Varian) of starch and 5-DOXYL-stearic acid: waxy corn starch at a ratio of 1:0.002 (a), waxy corn starch at a ratio of 1:0.004 (b), regular corn starch at a ratio of 1:0.004 (c).

showing more spin broadening than unmodified waxy corn starch (Fig. 1b), an indication of impeded penetration of the probe into the granule. Figure 3 further illustrates the effect of hydroxypropyl substitution for the series of laboratory-prepared modifications of waxy corn starch at increasing DS levels. Progressively increased spin broadening from that of waxy corn starch (Fig. 3a) occurs when increasing the DS from 0.055 to 0.085 to 0.133 (Fig. 3b–d). The commercially modified HPW at an unknown DS appears to have a high level of DS when Figure 2b is compared with Figure 3.

Spectra of Modified Regular Corn Starch

Figure 4 shows native unmodified and a series of hydroxypropyl substitutions of regular corn starch. The spectra for the substituted starches, like those of native unmodified regular corn starch, show

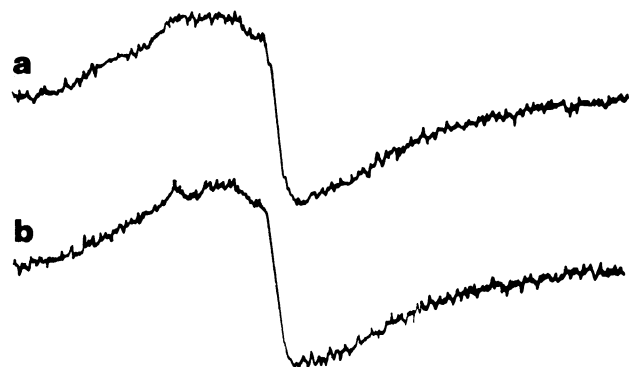


Fig. 2. Electronic spin resonance spectra (Varian) of commercially modified waxy corn starch and 5-DOXYL-stearic acid at a ratio of 1:0.004: acetylated (a), hydroxypropyl (b).

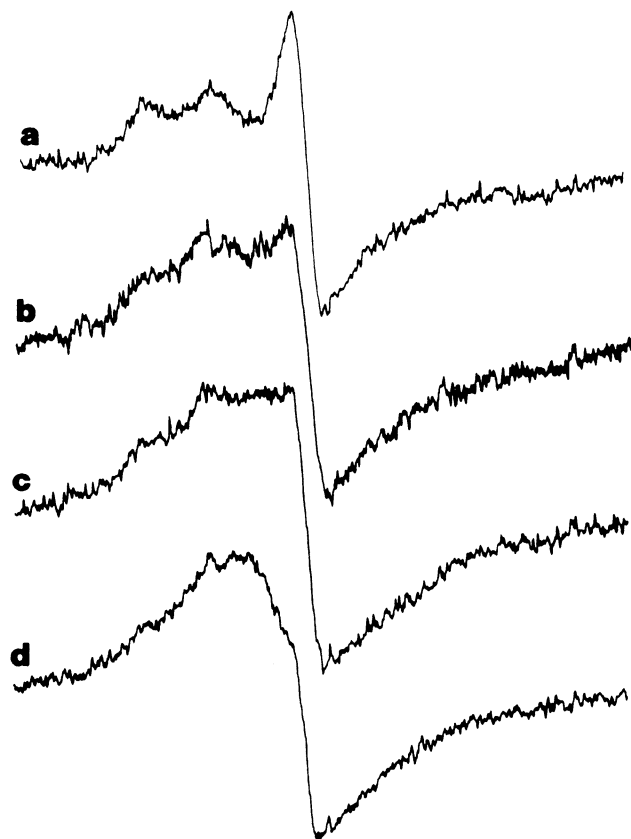


Fig. 3. Electronic spin resonance spectra (IBM) of native unmodified waxy corn starch and of laboratory prepared modifications of hydroxypropyl waxy corn starch and 5-DOXYL-stearic acid at a ratio of 1:0.004: native unmodified (a), DS 0.055 (b), DS 0.085 (c), DS 0.148 (d).

no spin broadening. As the levels of DS increase from 0.055 to 0.098 to 0.148, there is a progressive decrease in the slow motion component with a corresponding increase in the fast motion component in the low-field region of the spectra. Correspondingly, progressive changes are also noted in the high-field region of the spectra.

Ionic Substituions of Waxy Corn Starch

The cationic substitutions studied, a quaternary ammonium derivative of waxy corn starch at DS 0.036 and 0.052 had spectra identical to that of the unmodified waxy corn starch (Fig. 3a);

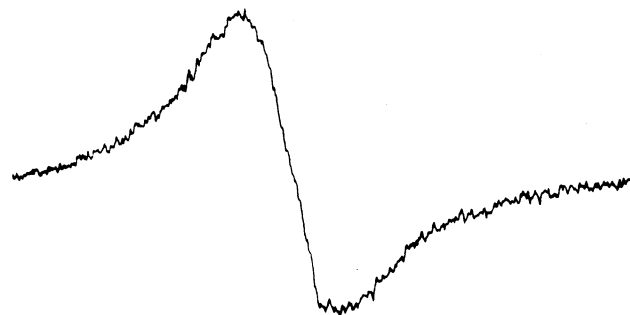


Fig. 6. Electronic spin resonance spectra (IBM) of a laboratory prepared modification of waxy corn starch octenylsuccinate at DS 0.015 and 5-DOXYL-stearic acid at a ratio of 1:0.004.

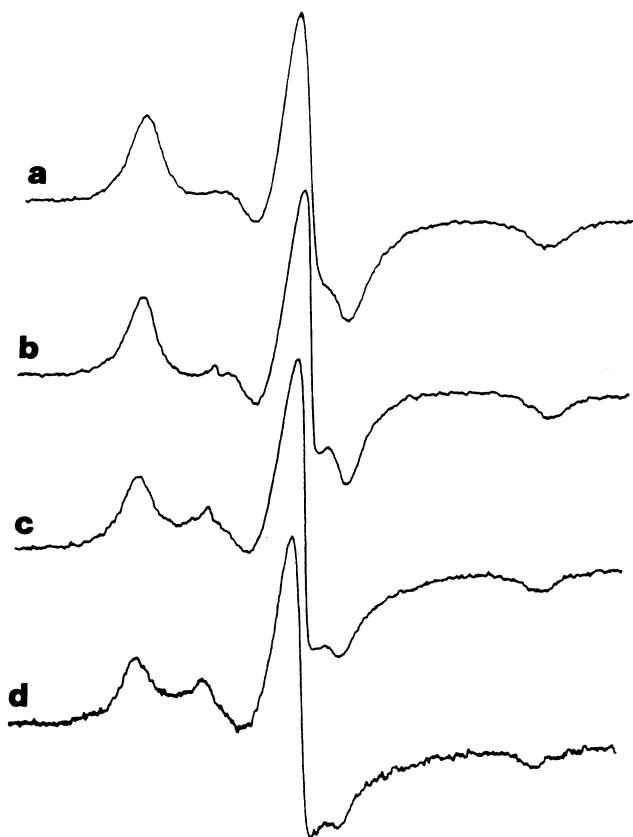


Fig. 4. Electronic spin resonance spectra (IBM) of native unmodified regular corn starch and of laboratory prepared modifications of hydroxypropyl regular corn starch and 5-DOXYL-stearic acid at a ratio of 1:0.004: native unmodified (a); DS 0.055 (b); DS 0.098 (c); DS 0.148 (d).

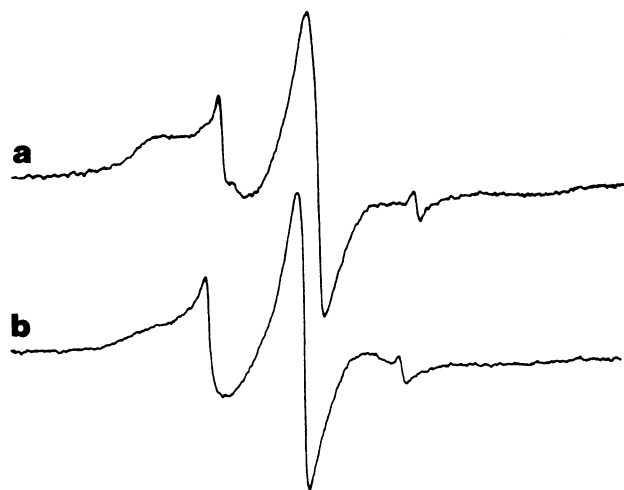


Fig. 5. Electronic spin resonance spectra (IBM) of laboratory prepared modifications of waxy corn starch phosphate monoester and 5-DOXYL-stearic acid at a ratio of 1:0.004: DS 0.0087 (a), DS 0.026 (b).

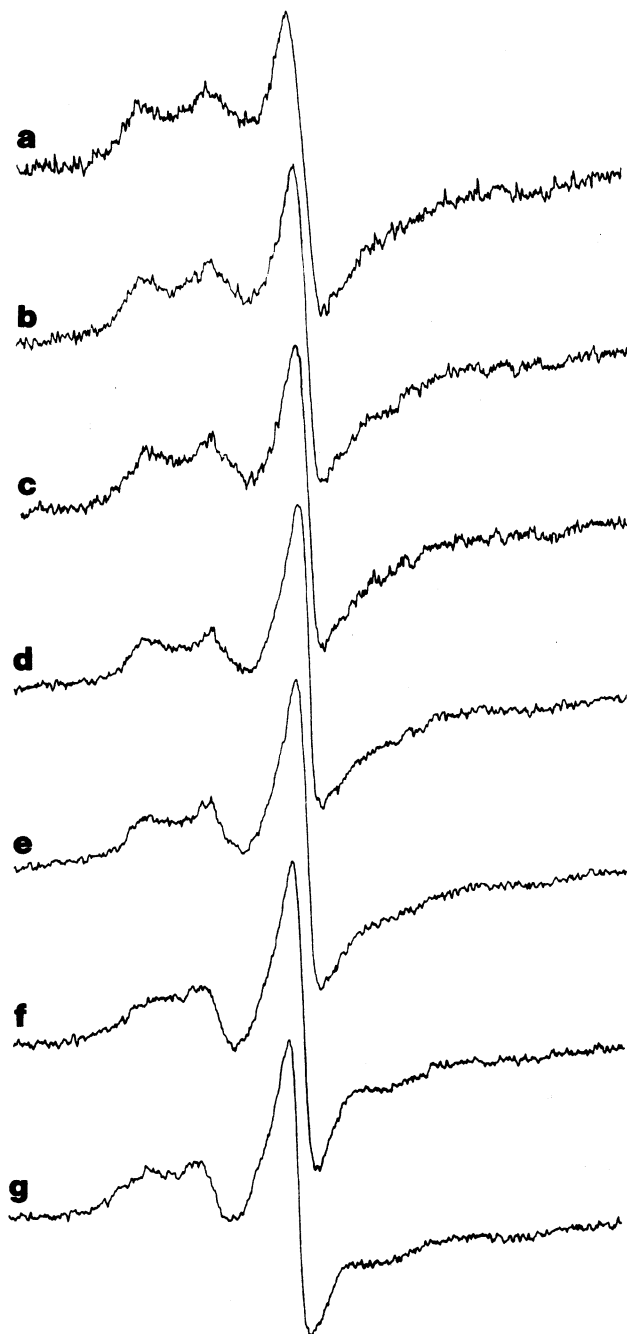


Fig. 7. Electronic spin resonance spectra (IBM) of native waxy corn starch and 5-DOXYL-stearic acid at a ratio of 1:0.004 at varying degrees of heat treatment: none (a), 40 (b), 45 (c), 50 (d), 55 (e), 75 (f), and 95°C (g).

thus the spectra are not shown. The anionic substitutions studied, a waxy corn starch phosphate monoester at DS of 0.0087 and 0.026, are shown in Figure 5. Conversely, this modification had spectra very dissimilar to that of the unmodified waxy corn starch. The spectra were neither like the powder pattern nor spin broadened spectra but appeared to show the three-line components of rapid isotropic motion of the nitroxide, which was superimposed on a slight powder pattern. This is an indication of a very little binding of the fatty acid probe.

Hydrophobic Substitution of Waxy Corn Starch

Figure 6 shows the unheated spectrum of the octenylsuccinate derivative of waxy corn starch with a DS of 0.015. The spectrum shows extreme spin broadening at very low DS when compared with the spin broadening for the nonionic hydrophilic substitutions.

Spectra of Heat Treated Native and Modified Waxy Corn Starch

Figure 7 shows gradual reduction in spin broadening from room temperature spectra of native waxy corn starch after heating to several different temperatures. Reduction in spin broadening was found to correspond to the amount of heating given.

Modified waxy corn starch also showed reduced spin broadening with heating. Figure 8 shows the spectra after heating at 95°C for the highest DS levels studied of hydroxypropyl waxy corn starch, quaternary ammonium waxy corn starch, waxy corn starch phosphate monoester, and the octenylsuccinate of waxy corn starch. For the spin broadened spectra of the hydroxypropyl and quaternary ammonium derivative there is a reduction in the amount of spin broadening (Fig. 8a and b). The phosphate monoester (Fig. 8c) showed reduction in the motionally narrow three-line component with replacement of a powder pattern that still showed much mobility. For the octenylsuccinate there was very little reduction in spin broadening (Fig. 8d).

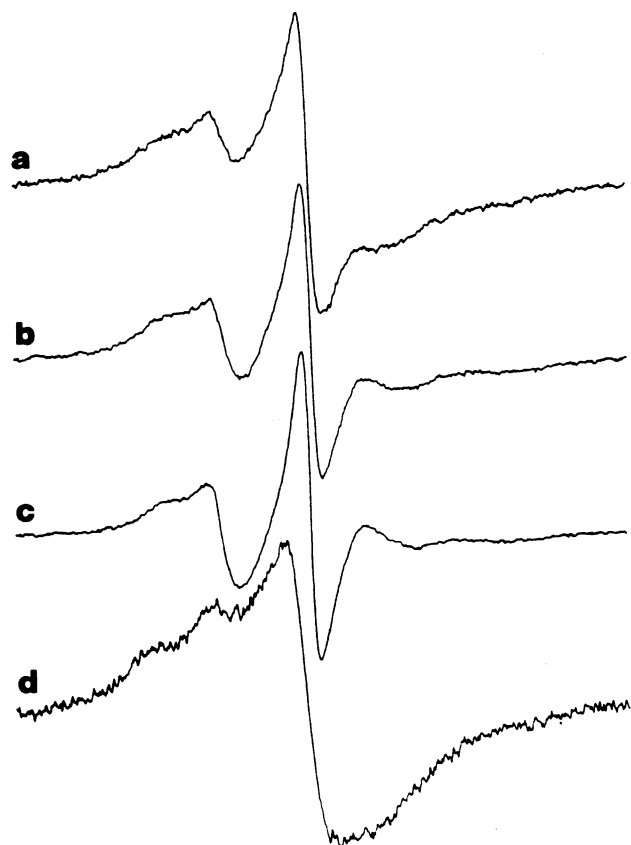


Fig. 8. Electronic spin resonance spectra (IBM) of modified waxy corn starches after heating at 95°C: hydroxypropyl, DS 0.085 (a); quaternary ammonium, DS 0.052 (b), phosphate monoester, DS 0.026 (c), octenylsuccinate, DS 0.015 (d).

ESR Spectra Affected by Salts

Figure 9 shows a series of starch samples (waxy corn starch and the laboratory-prepared modifications of hydroxypropyl waxy corn starch at increasing levels of DS) that were not washed with distilled water before probe addition. The same series of starches that were washed before probe addition (shown in Fig. 3) are quite dissimilar to those of Figure 9 in every case. Mineral analysis by ICP for washed and unwashed samples showed that the levels of sodium were reduced from 250 to 20 ppm, and the levels of potassium were reduced from 50 to <10 ppm after washing of the waxy corn starch. Sodium was reduced from 970 to 50 ppm and potassium from 20 to <10 ppm for the DS 0.133 hydroxypropyl waxy corn starch. The waxy corn starch was more spin broadened in the presence of salts, whereas the hydroxypropyl waxy corn starches showed elements of the three-line, motionally narrowed spectrum (which increased in the presence of salts) superimposed on a spin broadened type spectrum.

DISCUSSION

Three types of interactions of the fatty acid probe with the granular starch could be distinguished from the ESR spectra. The first type, when a powder pattern was obtained, was the relative strength of the complex seen by comparing fast and slow motion components of the ESR spectral line shape. Powder pattern spectra were obtained for regular and modified regular corn starch and for waxy and some modified waxy corn starches after heat treatment. In general, chemical modification led to more mobile powder pattern type spectra.

The second type of interaction seen when spin broadened spectra were obtained was the amount of surface absorption rather than granular penetration of the probe. This was noted by the degree of spin broadening, which for all modified starches differed from waxy corn starch. The nonionic hydrophilic substitutions inhibited

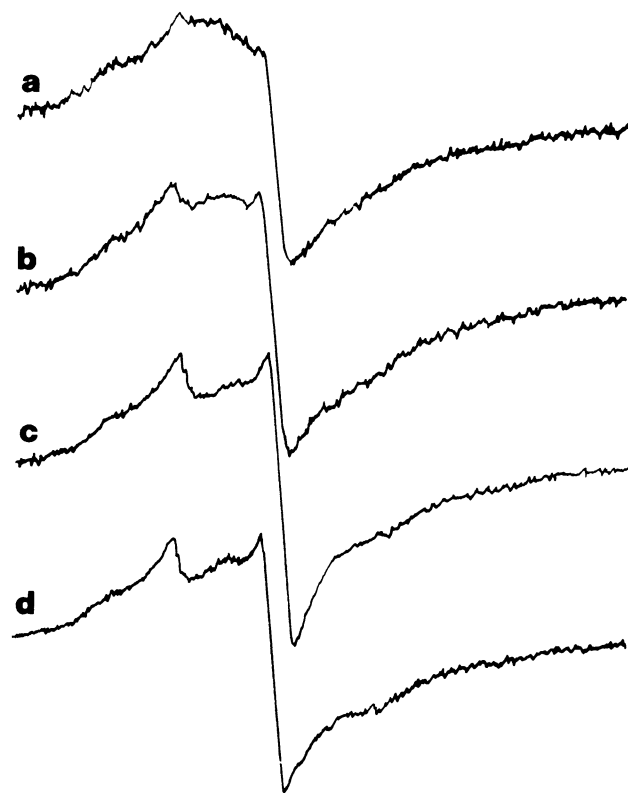


Fig. 9. Electronic spin resonance spectra (Varian) of unwashed native unmodified and unwashed laboratory prepared hydroxypropyl modifications of waxy corn starch with 5-DOXYL-stearic acid at a ratio of 1:0.004: native unmodified (a), DS 0.055 (b), DS 0.085 (c), DS 0.133 (d).

spin probe from entering the granule, leading to increased spin broadening. The cationic substitution did not seem to alter penetration of probe into the granule at the DS studied, and the spectra showed the same amount of spin broadening as native unmodified waxy corn starch. The hydrophobic substitution (the octenylsuccinate) bound lipid most exclusively to the outside of the granule with the most spin broadened spectra.

A third type of interaction was nonbinding of the probe. This was exhibited by the waxy corn starch phosphate monoester, which showed the three lines of rapid nitroxide motion indicating a repulsion of the probe. In comparison, the quaternary ammonium derivative of waxy corn starch did not demonstrate non-binding. This cationic substitution is large and has a net positive charge of one, whereas the phosphate monoester is much smaller and has a net negative charge of two. As the conjugate base of stearic acid has a net negative charge, it should be repelled by the anionic substitution but attracted by the cationic substitution. The starch samples containing salts also showed components of the motionally narrow three-line spectra.

In conclusion, reduction in binding by derivatized starch chains may be due to disruption of the native molecular conformation of both amylose and amylopectin, which decreased the strength of the starch-lipid complex. The reduction in binding in the presence of salts is likely the result of ionic interference with complex formation. Heating also decreased the strength of the complex formed after cooling, which most likely resulted from

altered molecular conformation. Decreased spin probe penetration into granular interior may be due to the greater branching in waxy corn starch and to the derivative side chains in modified corn starch creating steric hindrance to movement of the probe. Increased spin probe granule penetration after heating is the result of both greater granular surface area and greater ease of access of the fatty acid probes into the interior of the granule.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the fellowship support of this work by the Corn Refiner's Association, Inc., Washington, DC.

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[Received February 28, 1989. Revision received October 27, 1989. Accepted November 4, 1989.]