

# Hydration of Whey Protein-Wheat Starch Systems as Measured by Electron Spin Resonance<sup>1</sup>

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## ABSTRACT

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Hydration of whey protein concentrates (WPCs) individually and in combination with wheat starch was studied using a stable, nonhydrogen bonding, free radical probe and electron spin resonance measurement techniques. WPCs with two different protein-lactose ratios were used. Measurements were made at room temperature and after heating to 75 and 95°C. In WPC-water systems, some slowed motion was observed, as well as a partition of the probe into hydrophobic and hydrophilic

environments. The hydrophobic environment increased relative to the hydrophilic environment as a consequence of heating or increasing protein concentration. Spectra for the combined WPC-wheat starch systems showed elements of the individual components: namely, slowed motion (WPC and starch contributions) and hydrophobic-hydrophilic environment partition (WPC contribution). WPC and wheat starch did not appear to compete for water.

Whey protein products have been introduced into cereal-based formulations as substitutions for, or supplements to, other milk proteins, sucrose, or egg whites. In cakes, addition of whey proteins has been reported to result in decreased volume (de Goumois and Hanning 1953), increased crumb fragility (Guy 1982), and a more velvety crumb (Best 1967). The reasons for these changes are not fully understood because of the complexity of batters and the potential for interaction of whey components with batter components.

Studies of model cake batters containing nonfat dry milk solids showed that starch transitions as measured by differential scanning calorimetry, temperature profiles and water loss rates during baking, lipid-protein matrix development in the crumb as viewed by scanning electron microscopy, and overall cake characteristics were altered by the introduction of milk solids (Pearce et al 1984). The potential for interaction of the components of whey protein preparations with starch includes starch-lactose, starch-lipid, starch-mineral, and starch-protein interactions. Competition for water among the whey protein components, starch, flour proteins, and sugars may also occur.

Recently, electron spin resonance (ESR) has been used to study hydration and lipid-binding properties of starch and gluten

(Biliarderis and Vaughan 1987; Nolan et al 1986; Pearce et al 1985, 1987a,b, 1988; Windle 1985) and water and protein mobility in casein (LeMeste and Duckworth 1988).

In the current study, the hydration of whey protein concentrates (WPCs) and lactose was studied using the stable, nonhydrogen bonding, free radical probe, TEMPO. Two WPCs with different protein-lactose ratios were used. The effects of heating and protein concentration of WPCs were studied. Hydration of combinations of WPCs, lactose, and wheat starch were also studied, and the effects of heating were determined.

## MATERIALS AND METHODS

Reagent grade D(+)-lactose monohydrate powder (J. T. Baker, Phillipsburg, NJ) and wheat starch (Aytex P, General Mills, Minneapolis, MN) were used.

A high-protein, low-lactose whey protein concentrate (manufacturer's analysis, 75% protein, <0.5% lactose, 7% fat, and 3% ash) and a medium-protein, high-lactose whey protein concentrate (manufacturer's analysis, 50% protein, 34% lactose, 5% fat, and 3% ash) were obtained from Express Foods, Louisville, KY. Protein contents determined as total Kjeldahl nitrogen and using the conversion factor of 6.38 for whey products were 74.6% and 52.6%, respectively.

## Spin Probe

A stock solution of water and TEMPO (2,2,6,6-tetramethylpiperidinyloxy, Aldrich Chemical, Milwaukee, WI) (1:0.001, w/w) was prepared by slurring the probe with water for 24 hr at room temperature.

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### Sample Preparation

Lactose-water-TEMPO solutions were prepared by combining lactose and the water-TEMPO stock solution in the ratio of 0.4:2.0 (w/w). Samples were magnetically stirred for 12 hr prior to determination of ESR spectra.

WPC-water-TEMPO systems were prepared by combining WPC samples with water-TEMPO stock solution and magnetically stirring for 12 hr. The amount of the high-protein, low-lactose WPC added to 2 ml of water-TEMPO stock solution was varied from 0.3 to 1.0 g to give a range in protein content from 9.7 to 24.9%. Amounts of the medium-protein, high-lactose WPC were varied from 0.45 g to 1.5 g to give a range of protein content from 9.7 to 22.5%.

In another series of experiments, lactose was added to the high-protein, low-lactose WPC to give protein-lactose ratios similar to those in the medium-protein, high-lactose system. Amounts of the high-protein, low-lactose WPC added to 2 ml of the water-TEMPO stock solution were varied from 0.3 to 1.0 g and amounts of lactose from 0.15 to 0.51 g. This resulted in protein/lactose contents from 9.1%/6.12% to 21.3%/14.5%. All samples were magnetically stirred for 12 hr.

Hexane-extracted WPC was prepared by magnetically stirring high-protein, low-lactose WPC with hexane (99+%, Aldrich Chemical, Milwaukee, WI) in the ratio of 0.4:1 (w/v) for 24 hr at room temperature. The resulting slurry was filtered, washed four times with 50-ml portions of hexane, and air-dried.

Portions of hexane-extracted WPC were then slurried with 2 ml of water-TEMPO stock solution for 12 hr. Amounts of hexane-extracted WPC were also varied from 0.3 to 1.0 g.

### Starch-Containing Systems

Wheat starch-water-TEMPO systems were prepared by magnetically stirring wheat starch-water-TEMPO in the ratio of 1:2:0.002 for 24 hr.

Lactose-starch-water-TEMPO solutions and WPC-starch-water-TEMPO systems in the ratio of 0.4:1:2:0.002 were prepared by stirring for 24 hr.

For centrifugation studies, aliquots of lactose-starch-water-TEMPO solutions or WPC-starch-water-TEMPO were centrifuged at  $4,250 \times g$  for 7 min. Supernatants and the centrifugate were transferred to separate capillary tubes and ESR spectra determined.

The order in which wheat starch and lactose or WPC were added to the water-TEMPO stock solution was varied in another set of experiments to determine whether the probe was preferentially partitioned with the starch, the WPC, or the lactose. In the first part of the experiment, lactose or WPC was slurried with the water-TEMPO stock solution for 12 hr, wheat starch was added, and the mixture slurried for 24 hr. In the second part of the experiment, wheat starch and water-TEMPO probe were slurried for 12 hr, then lactose or WPC was added, and the mixture was slurried for 24 hr. Ratios of WPC-wheat starch-water-TEMPO or lactose-wheat starch-water-TEMPO were 0.4:1:2:0.002.

### Heating Studies

An aliquot of the sample was transferred to a 2-mm capillary tube and the tube was heat sealed. 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

An aliquot of each sample was transferred to a 2-mm capillary tube. The tube was then placed in a 5-mm nuclear magnetic resonance tube and spectra 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.

Correlation times were calculated only when a simple three-line spectrum was present (Fig 1). All calculations were made using Kivelson theory (Kivelson 1960, Stone et al 1965, Pearce

et al 1985). All correlation times were calculated assuming isotropic motion of the nitroxide radical.

When a high-field doublet was present, the fraction of the spin probe in the more hydrophobic environment ( $f$  value) was calculated from the amplitude of lines  $H$  and  $P$  (Fig. 2) as

$$f = H/(H + P)$$

(McConnell et al 1972, Pearce et al 1988).

## RESULTS

The ESR spectrum for water-TEMPO is shown in Figure 1. The three-line spectrum is typical of freely spinning free radicals. Correlation times were 0.01 nsec, as previously reported (Pearce et al 1985). Spectra for lactose-water-TEMPO (0.4:2:0.002) at room temperature and after heating to 75 and 95°C also showed three-line spectra with correlation times of 0.01 nsec.

### WPC-Water-TEMPO

Spectra for the high-protein, low-lactose WPC at room temperature and after heating to 75 and 95°C are shown in Figure 2. Spectra for the medium-protein, high-lactose WPC showed similar characteristics.

The amplitude of the high-field line is reduced, indicating slowed motion of the probe. In addition, a high-field doublet is present. Correlation times were not calculated because of the presence of the doublet.

The doublet is attributed to the presence of both hydrophobic and hydrophilic environments (McConnell et al 1972, Pearce et al 1988). The relative distribution of TEMPO in the two environments, expressed as the  $f$  value, is summarized in Figure 3 for the two WPC preparations. For both WPCs, the  $f$  value increased with increasing concentration. It also increased after heating to 75 or 95°C (Fig. 3A and B). Addition of lactose to the high-protein, low-lactose WPC to give protein-lactose ratios similar to those found in the medium-protein, high-lactose powder resulted in  $f$  values that were similar to those for the two WPCs. The  $f$  values also increased as concentration was increased and after heating (Fig. 3C). Hexane extraction of the high-protein, low-lactose WPC did not change the  $f$  values over the concentration range studied or the response to heating.

### Wheat Starch-Water-TEMPO

Spectra for wheat starch-water-TEMPO (1:2:0.002) at room temperature and after heating to 75 and 95°C are shown in Fig. 4A-C. Spectra show three-line spectra with reduced high-field amplitudes. Correlation times were  $0.16 \pm 0.03$  nsec,  $0.23 \pm 0.03$  nsec, and  $0.25 \pm 0.04$  nsec for samples at room temperature, and after heating to 75 and 95°C, respectively. These values are similar to those reported previously (Pearce et al 1985). The values for heated samples indicate slightly slowed motion relative to unheated samples, but they are not considered significantly different from those for unheated samples (Pearce et al 1985).

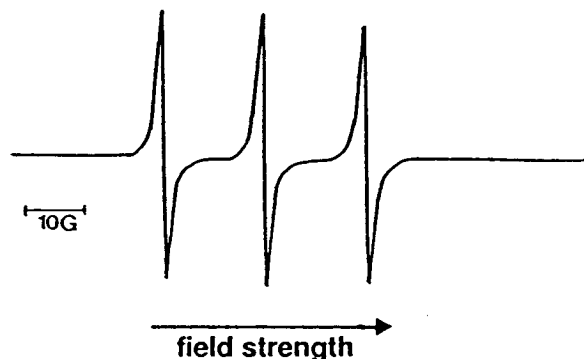


Fig. 1. Electron spin resonance spectrum for water-TEMPO (1:0.001).

### Lactose-Wheat Starch-Water-TEMPO

Spectra for lactose-wheat starch-water-TEMPO (0.4:1:2:0.002) (Fig. 4C-F) showed three-line spectra with reduced high-field amplitudes similar to those for wheat starch-water-TEMPO samples. The correlation times for samples at room temperature and after heating to 75 and 95°C were  $0.11 \pm 0.02$  nsec,  $0.29 \pm 0.05$  nsec, and  $0.30 \pm 0.03$  nsec, respectively, and are similar to those for similarly treated samples containing only wheat starch-water-TEMPO. The order of addition of lactose and starch to the water-TEMPO solution did not affect the correlation times.

Centrifugation of the lactose-wheat starch-water-TEMPO system resulted in separation into a clear supernatant and a bottom layer that was primarily wheat starch. ESR signals were detected for both layers. Average correlation times for the supernatant were  $\leq 0.01$  nsec, which is typical of water-TEMPO, and 0.3 nsec for the bottom layer, which is typical of this wheat starch-water-TEMPO system. Similar results were reported by Pearce et al (1985) for wheat starch-water-TEMPO systems without added lactose. These experiments indicate that the presence of lactose at the concentration used in these experiments did not alter the mobility of TEMPO from that found in the wheat starch-water system.

### WPC-Wheat Starch-Water-TEMPO

Spectra for the high-protein, low-lactose WPC-wheat starch-water-TEMPO system (0.4:1:2:0.002) are shown in Figure 5 for measurements made at room temperature and after heating to 75 and 95°C. Medium-protein, high-lactose WPC-wheat starch-water-TEMPO spectra showed similar characteristics. All spectra showed elements that were present in spectra for wheat starch and whey protein systems individually, namely, reduced high-field height, which occurred with either WPC or starch indi-

vidually, and a high-field doublet, which was typical of the protein systems. Table I shows  $f$  values for the high-field doublets. In each case, the  $f$  value tended to increase with heating.

The composite nature of the spectra for WPC-wheat starch-water-TEMPO systems was also shown by the centrifugation experiments in which characteristic whey protein-water-TEMPO spectra were found for the supernatants and wheat starch-water-TEMPO spectra for the bottom layer.

The order in which water-TEMPO was slurried with whey protein versus wheat starch did not affect the resulting spectra. Addition of lactose to the high-protein, low-lactose powder did not change the shape of the spectra from those shown for the high-protein, low-lactose powder (Fig. 5) or the  $f$  values (Table I).

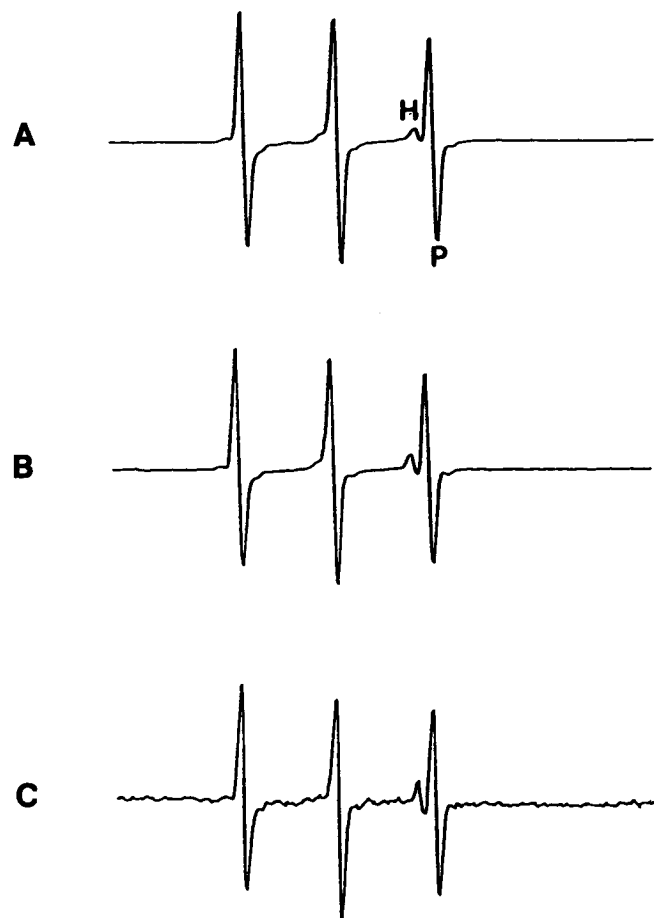


Fig. 2. Electron spin resonance spectra for high-protein, low-lactose whey protein concentrate-water-TEMPO (0.4:2:0.002) at A, room temperature; B, after heating to 75°C; and C, after heating to 95°C.

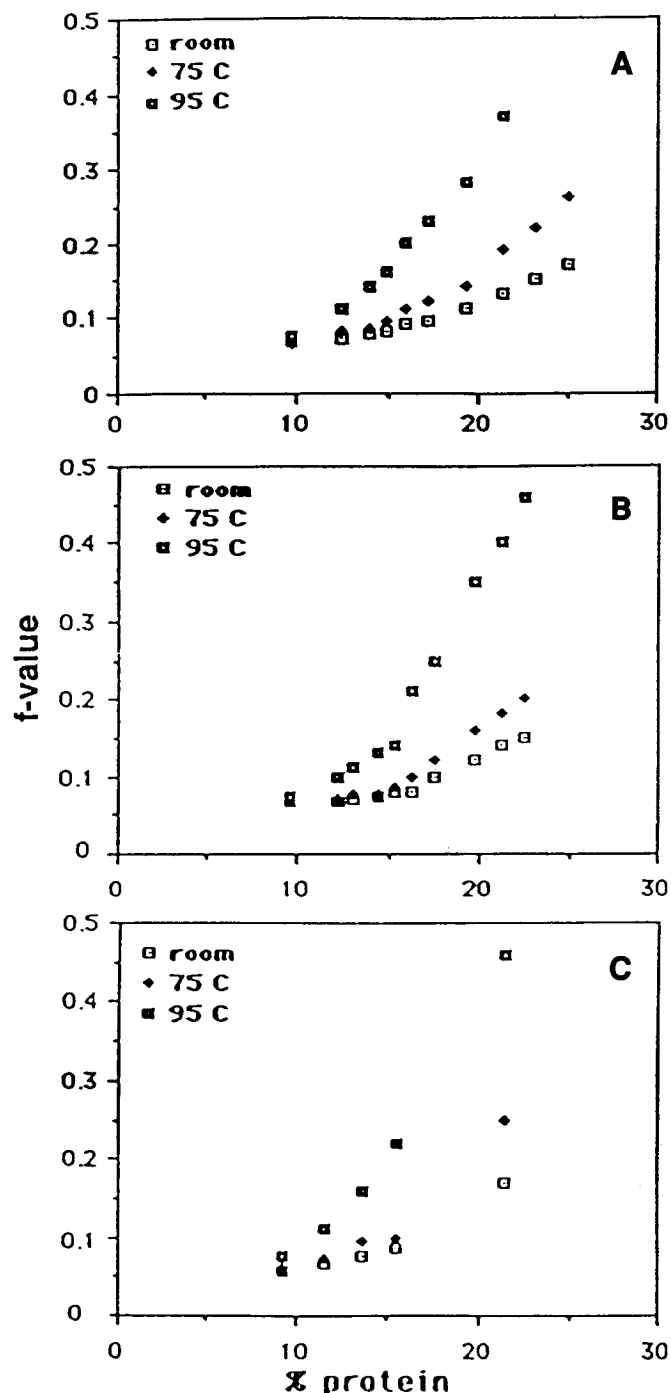


Fig. 3. Electron spin resonance spectra  $f$  values for whey protein concentrate (WPC)-water-TEMPO as a function of protein concentration and temperature. A, High-protein, low-lactose WPC; B, medium-protein, high-lactose WPC; C, high-protein, low-lactose WPC with added lactose.

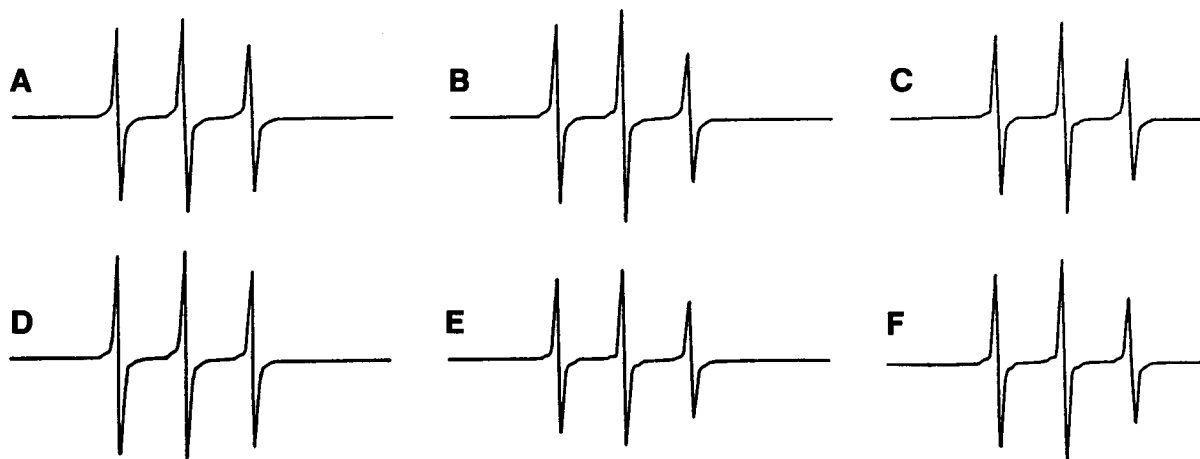


Fig. 4. Electron spin resonance spectra for wheat starch-water-TEMPO (1:2:0.002) and lactose-wheat starch-water-TEMPO (0.4:1:2:0.002). A, wheat starch-water-TEMPO at room temperature; B, wheat starch-water-TEMPO after heating to 75°C; C, wheat starch-water-TEMPO after heating to 95°C; D, lactose-wheat starch-water-TEMPO-room temperature; E, lactose-wheat starch-water-TEMPO after heating to 75°C; F, lactose-wheat starch-water-TEMPO after heating to 95°C.

### DISCUSSION

The ESR results for the WPC-water-TEMPO systems show that motion of the probe is slowed somewhat in the presence of WPCs. In addition, the presence of the high-field doublet showed that the probe was distributed between hydrophilic and hydrophobic environments. The hydrophobic environment encountered by the probe in WPC-water-TEMPO (a nonhydrogen bonding, nonreactive probe) systems may be due to the hydrophobic groups in the protein or to the presence of lipids. Hexane extraction of free lipids did not change the ratios of hydrophobic to hydrophilic environments, so that if lipids contribute to the hydrophobic environment, they are probably tightly bound to the protein. Denaturation may contribute to the hydrophobic environment through conformational changes that expose additional hydrophobic groups. Heating the WPC-water-TEMPO systems to 75 or 95°C resulted in increased hydrophobicity as measured by the ratio of hydrophobic/hydrophilic environments. Evaluation of the surface hydrophobicity of whey proteins based on the binding of fluorescent probes (Voutsinas et al 1983; Mangino et al 1987, 1988; Haque and Kinsella 1988) has generally shown decreases in surface hydrophobicity as a result of heating individual whey proteins or as a result of heat treatments of whey preparations during or after processing. Other measures of denaturation such as protein solubility (de Wit et al 1988, Li-Chan 1983) also show changes that are interpreted as being the result of conformational changes. Changes in surface hydrophobicity during processing represent the net result of complex interactions among the several components of the whey. The change in these complexes during subsequent heating is the characteristic being measured in our ESR experiments with heated samples. They are indicative of the potential for further changes that might occur when WPCs are incorporated into other systems.

The  $f$  values for the WPC-water-TEMPO systems also increased as protein concentration was increased. Nuclear magnetic resonance studies of whey protein concentrates (Lambelet et al 1988) have shown increases in the proton transverse relaxation rate as the protein concentration was increased. Their results were interpreted as indicating an exchange between protons in water bound to protein and free bulk water. Relaxation rates decreased as temperature was increased to 55°C and then increased as the WPC-water system was heated to 90°C. In ESR experiments such as those reported here, the distinction between bound and free water is not made. Only the mobility of the probe in the several microenvironments it encounters is considered.

In the starch-water-TEMPO systems, the different microenvironments may be due to changes in the microviscosity at the starch granule surface or interior close to the amylose or amylopectin regions (Pearce et al 1985). Some slowed motion

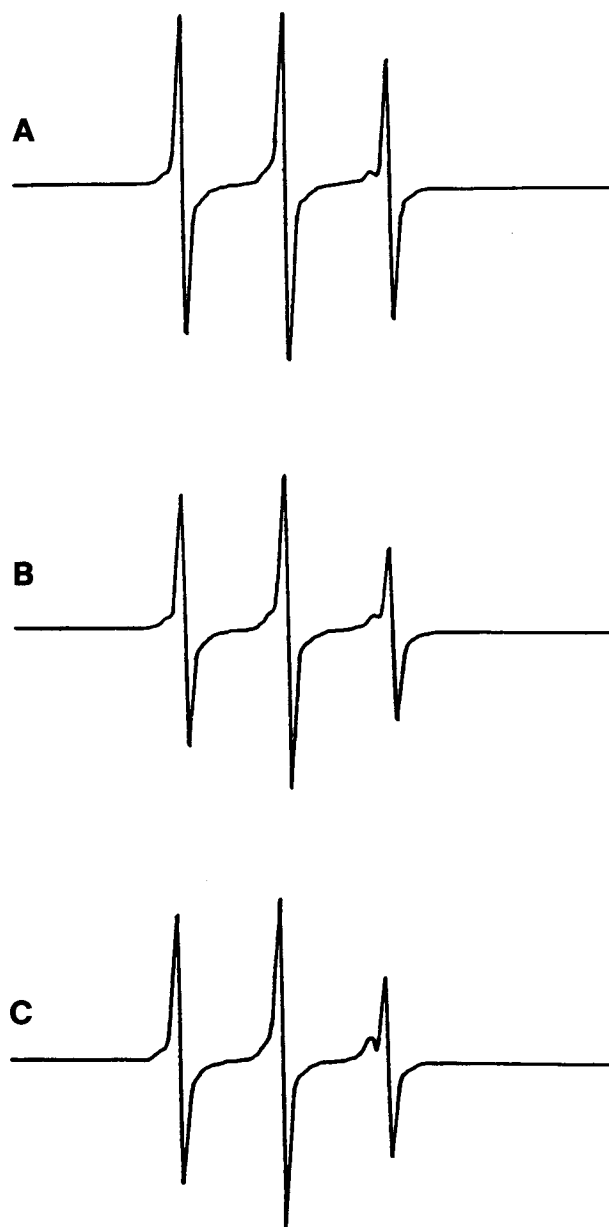


Fig. 5. Electron spin resonance spectra for high-protein, low-lactose whey protein concentrate-wheat starch-water-TEMPO (0.4:1:2:0.002) at A, room temperature; B, after heating to 75°C; and C, after heating to 95°C.

**TABLE I**  
**Mean<sup>a</sup> *f* Values for Wheat Starch-Water-TEMPO Systems**  
**Containing Whey Protein Concentrates (WPC)**  
**with Different Protein-Lactose Ratios**

System	Temperature		
	Ambient <sup>b</sup>	75°C	95°C
High-protein, low-lactose WPC wheat starch	0.10 ± 0.00	0.15 ± 0.01	0.19 ± 0.02
Medium-protein, high-lactose WPC wheat starch	0.09 ± 0.02	0.12 ± 0.01	0.16 ± 0.03
High-protein, low-lactose WPC lactose mixture wheat starch	0.08 ± 0.01	0.11 ± 0.02	0.12 ± 0.01

<sup>a</sup> Mean of three samples.

<sup>b</sup> Ambient laboratory temperatures were around 21°C.

of the probe in the presence of starch was found both in the presence and absence of WPC, and this is attributed to changes in microviscosity rather than in bulk viscosity. Superimposed on this was the hydrophobic/hydrophilic character of the whey proteins. Whey proteins and starch did not appear to compete for water for the following reasons. The characteristics of the ESR spectra of wheat starch and whey proteins individually were present in the combined system; they were not affected by the order of addition of starch and whey proteins to the water-TEMPO; and characteristic individual spectra could be obtained when the mixture was separated by centrifugation.

Lactose alone had little effect on the water microenvironment. In combination with wheat starch, the correlation times were similar to those found for other sugars including maltose and sucrose (Johnson et al 1990). Addition of free lactose to the low-lactose WPC did not change the hydration properties as compared to those of the WPC processed with lactose present.

In earlier studies, gluten-water-TEMPO systems also showed the presence of hydrophobic/hydrophilic environments, and the relative proportion of the hydrophobic environment increased with heating (Pearce et al 1988). Thus, in a batter or dough in which both gluten and whey proteins are present, the hydrophobic environment might be expected to be increasing relative to the hydrophilic environment during heating with concomitant changes in the potential for interactions with other components of the batter.

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