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L. E. Pearce

E. A. Davis

J. Gordon

W. G. Miller

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APPLICATION OF ELECTRON SPIN RESONANCE TECHNIQUES
TO MODEL STARCH SYSTEMS

L. E. Pearce¹, E. A. Davis¹, J. Gordon¹ and W. G. Miller²

¹ University of Minnesota,
Department of Food Science and Nutrition,
1334 Eckles Avenue, St. Paul, MN 55108

² Department of Chemistry,
225 Pleasant Street S.E., Minneapolis, MN 55455

Abstract

Starch model systems were examined by electron spin resonance (ESR) techniques with either 16-DOXYL-stearic acid or TEMPO spin probes in water or hexane. Room temperature starch-water-16-DOXYL-stearic acid spectra showed strong adsorption occurred between the starch and probe. Starch-water-TEMPO spectra at room temperature did not show strong adsorption between starch and probe, but did show some slowed motion of the probe as a result of different local environments experienced by the probe within the starch granule. Starch-water-probe spectra from systems heated from 45-95° C showed no major differences from unheated samples. Also, no major spectral differences existed for each starch system combination studied: wheat starch, hexane extracted wheat starch, waxy cornstarch, or high amylose cornstarch. Spectra from unheated wheat starch-hexane-probe systems did not show that starch-probe interactions occurred.

Introduction

Electron spin resonance (ESR) emerges as an attractive technique used to explore components of baked systems and their interactions at the molecular level. ESR techniques have been used to examine the conformation of amylose in aqueous solution (Ebert, 1984) and lipid-protein interaction in gluten (Nishiyama et al., 1981). ESR techniques can also be used to examine starch-water interactions and starch-water-fatty acid type interactions. The use of stable free radicals, particularly nitroxides as spin probes or spin labels is based on the change of spectral line shape with motion. Thus a spin probe either adsorbed or in a highly viscous medium will have an entirely different line shape from that of the probe in a solvent (Berliner, 1976; Berliner, 1979). In order to better understand these interactions, hexane can be used as an alternate solvent and TEMPO and 16-DOXYL-stearic acid can be used as spin probes. Water and hexane differ in their dielectric properties. Water has a dielectric constant of 80.37 and hexane has a dielectric constant of 1.890 at 20° C.

The immobilization of spin labels has been shown through use of spin probes of varying functionality to be through the probe side chain and not through the nitroxide moiety (Miller, 1979). In relationship to starch, it is attractive to choose two probes of differing hydrogen bonding and hydrophobic-hydrophilic properties to better understand the starch-water interactions relative to starch-water-fatty acid type interactions (such as are found with stearic acid type monoglycerides).

In this study TEMPO, a non-hydrogen bonding, more hydrophilic probe, and 16-DOXYL-stearic acid, a more hydrophobic probe were used. Water and hexane were used as solvents; the two solvents have the potential to interact differently with starch and probe. Starches examined were wheat starch, high (70%) amylose cornstarch, waxy cornstarch, and hexane extracted wheat starch. Each starch-probe combination was suspended in solvent in a starch:solvent:probe weight ratio of 1:2:0.002.

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Direct inquiries to E.A. Davis.
Telephone number: (612) 373-1158.

Key Words: Starch electron spin resonance,
electron spin resonance starch, starch structure.

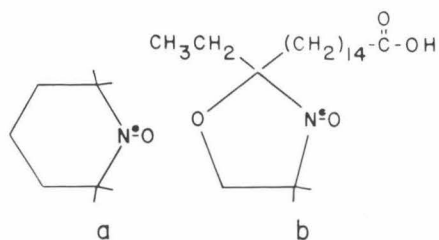


Fig.1. Structure of spin probes:

- (a) TEMPO;
 (b) 16-DOXYL-stearic acid.

Materials and Methods

Spin Probes

The structures of the spin probes are shown in Figure 1. The two probes, TEMPO and 16-DOXYL-stearic acid, were obtained from Aldrich Chemical Co. All probes were used as received and prepared for use in a probe:solvent weight ratio of 1:1000. Probes in water were magnetically stirred for 24 h before use. Probes in hexane were stirred for 2 h due to increased probe solubility in hexane. The solubility of 16-DOXYL-stearic acid in water was less than 1 part per 1000; i.e., the system was two phase.

Solvents

The two solvents used were glass distilled water and reagent grade hexane, 99+% hexane, from Aldrich Chemical Co.

Starches

The starches used were: wheat starch (Aytex P, General Mills, Inc.); waxy corn starch (Amioca, American Maize-Products Co.); and high (70%) amylose cornstarch (Amaizo Amylomaize™ VII, American Maize-Products, Co.). The wheat starch was also evaluated after room temperature hexane extraction for removal of surface lipids from the granules (Johnson and Hosoney, 1979).

Sample Preparation

Starch-water-probe

Room temperature adsorption studies were conducted similar to the method of Liang et al. (1980). Starch:water:probe, 1:2:0.002 was slurried with stirring for 24 h at room temperature. Starch and water were then separated by centrifugation. The separated starch was washed with 2.5 ml glass distilled water for 2 min and again separated. This process was continued until either no ESR signal could be detected in the supernatant or twenty washings were completed. The dilution of any spin probe in the aqueous phase external to the starch granules was estimated to be a factor of 5 or 6 per washing by this procedure. The pH of starch-water-probe slurries ranged from 5.49 - 5.83 indicating the systems were saturated with carbon dioxide and, as expected, not changed by stearic acid due to its low solubility. The ESR spectrum of each probe was also determined before addition of starch and in the dry state (neat spin label).

Heating studies were conducted on the slurried starch-water-probe system. Starting at 45° C the samples were heated for 4 min, cooled to room temperature, the ESR spectrum recorded,

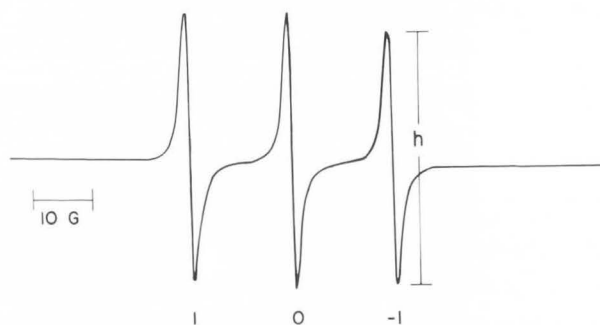


Fig.2. Type of 3-line ESR spectra obtained; h is peak to peak line height, h(0) is the central line, h(1) is the low field line and h(-1) is the high field line.

and the process repeated at 5° increments to 95° C. Temperature was monitored with a Digital Thermocouple Thermometer Model 8529-00 (Cole-Parmer Instruments Co.).

Starch-hexane-probe

Wheat starch-hexane-probe systems were slurried at room temperature for 2 h with stirring in order to determine whether probe binding to starch was influenced differently when the probe was dissolved in a hydrophobic solvent whose properties are very different from water. ESR spectra were obtained at room temperature for the: starch-hexane-probe slurry; separated starch from hexane; and hexane supernatant. Wheat starch was washed with hexane and spectra obtained from the separated starch and hexane supernatant. The dilution factor per wash was similar to that in the starch:water:probe system.

ESR Spectra

Spectra were collected using a Varian E-3 spectrometer at about 9.33 GHz at room temperature using 2 mm glass tubes for sample cells. No attempt was made to exclude oxygen. The spectra were recorded in the vicinity of 3.2 G with attenuation power low enough to avoid any saturation.

Correlation times (τ) were collected for three-line spectra (Fig. 2) based on the Kivelson theory (Kivelson, 1960) using peak-to-peak line height (h) ratios of first derivative spectra and the line width of the central line $[T_2(0)]^{-1}$ (Stone et al., 1965). Thus

$$\tau = 4 \left[\left(\frac{h(0)}{h(1)} \right)^2 + \left(\frac{h(0)}{h(-1)} \right)^2 - 2 \right] b^{-2} [T_2(0)]^{-1}; \quad (1)$$

where $h(M_N)$ are the line heights corresponding to the nitrogen nuclear spin state ($M_N = +1, 0, -1$) and b is a constant determined from the components of the electron-nuclear hyperfine tensor, given by

$$b = (4\pi/3) [A_{zz} - \frac{1}{2}(A_{xx} + A_{yy})]. \quad (2)$$

All correlation times were calculated assuming isotropic motion of the nitroxide. Correlation times were calculated only when a simple 3 line spectrum was observed.

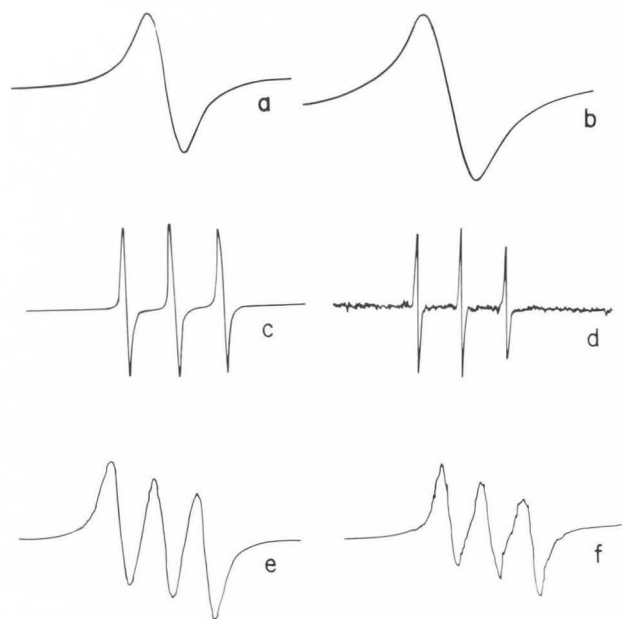


Fig. 3. ESR spectra of TEMPO when neat (a), or in dilute solution in water (c), or hexane (e). Corresponding spectra for 16-DOXYL-stearic acid are shown in (b), (d) and (f); (d) is the spectrum of the supernatant, i.e., the saturated solution.

Results and Discussion

Typical ESR spectra for TEMPO neat, 16-DOXYL-stearic acid neat, TEMPO in water, a saturated solution of 16-DOXYL-stearic acid in water, TEMPO in hexane, and 16-DOXYL-stearic acid in hexane can be found in Figure 3. Both probes run neat had similar line shapes consisting of one very broad line (Fig. 3a and 3b), a result of spin broadening due to the high spin concentration. The probes in water (Fig. 3c and 3d) both had a sharp 3-line spectrum. However, 16-DOXYL-stearic acid (Fig. 3d), ($\tau = 1 \times 10^{-10}$ sec) had a decreased high field line height indicative of motion slightly slower than TEMPO in water (Fig. 3c), ($\tau < 10^{-11}$ sec) where all three lines were approximately the same height. Three line spectra, broader than those in water, were recorded for each probe in hexane (Fig. 3e and 3f). Line broadness in hydrocarbon solvents has been attributed to oxygen broadening (Jost and Griffith, 1976). When nitrogen gas was bubbled through hexane systems sharp three line spectra were recorded.

Unheated starch experiments with water

Figures 4 and 5 contain typical spectra of starch and supernatant separated from starch-water-probe systems. Also, typical spectra are included for starch washed repeatedly with water and the supernatant from these washes.

TEMPO

Starch separated without any washing from the 24 h slurry of starch-water-TEMPO had a sharp 3-line spectrum (Fig. 4a) with some slowed probe motion. Slowed motion for TEMPO in the spun down starch fraction can be seen from a comparison between TEMPO in water (Fig. 3c) ($\tau < 10^{-11}$ sec)

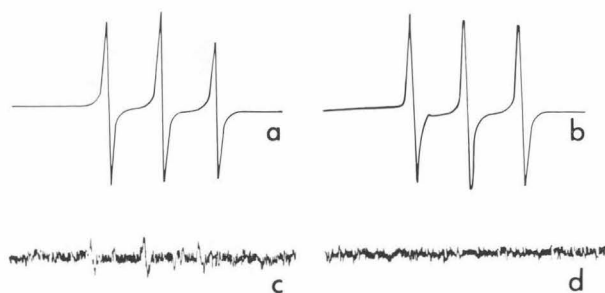


Fig. 4. Typical ESR spectra for starch-water-TEMPO: (a) Spun down starch fraction, initial slurry; (b) Supernatant, initial slurry; (c) Spun down starch fraction, 8th wash; (d) Supernatant, 8th wash.

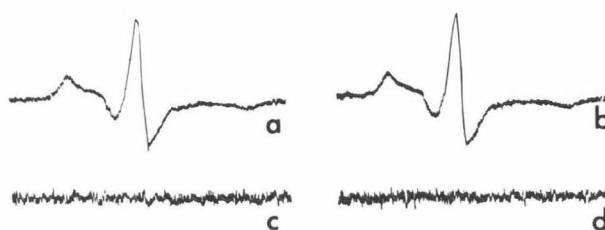


Fig. 5. Typical ESR spectra for starch-water-16-DOXYL-stearic acid. (a) Spun down starch fraction, initial slurry. (b) Spun down starch fraction, 18th wash. (c) Supernatant; initial slurry. (d) Supernatant, initial 18th wash.

and TEMPO in the starch fraction from the slurry (Fig. 4a) ($\tau = 1 \times 10^{-10}$ sec). A sharp 3-line spectrum similar to that seen for TEMPO in water was obtained from the first supernatant (Fig. 4b). The τ value was $\tau < 10^{-11}$ sec indicating probe motion was not slowed. Subsequent washings and separations of starch from supernatant gave similar results except for gradual diminution in spectral intensity. No ESR signal was detected after the 8th wash in the supernatant (Fig. 4d). The ESR signal for the 8th wash starch fraction (Fig. 4c) was so small that τ could not be calculated. Eventually the ESR signal disappeared from the starch fraction with further washing of the starch. With a dilution factor of 5 or 6 per wash, it was calculated that the ESR signal should have disappeared by the 6th wash due to an undetectable concentration of spin probe remaining in the sample ($< 10^{-6}$ M). The eight washings actually required for the ESR signal to disappear from the spun down starch fraction was in close agreement with the theoretical calculated number of washings, allowing for experimental error.

The slowed TEMPO motion at room temperature for the starch fraction indicates that TEMPO in water interacts with the starch granule prior to heating. The majority of ESR signal in the spun down starch fraction was from probe in the aqueous phase around the starch granules, but since τ was measurably slowed in the composite spectrum, it suggests that there was some penetration of probe and water in the granules.

Since TEMPO washed out of the starch fraction easily, a strong binding or adsorption did not occur. The slowed motion was attributed to differences in the local environment around TEMPO in the starch granule.

16-DOXYL-stearic acid

The spectra from a spun down wheat starch-water-16-DOXYL-stearic acid sample (Fig. 5a) were markedly different from those of the 3-line spectrum 16-DOXYL-stearic acid in water (Fig. 3d) or of the neat probe (Fig. 3b). Instead of the 3-line spectrum, a dilute-spin broad line powder pattern occurred indicating greatly slowed motion. After 18 washings, the line shape and intensity of the spectra of the starch fraction remained unchanged (Fig. 5b). The supernatants from the original slurry (Fig. 5c) or from any of the 18 washings (Fig. 5d) had a spin concentration too low to detect ($< 10^{-6}$ M), much lower, in fact, than that in a saturated solution of the spin probe.

The occurrence and persistence of a dilute spin powder pattern spectrum from the starch fractions combined with the absence of any ESR activity in the supernatants indicates a very strong adsorption, binding, or simultaneous adsorption and binding of the stearic acid probe. The interactions could have occurred either at the surface or in the interior of the starch granule.

To help answer this question, the following calculation was made and is summarized in Table 1. Total surface area for the amount of starch in the sample (2.5 g) was calculated assuming a spherical shape, density of 1.5 g/cm^3 (Banks and Greenwood, 1975), and diameters of 1, 10, and $100 \mu\text{m}$. The surface area occupied per molecule of 16-DOXYL stearic acid and the distance between molecules were estimated assuming adsorption and binding of all of the probe molecules on the starch surface. This calculation shows that the distance between 16-DOXYL stearic acid molecules on a sample of starch granules with diameters between 10 and $100 \mu\text{m}$ would range from 4.1 to 1.3 Å. Distances of this magnitude should result in a broadened ESR spectrum similar to that of the neat probe (Fig. 3b). The spectra actually observed for starch with diameters ranging from 2 to $40 \mu\text{m}$ (Goebel et al. 1984), however, were a dilute spin broad line powder pattern indicating that the probe molecules were more widely spaced. Such a spacing would be possible if the probes were adsorbed or bound in the interior rather than only on the surface.

In a separate experiment the starch-water-16-DOXYL-stearic acid slurry was sampled at 15 min intervals to determine the time period required for the powder pattern spectrum to appear. In this experiment care was taken to insure that the "chunks" of spin probe in the spin probe-water dispersion were not sampled. The powder pattern was obtained after 90 min of slurring.

Some discussion exists as to whether starch granules have a lipid membrane or surface lipid strongly adhered to the granule surface. The ease by which 16-DOXYL-stearic acid binds to starch does not support a lipid surface. Studies show that hydrophobic probes take a much longer time to transfer into the membrane as found in classic membrane-probe studies (McConnell, 1976).

Heated Starch Experiments with water

TEMPO

Typical spectra of wheat starch-water-TEMPO systems heated at different temperatures are shown in Fig. 6. The spectrum run immediately after the slurry was prepared without heating (Fig. 6a) showed slowed motion the same as the slowed motion seen for starch fractions slurried with TEMPO in the washing experiment (Fig. 4a). Spectra from samples heated at higher temperatures (Fig. 6b and 6c) also showed slowed motion of the probe with τ ranging from 2×10^{-10} to 4×10^{-10} sec. This motion was slightly slower than room temperature τ ranging between 1×10^{-10} and 2×10^{-10} sec but was not considered significantly different from the motion calculated from unheated systems.

16-DOXYL-Stearic Acid Heating Experiments

Typical spectra from wheat starch-water-16-DOXYL-stearic acid systems at room temperature and after heating are shown in Fig. 7. The spectrum of the room temperature slurry (Fig. 7a) showed a broad line powder pattern indicating greatly slowed motion. After heating at different temperatures only minor changes were observed in the line shape of the spectra (Fig. 7b and 7c). The small changes seen are typical of changes expected from local environment differences between starch and water (such as viscosity changes). The most significant feature of this experiment was that strong probe adsorption occurred at room temperature and remained unchanged with heating.

Other Starches

The other four starch types studied showed only small differences for the same probe system after mixing, separation of starch from supernatant, washed starch or heated starch systems. Therefore the typical spectra presented in Figures 2-6 for wheat starch are representative of the results seen for the other starches as long as the treatment was the same.

Hexane as a solvent

Figures 8a and 8b show spectra of wheat starch with hexane using TEMPO and 16-DOXYL-stearic acid as probes respectively. These spectra are identical to those found for probes and hexane (Fig. 3e and 3f). These results indicate that binding or adsorption on starch of either probe did not occur when slurried with hexane.

The spectra of starch initially separated from supernatant (Fig. 8c and 8d) and with successive hexane washes (Fig. 8g and 8h) show a less intense spectrum for each probe type with repeated washings. The supernatant from each successive wash also shows a less intense probe signal as the probe concentration remaining in the starch decreases. Spectra of the 10th wash supernatant (Fig. 8i and 8j) show a much less intense ESR signal than supernatant from the initial slurry (Fig. 8e and 8f). Thus the binding of the stearic acid probe either needs a more hydrophilic solvent such as water to facilitate the binding or a solvent such as water for which the stearic acid probe has a lower solubility.

Conclusions and Implications

The strong starch-16-DOXYL-stearic acid

Starch Electron Spin Resonance

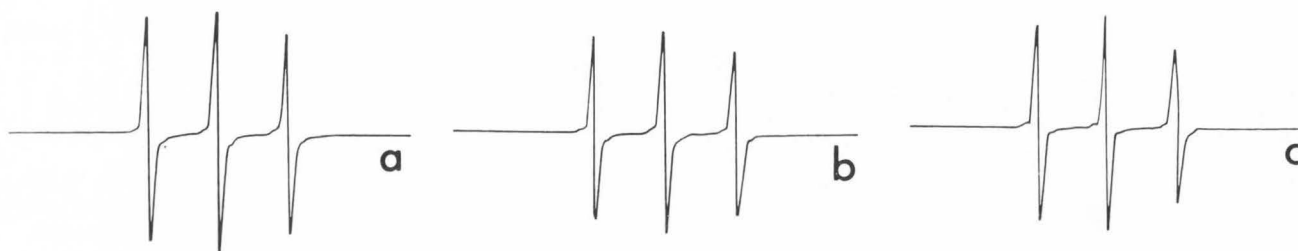


Fig.6. Typical ESR spectra for starch-water-TEMPO:
(a) Unheated; (b) Heated to 45°C; (c) Heated to 95°C.

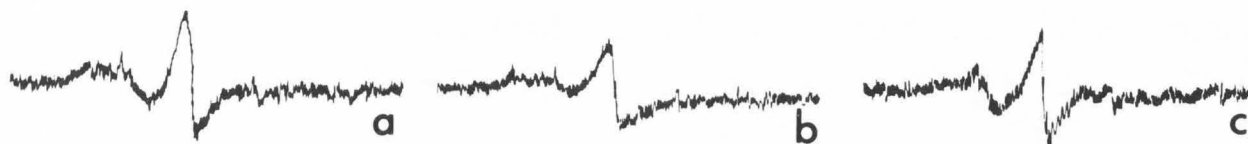


Fig.7. Typical ESR spectra for starch-water-16-DOXYL-stearic acid:
(a) Unheated; (b) Heated to 45°C; (c) Heated to 95°C.

interactions were found to take place in the starch granules at room temperature in aqueous systems and were not changed when starch was heated up to 95° C. TEMPO although motionally slowed did not result in strong binding with starch. Slowed probe motion was attributed to differences in the local environments experienced by the probe within the starch granule. No major differences existed for each starch system combination studied: wheat starch, hexane extracted wheat starch, waxy cornstarch or high amylose corn starch. Spectra from unheated wheat starch-hexane-probe systems do not show that starch-probe interactions took place.

These results suggest that interactions such as adsorption or binding can occur during product formulation at room temperature and early in formulation with long chain saturated fatty acid type molecules. Knowledge of how and in what way the interactions take place would allow the processor to alter the formulation and utilize techniques to control which reactions and at what time during processing these reactions take place. Encapsulation would be an example of such a procedure. Rotational correlation coefficients at room temperature and for heated and cooled samples were similar. This indicates that water and spin probe enter the starch granule at room temperature.

Table 1. Calculated Starch and 16-DOXYL-Stearic Acid Parameters.

Parameter	Starch Granule Diameter (μm)		
	1	10	100
Starch Surface Area (A^2)	1×10^{21}	1×10^{20}	1×10^{19}
$\text{A}^2/\text{Stearic Acid Molecule}$	166.7	16.7	1.7
Distance between molecules (A)	12.9	4.1	1.3

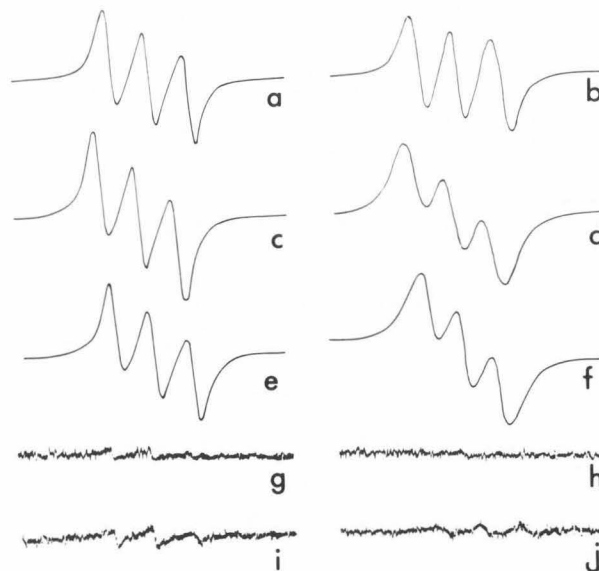


Fig.8. Typical ESR spectra for starch-hexane-TEMPO (a,c,e,g,i) and starch-hexane-16-DOXYL-stearic acid (b,d,f,h,j)
(a,b) Unfractionated;
(c,d) Starch fraction, initial slurry;
(e,f) Supernatant, initial slurry;
(g,h) Starch fraction, 10th wash;
(i,j) Supernatant, 10th wash.

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References

- Banks, W, Greenwood, CT (1975) The Structure and Biosynthesis of the Starch Granule, in: Starch and its Components, Halsted Press, New York, 242 - 305.
- Berliner, LJ, (Ed.) (1976) Spin Labeling: Theory and Applications. Academic Press, New York.
- Berliner, LJ, (Ed.) (1979) Spin Labeling II: Theory and Applications. Academic Press, New York.
- Ebert, B (1984) Helical Conformation of Amylose in Aqueous Solution. II. Electron Spin Resonance, Fluorescence Depolarization, and Sedimentation Measurements. *Biopolymers* 23, 2543-2557.
- Goebel, NK, Grider, J, Davis, EA, Gordon, J (1984) The Effects of Microwave Energy and Convection Heating on Wheat Starch Granule Transformations. *Food Microstructure* 3, 73-82.
- Johnson, AC, Hosenev, RC (1979) Chlorine Treatment of Cake Flours. III. Fractionation and Reconstitution Techniques for Cl₂ - Treated and Untreated flours. *Cereal Chem.* 56, 443 - 445.
- Jost, P, Griffith, OH (1976) Instrumental Aspects of Spin Labeling, in: Spin Labeling: Theory and Applications, L. J. Berliner (Ed.), Academic Press, New York, 251-272.
- Kivelson, D (1960) Theory of ESR Linewidths of Free Radicals. *J. Chem. Phys.* 33, 1094-1106.
- Liang, TM, Dickenson, PM, Miller, WG (1980) Conformation and Mobility of Polymers Adsorbed on Oxide Surfaces by ESR Spectroscopy, in: Polymer Characterization by ESR and NMR, A. E. Woodward and F.A. Bovey (eds.), ACS Symposium Series 142, American Chemical Society, Washington D.C., 3-18.
- McConnell, HM (1976) Molecular Motion in Biological Membranes, in: Spin Labeling: Theory and Applications, L.J. Berliner (ed.), Academic Press, New York, 525-560.
- Miller, WG (1979) Spin-Labeled Synthetic Polymers, in: Spin Labeling II. Theory and Applications, L.J. Berliner (ed.), Academic Press, New York, 173-221.
- Nishiyama, J, Kuninori, T, Matsumoto, H, Hyono, A (1981) ESR Studies on Lipid-protein Interaction in Gluten. *Agric. Biol. Chem.* 45, 1953-1958.
- Stone, TS, Buckman, T, Nordis, PL, McConnell, HM (1965) Spin labeled Biomolecules. *Proc. Natl. Acad. Sci. USA* 54, 1010 - 1017.

Discussion with Reviewers

- W. R. Croasmun: What more detailed information about the motional state of the probe can be obtained from the lineshape of the ESR spectra of the starch-water-16-DOXYL-stearic acid system?
- Authors: The powder pattern spectra obtained for starch-water-16-DOXYL-stearic acid systems resulted from very strong probe adsorption,

probe binding, or a combination of probe adsorption and binding with starch. The major focus of this study was to determine whether starch-probe interactions occurred. This could be determined from visual analysis of the spectral line shape. It is possible to also calculate rotational correlation coefficients for these types of spectra which would provide quantitative information on the degree of probe immobilization. Calculation of the rotational correlation coefficient would not provide information necessary for the objectives of this study but could be useful to determine differences in the degree of probe immobilization for different starches.

C. Hosenev: Although not specifically mentioned, I assume the 16-DOXYL-stearic acid probe is bound by amylose. Yet you report that the probe gave identical results with waxy and high amylose starches. How is this explained?

Authors: The powder pattern spectra recorded for both waxy and high amylose cornstarch are indicative of strong binding of the probe, adsorption of the probe, or a simultaneous combination of these two different phenomena. It is possible that the binding and adsorption occurred with the amylose, amylopectin, and lipid components of the high amylose starch. Small spectral differences indicated by a slightly less broad powder pattern were evident for the waxy starch and could have resulted from absence of the amylose binding or adsorption contribution to the spectra. Further work is being conducted to answer this question.

C. Hosenev: I do not understand your statement concerning a lipid membrane on starch granules. I know of no evidence of such a membrane.

Authors: The issue of whether a lipid membrane exists on starch granules is a controversy having supportive evidence in the literature for either position. Gracza cites numerous studies that suggest the presence of lipid or lipoprotein membranes on starch granules and that the membrane is derived from the amyloplast in the developing plant (Gracza, R. Minor constituents of starch. In: *Starch: Chemistry and Technology*, R. L. Whistler and E. F. Paschall (eds.), Academic Press, New York, 1965; 105-131). Simmonds also observed remnants of amyloplast membranes surrounding starch granules (Simmonds D. H. Morphological and molecular aspects of wheat quality. *Wallerstein Laboratories Communications* XXXIV, 1971; 17-31). Conversely, Cloke et al. (Cloke J.D., Gordon J., and Davis E.A. Freeze-etch of emulsified cake batters during baking. *Food Microstructure* 1, 1982; 177-187) and Davis and Gordon (Davis E.A. and Gordon J. Applications of low temperature microscopy of food systems. *J. of Microsc.* 112, 1978; 205-214) found no evidence of a starch granule membrane in freeze-etch and ultra-low temperature microscopy studies of model systems.