Lipid Binding in Whey Protein-Wheat Starch Systems as Measured by Electron Spin Resonance[1]

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ABSTRACT

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Lipid binding by whey protein concentrates (WPCs) and WPCs in combination with wheat starch was studied with electron spin resonance (ESR) measurements using 16-DOXYL-stearic acid as the free radical probe. Samples containing WPC, water, and probe showed broadened three-line spectra representing the probe at different mobilities. The whey protein concentrate containing higher levels of lactose showed an additional fast component in the high-field region that could not be reproduced by adding lactose to the low-lactose powder. In combined wheat starch and WPC systems, more of the probe was in faster motion when using high-protein, low-lactose WPC than was observed in the absence of the WPCs, but this was not observed when the medium-protein, high-lactose WPC was used. After heating to 95°C, similar differences in response to WPCs were found. Centrifugation experiments showed, however, that a portion of the probe remained with the whey protein concentrates in addition to that bound to the wheat starch.

Whey protein concentrates (WPCs) have been used in cereal-based formulated systems such as batters and doughs with moderate success. Their foaming and emulsifying properties are well known, and cake systems have been suggested as model systems for evaluating water binding, cohesion, elasticity, emulsification, and foaming properties of whey protein concentrates (Harper 1984).

The contributions of starch to the structure of cakes are also well recognized, and the interactions of batter components with starch have been studied in this context. Starch-lipid interactions including starch-emulsifier interactions have been shown to be important in determining functional properties of starch in many cereal-based formulated foods such as batters and doughs.

Recently, electron spin resonance (ESR) using stable free radical spin probes containing stearic acid has been used to study starch-lipid interactions (Pearce et al 1985, 1987a; Nolan et al 1986; Biliaderis and Vaughan 1987), gluten-lipid interactions (Nishiyama et al 1981, Pearce et al 1987b), and gluten-starch-lipid interactions (Pearce et al 1987b). The effects of incorporating other batter components, such as WPCs, have not been studied, however.

In ESR studies of the hydration of whey proteins (Schanen et al 1990), an increase in the proportion of a nonhydrogen-bonding, stable free radical probe in the hydrophobic environment

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relative to the hydrophilic environment occurred with heating. The implications of these changes in terms of lipid binding are examined in the present study. This will aid in understanding more precisely how emulsification is enhanced in such WPC containing systems.

Accordingly, in this study, lipid binding by WPCs individually and in combination with wheat starch was investigated using 16-DOXYL-stearic acid as the lipid probe. Two WPCs with different protein and lactose concentrations were used. The effects of heating the WPC-water-probe and WPC-wheat starch-water-probe systems were examined.

MATERIALS AND METHODS

Lactose, wheat starch, and WPCs were the same as those used for the hydration studies (Schanen et al 1990).

A high-protein, low-lactose and a medium-protein, high-lactose whey protein concentrate (Express Foods, Louisville KY) were used. Manufacturers' analysis for the preparations were: 75% protein, <0.5% lactose, 7% fat, and 3% ash; and 50% protein, 34% lactose, 5% fat, and 3% ash, respectively. Protein contents of the WPCs were determined by the authors as total Kjeldahl nitrogen using a conversion factor of 6.38 and found to be 74.6 and 52.6%, respectively. In addition to the two WPCs described above, lactose was added to the high-protein, low-lactose WPC to produce a mixture similar in protein and lactose content to that of the medium-protein, high-lactose WPC.

Spin Probe

The probe, 16-DOXYL-stearic acid, was obtained from Aldrich Chemical Co. (Milwaukee, WI). A water-spin probe stock solution (water-spin probe ratio, 1,000:1) was prepared by slurrying the probe with water for 24 hr at room temperature as described by Pearce et al (1985). A two-phase system resulted due to the low solubility of 16-DOXYL-stearic acid. Care was taken to exclude any undissolved probe in the samples in order to obtain the three-line spectra of the solution as described by Pearce et al (1985).

Preparation of Samples for Electron Spin Resonance Measurements

The ratios of lactose, WPC, or wheat starch to the stock probe solution and the ratios of their combinations are shown in Table I.

Two different procedures were used to prepare samples for analysis in the experiments in which the order of addition of wheat starch, WPC, or lactose was added to the stock probe solution. In the first procedure, lactose or WPC was slurried with the water-spin probe stock solution for 12 hr, wheat starch was added, and the mixture was slurried for 24 hr. In the second procedure, wheat starch and water-spin probe were slurried for 12 hr, then lactose or WPC was added, and the mixture was slurried for 24 hr.

For centrifugation studies, aliquots of WPC-wheat starch-water-spin probe samples and lactose-wheat starch-water-spin probe samples prepared as described in Table I were centrifuged for 7 min at 4,250 × *g*. Supernatants and the bottom layer were separated, aliquots were transferred to capillary tubes, and their ESR spectra were determined.

TABLE I
Ratios of Lactose, Whey Protein Concentrate, and Wheat Starch to Spin Probe-Water Stock Solutions and Slurrying Times

Sample	Ratio of Sample to Spin Probe-Water Stock Solution[a]	Slurrying Time (hr)
Lactose	0.4:2	12
WPC[b]	0.4:2	12
Wheat starch	1:2	24
Lactose-wheat starch	0.4:1:2	24
WPC[b]-wheat starch	0.4:1:2	24

[a] Stock solution ratio of spin probe to water, 1:1,000.
[b] Whey protein concentrate (WPC): high-protein, low-lactose concentrate; medium-protein, high-lactose concentrate; and high-protein, low-lactose concentrate with lactose added.

Heating Studies

An aliquot of the sample was transferred to a 2-mm (i.d.) capillary tube with a syringe. The capillaries were heated for 4 min in either a 75 or 95°C water bath and then quenched for 4 min in a room temperature water bath prior to ESR measurements.

ESR Spectra

Capillary tubes (2-mm i.d.) containing the samples were placed in 5-mm nuclear magnetic resonance spectroscopy sample tubes, and spectra were determined on a Varian E-3 spectrophotometer at about 9.34 GHz at room temperature. Spectra were centered at 3,235 G with a scan range of $\pm 0.5 \times 10^2$. Attenuation power was low enough to avoid saturation.

Apparent correlation times (τ) were calculated only when simple three-line spectra were present. All calculations were made using Kivelson theory (Kivelson 1960, Stone et al 1965) assuming isotropic motion of the probe as described previously (Pearce et al 1985).

RESULTS

Lactose and WPC Studies

ESR spectra for the water-16-DOXYL-stearic acid system showed sharp three-line spectra with correlation times of 0.1 nsec. This correlation time, which indicated a nonassociation of the probe and its environment, serves as a baseline to which samples containing additional components can be compared.

Lactose-water-16-DOXYL-stearic acid. ESR spectra for water-16-DOXYL-stearic acid in the presence of lactose showed a sharp three-line spectrum (Fig. 1) with a correlation time of 0.1 nsec. The correlation times were not changed by heating to 75 and 95°C. The similarities between spectra for the lactose-containing system and spectra for the water-16-DOXYL-stearic acid system showed that lactose, at the concentration used in these experiments, did not affect the mobility of the 16-DOXYL-stearic acid probe.

WPC-water-16-DOXYL-stearic acid. Spectra of the WPC-water-16-DOXYL-stearic acid systems are shown in Figure 2 and apparent correlation times in Table II. Spectra were characterized by a broadened three-line pattern, which represents 16-DOXYL-stearic acid of differing mobilities. The buildup of intensity in the low-field line indicates some slow motion of the probe and possibly a small amount of probe that is completely immobilized. The broadened three-line spectrum indicates, however, that most of the spin probe is not completely immobilized. Spectra of the medium-protein, high-lactose WPC-water-16-DOXYL-stearic acid samples show, in addition to the broadened three-line spectrum, a fast motion component in the high-field region (F in Fig. 2d–f). This component was present both at room temperature and after heating. The fast component was not present in the high-protein, low-lactose WPC and could not be produced by the addition of lactose to the low-lactose WPC (Fig. 2a–c vs. 2g–i).

Apparent correlation times for the high-protein, low-lactose WPC-water-16-DOXYL-stearic acid samples were 1.3, 1.7, and

Fig. 1. Electron spin resonance spectrum for lactose-water-16-DOXYL-stearic acid (0.4:2:0.002) at room temperature.

1.5 nsec, for unheated samples and after heating to 75 and 95°C, respectively (Table II). Apparent correlation times for the high-protein, low-lactose WPC-water-16-DOXYL-stearic acid samples to which lactose was added were in the same range. The correlation times of 1.3–1.7 nsec indicate slowed motion relative to the probe in water or in water-lactose systems. These correlation times were not changed by heating. Apparent correlation times were not calculated for the medium-protein, high-lactose system, because the presence of the fast component in the high-field region of the spectrum made measurement of peak heights inaccurate.

Studies of Wheat Starch in Combination with Lactose and WPC

Wheat starch-water-16-DOXYL-stearic acid. Wheat starch-water-16-DOXYL-stearic acid spectra show a broad line powder pattern at room temperature and after heating (Fig. 3). Small changes in the slow motion (A) and fast motion (B) components of the low-field region occur with heating. The slight increase in the amplitude of B after heating indicates that more of the probe is now in faster motion. These results are consistent with results previously reported by Pearce et al (1985, 1987a,b) and are included for comparison with the results when lactose or WPCs are added to the system.

Lactose-wheat starch-water-16-DOXYL-stearic acid. With lactose added to the system, the spectra show the broad line powder patterns at room temperature and after heating (Fig. 4a–c) that are characteristic of wheat starch-water-16-DOXYL-stearic acid alone (Fig. 3a–c). The spectra were not affected by the order in which lactose and wheat starch were added to the water-spin probe solution. Spectra for the supernatant after centrifugation showed no signal (Fig. 4d), and the broad line powder pattern was recovered in the starch layer (Fig. 4e). Similar results were reported by Pearce et al (1985) for wheat starch-water-16-DOXYL-stearic acid and demonstrate that the strong binding of the probe to the wheat starch was not affected by the presence of lactose.

WPC-wheat starch-water-16-DOXYL-stearic acid. Spectra of WPC-wheat starch-water-16-DOXYL-stearic acid are shown in Figure 5. The broad line powder pattern, indicating immobilized spin probe, was present for each of the WPC-wheat starch systems both before and after heating. Small differences between systems containing WPCs and those containing only wheat starch were found in regions A and B and the high-field region, however.

The amplitude of the B component in the spectra of the high-protein, low-lactose WPC-wheat starch-water-16-DOXYL-stearic acid system (Fig. 5a–c) was higher than that for the wheat starch-water-16-DOXYL-stearic acid system, indicating that more of the probe was in faster motion when the WPC was present. The amplitude of the B component in this WPC-containing system was slightly reduced relative to the A component after heating to 75°C, but after heating to 95°C, the spectrum was similar to the room temperature spectrum. Therefore, for both the unheated system and the system heated to 95°C, more of the probe appeared to be in faster motion in the presence of this WPC than in its absence. Addition of lactose to the high-protein, low-lactose WPC system did not change the room temperature spectrum (Fig. 5a vs. 5g), but after heating, the spectra resembled those for the wheat starch in the absence of WPC (Fig. 5h and i vs. Fig. 3b and c).

The relative amplitudes of the A and B components of the spectra for the medium-protein, high-lactose wheat starch-water-16-DOXYL-stearic acid (Fig. 5d–f) resemble those for the wheat starch-water-16-DOXYL-stearic acid system (Fig. 3a-c) more closely than did those for the higher protein WPC (Fig. 5a-c). This result suggests that the faster motion fraction of the probe

TABLE II
Apparent Correlation Times for High-Protein, Low-Lactose WPC,[a] With and Without Added Lactose

System[b]	Correlation Time (nsec)		
	Room Temperature	75°C	95°C
High-protein, low-lactose WPC	1.3 ± 1.2	1.7 ± 0.6	1.5 ± 0.2
High-protein, low lactose WPC with added lactose	1.4 ± 0.1	1.4 ± 0.3	1.3 ± 0.4

[a] Whey protein concentrate.
[b] Ratio of WPC-water-16-DOXYL-stearic acid = 0.4:2:0.002.

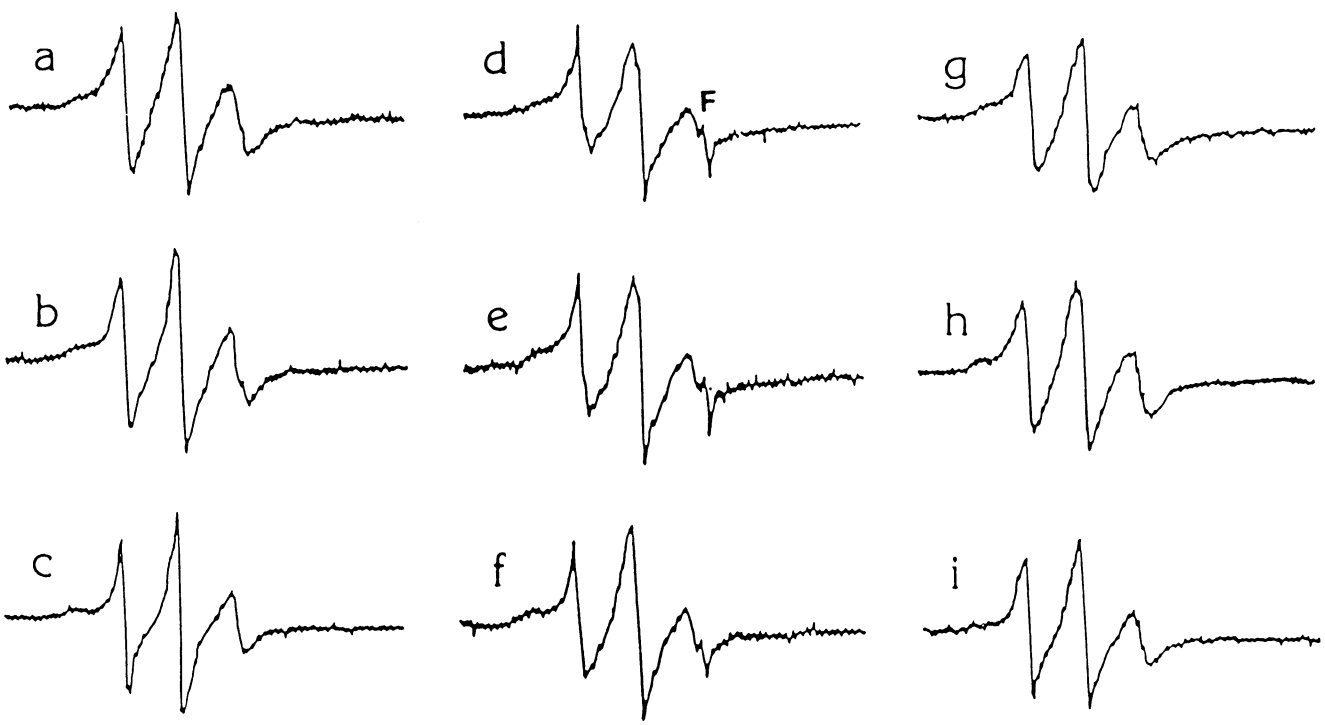

Fig. 2. Electron spin resonance spectra for WPC-water-16-DOXYL-stearic acid (0.4:2:0.002) at room temperature, and after heating to 75 and 95°C. **a,** High-protein, low-lactose whey protein concentrate (WPC) at room temperature. **b,** High-protein, low-lactose WPC at 75°C. **c,** High-protein, low-lactose WPC at 95°C. **d,** Medium-protein, high-lactose WPC at room temperature. **e,** Medium-protein, high-lactose WPC at 75°C. **f,** Medium-protein, high-lactose WPC at 95°C. **g,** High-protein, low-lactose WPC with added lactose at room temperature. **h,** High-protein, low-lactose WPC with added lactose at 75°C. **i,** High-protein, low-lactose WPC with added lactose at 95°C.

observed in the high-protein, low-lactose WPC system was not present in the medium protein WPC system.

Spectra typical of those for WPC-water-16-DOXYL-stearic acid samples were recovered in the supernatant layers after centrifugation (Fig. 6a and b, 6d and e). Spectra typical of wheat starch-water-16-DOXYL-stearic acid were found for the starch layer (Fig. 6c and f). Thus, some of the 16-DOXYL-stearic acid remained associated with WPCs, a result that contrasts with the observation that all of the 16-DOXYL-stearic acid remains associated with starch when only starch or starch and lactose are present. The order in which the WPCs and wheat starch were added to the water-spin probe stock solution did not affect the characteristics of the spectra demonstrating that the probe was not preferentially bound to one of the components.

DISCUSSION

ESR spectra suggest that, in the presence of WPCs, the motion of 16-DOXYL-stearic acid is slowed somewhat. It encounters several different microenvironments, and some of the probe may be immobilized although most of it is not completely immobilized. The slowed motion, as shown by correlation times, was not greatly affected by heating. Hydration studies (Schanen et al 1990) showed that the nonhydrogen-bonding probe, TEMPO, encountered more hydrophobic environments relative to the hydrophilic environments after the WPCs were heated. However, heating of WPCs did not appear to result in more slowed motion of the 16-DOXYL-stearic acid as might be expected on the basis of such changes in the hydrophobic-hydrophilic environments. The composite nature of spectra for the WPC-water-16-DOXYL-stearic acid systems, which shows the presence of the several microenvironments even at room temperature, may obscure the temperature-dependent effects on the hydrophilic-hydrophobic environments shown in the hydration studies.

These WPCs have good emulsification properties (Schanen 1988). Both the presence of hydrophilic-hydrophobic environments demonstrated in the hydration studies and the distribution of the lipid probe among several microenvironments are important properties of WPCs related to the ability of WPCs to move into water-oil interfaces in emulsions that will have oil-WPC-water environments.

The extent to which the ESR spectra for wheat starch-water-probe were modified by the addition of WPCs depended on the type of WPC being added. In the high-protein, low-lactose WPC-wheat starch system, the presence of this WPC resulted in an increase in the faster component in the wheat starch spectrum both before heating and after heating to 95°C. After heating to 75°C, the probe was immobilized to the same extent as in the absence of WPC. In the medium-protein, high-lactose WPC-wheat starch system, the probe appeared to be immobilized to the same extent as in the absence of WPC at all temperatures.

The composite nature of the room temperature spectra for both WPC-wheat starch-water-16-DOXYL-stearic acid systems was

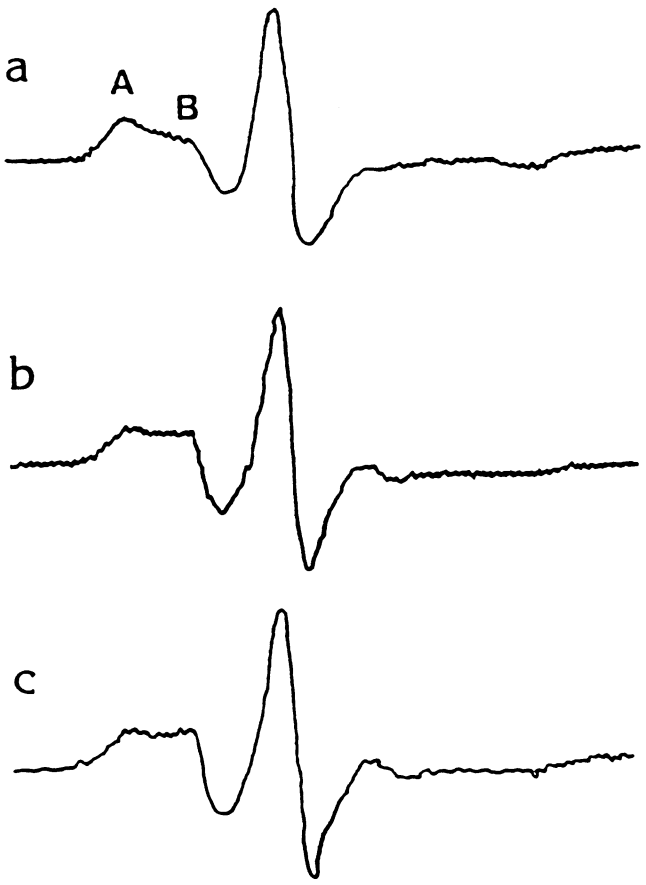

Fig. 3. Electron spin resonance spectra for wheat starch-water-16-DOXYL-stearic acid (1:2:0.002) at room temperature and after heating to 75 and 95°C. **A** is the slow component, **B** is the fast component. **a,** Room temperature, **b,** 75°C, and **c,** 95°C.

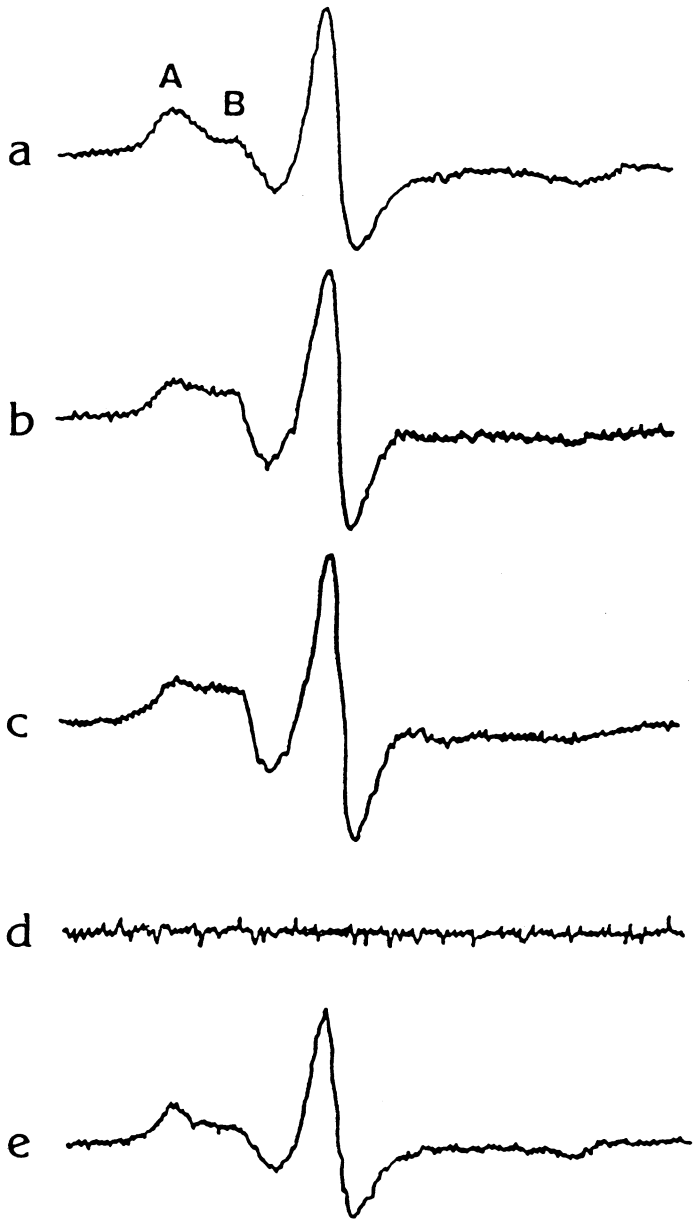

Fig. 4. Electron spin resonance spectra for lactose-wheat starch-water-16-DOXYL-stearic acid (0.4:1:2:0.002). **a,** Room temperature. **b,** After heating to 75°C. **c,** After heating to 95°C. **d,** Supernatant after centrifuging. **e,** Spun-down fraction after centrifuging.

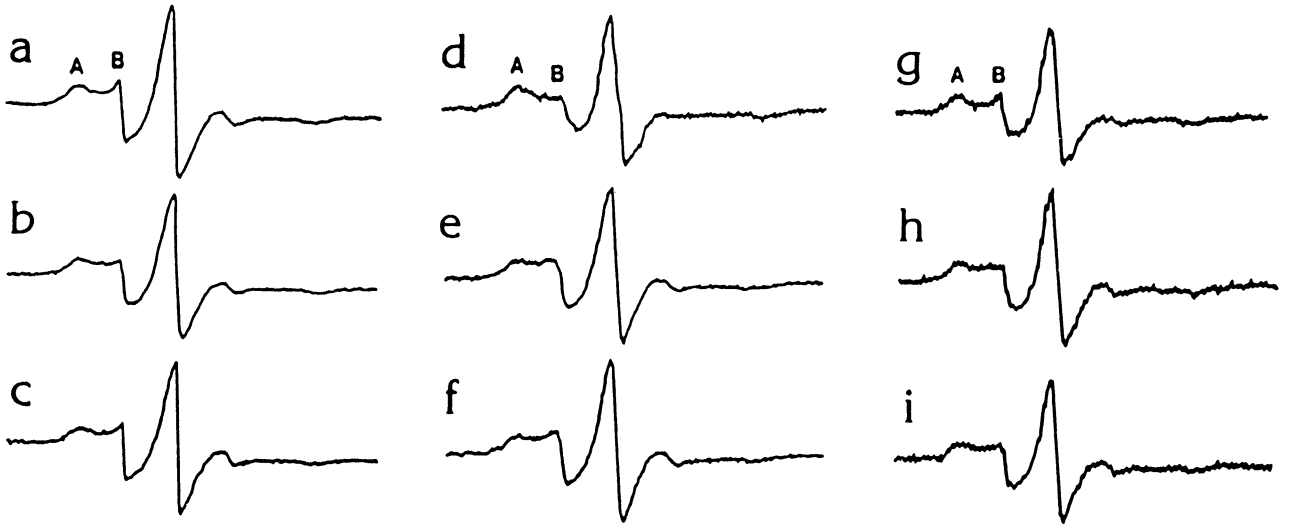

Fig. 5. Electron spin resonance spectra for whey protein concentrate (WPC)-wheat starch-water-16-DOXYL-stearic acid (0.4:1:2:0.002) at room temperature and after heating to 75 and 95°C. **a,** High-protein, low-lactose WPC at room temperature. **b,** High-protein, low-lactose WPC at 75°C. **c,** High-protein, low-lactose WPC at 95°C. **d,** Medium-protein, high-lactose WPC at room temperature. **e,** Medium-protein, high-lactose WPC at 75°C. **f,** Medium-protein, high-lactose WPC at 95°C. **g,** High-protein, low-lactose WPC with added lactose at room temperature. **h,** High-protein, low-lactose WPC with added lactose at 75°C. **i,** High-protein, low-lactose WPC with added lactose at 95°C.

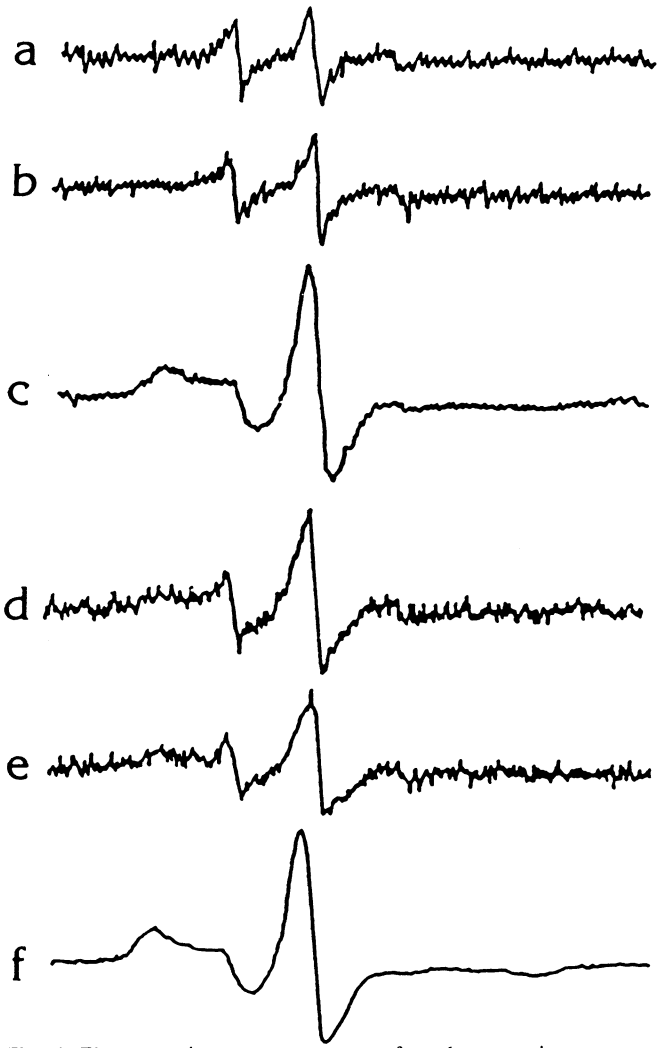

Fig. 6. Electron spin resonance spectra for whey protein concentrate (WPC)-wheat starch-water-16-DOXYL-stearic acid after centrifugation. **a,** High-protein, low-lactose WPC, top layer of supernatant. **b,** High-protein, low-lactose WPC, middle layer of supernatant. **c,** High-protein, low-lactose WPC, spun-down fraction. **d,** Medium-protein, high-lactose WPC, top layer of supernatant. **e,** Medium-protein, high-lactose WPC, middle layer of supernatant. **f,** Medium-protein, high-lactose WPC, spun-down fraction.

shown also by the centrifugation studies. The broadened three-line spectrum of WPC-water-16-DOXYL-stearic acid was recovered as well as the powder pattern of wheat starch-water-16-DOXYL-stearic acid. Thus, some of the probe remained in environments associated with WPC as well as in those associated with wheat starch. In the absence of WPC (Pearce et al 1985) or in presence of lactose, all of the probe was associated with the starch. These results, together with the results of the experiments in which the order that wheat starch and WPC were added to the probe was varied, suggested that the probe was not bound preferentially to one component at the expense of the other component. Consequently, the emulsification properties of WPCs related to WPC-lipid interactions would not interfere with starch-lipid interactions that contribute to starch functional properties in systems, such as batters, in which both properties contribute to final product quality.

The composition of the WPCs affected their binding properties, with a fast component in the field region being present in the medium-protein, high-lactose WPC spectra. This spectrum could not be reproduced by the addition of lactose to the high protein powder. Thus, in the medium-protein, high-lactose WPC, which were produced by adding back lactose prior to drying, protein-lactose interactions may have occurred that contributed to the fast motion components.

Binding of probes such as heptane, *cis*-parinaric acid, and 8-anilino-1-napthalene sulfonic acid has been used as a measure of hydrophobicity in whey proteins (Voutsinas et al 1983; Mangino et al 1987, 1988; Haque and Kinsella 1988). The effects of heating on the binding of these probes by WPCs have been variable. In general, however, the trend in the WPCs after processing is toward lower binding, which is interpreted as a decrease in hydrophobicity upon heating. In our case, using both TEMPO and 16-DOXYL-stearic acid, the pattern is an increase in the hydrophobic environment relative to the hydrophilic environment for TEMPO and little change in the environment for 16-DOXYL-stearic acid. These results suggest that each method evaluates different aspects of the hydrophobicity and binding properties of whey proteins.

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