

Dielectric Properties and Water Mobility for Heated Mixtures of Starch, Milk Protein, and Water¹

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ABSTRACT

Cereal Chem. 72(1):64-69

The dielectric properties of hydrated whey protein isolate (WPI), Ca-caseinate, and wheat starch, alone or in combination, were measured at ambient temperature and during heating to 90°C. At lower moisture contents and ambient temperature, WPI exhibited higher dielectric properties than starch, whereas Ca-caseinate had a lower dielectric constant than either WPI or starch. At higher moisture contents, the dielectric properties were similar. At moisture contents of 30–80%, WPI showed increasing microwave absorption properties with increasing temperature; at higher moisture contents, microwave absorption by Ca-caseinate decreased with temperature. Adding WPI affected the dielectric loss and

absorptivity of starch during heating but such an effect was not evident with Ca-caseinate. The dielectric properties of these systems were compared with their hydration properties by electron spin resonance using TEMPO, a water and oil-soluble noninteractive probe to correlate water mobility results to dielectric relaxation phenomena. All systems were equally effective in slowing the motion of TEMPO. However, their dielectric properties differed, indicating that the dielectric properties are not only influenced by the water but by the macromolecules present as well.

Milk protein ingredients are used in many applications by the food industry because they provide a wide range of functional properties and have high nutritional value. The influence of milk proteins in various food systems has been extensively studied. In one study, Pearce et al (1984) found that the adding nonfat dry milk solids to cake batter influenced properties such as air cell size, foam stability, and lipid emulsification. Other studies have also addressed the functionality of milk proteins and their incorporation into various products (Kinsella 1982). What is not clear, however, is the relationship between the functional properties and the physicochemical interactions of the proteins with other ingredients that cause functionality modification. In cereal-based products, such interactions include interactions between milk proteins, starch, lipids, and water. With the advance of microwave-heated products, a better understanding of the dielectric behavior of milk proteins, alone and in combination with starch,

is important to controlling cereal product quality.

Dielectric properties, the interaction of a material with electromagnetic radiation, can be expressed as: dielectric constant (k'), a measure of the ability to store the electromagnetic radiation, which is related to polarity; dielectric loss (k''), a measure of the ability of a material to transform or dissipate the electric energy into heat; and absorptivity ($1/R_i$), a measure of how the waves will be affected when they enter the material, defined as the inverse of the penetration depth. The penetration depth provides information on how far a wave will penetrate into a material before it is reduced to 1/e of its original intensity (Mudgett 1986).

In heating by electromagnetic energy, water is the primary component of a food system that interacts with electromagnetic radiation at 2,450 MHz. Water mobility has been assessed by many methods for various systems. Electron spin resonance (ESR) using the noninteractive probe TEMPO has been used to assess water mobility in gluten and water systems (Pearce et al 1988) and whey protein concentrate and water systems (Schanen et al 1990). In both studies, a splitting in the high field line of a typical three-line TEMPO spectrum indicated a partitioning of the probe into two water environments with differing mobility. Water self-diffusion coefficients (D) and dielectric properties have been also obtained (Umbach et al 1992) for conventional and microwave-

¹Paper 20,770 of the Minnesota Agricultural Experiment Station projects 18-027 and 18-063. Additional support from the Minnesota-South Dakota Dairy Foods Research Center.

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heated starch and gluten systems. No difference between the D values was found between the conventional and microwave-heated samples. The k' and D were both affected by the moisture content, but k'' was not. Both properties were dependent on the ratio of starch to gluten for the heated samples. In another study, the dielectric properties of modified corn starches were examined (Miller et al 1991). The k' of modified and unmodified corn starches remained constant during heating, whereas k'' and $1/R_i$ of certain modified corn starches increased. Also, the degree of substitution influenced the dielectric behavior of the corn starches.

The mobility of water molecules in the vicinity of a macromolecule can affect the dielectric properties of a system. The k' is most affected, but the k'' and $1/R_i$ depend on water mobility also, as well as on the nature of the macromolecules and the relaxation of particular side chains. Although water relaxation and water mobility have been addressed by a number of scientists, food systems have not been widely characterized by their dielectric properties. In this study, the dielectric properties of commercial whey protein isolate (WPI), Ca-caseinate, wheat starch, and their combinations were studied at ambient temperature and during heating to 90°C. The hydration properties of these systems were also studied by ESR using the water and oil-soluble noninteracting probe TEMPO to correlate water mobility results to dielectric relaxation phenomena.

MATERIALS AND METHODS

Materials

WPI (Bi-Pro, Le Sueur Isolates, Le Sueur, MN) isolated from cheese whey by ion-exchange chromatography was used. The WPI suspension was ultrafiltered and freeze-dried. The isolate contained 95% undenatured protein (manufacturer's analysis: protein >95%, ash <3%, moisture 5%, fat 1%, lactose <1%). Ca-caseinate (Alanate 309) was provided by New Zealand Milk Products (Santa Rosa, CA) (manufacturer's analysis: protein 90.5%, ash 4.1%, moisture 4.2%, fat 1.1%, lactose 0.1%). Wheat starch (Aytex-P) was obtained from Ogilvie Mills (Minneapolis, MN). The probe TEMPO (2,2,6,6-Tetramethyl piperidine-1-oxyl) (Fig. 1) used in the ESR experiments was obtained from Aldrich Chemical Co (Milwaukee, WI).

Dielectric Properties

The k' and k'' were measured at 2,450 MHz using the cavity perturbation method (ASTM 1970) with a network analyzer (model HP 8357A, Hewlett Packard, Santa Rosa, CA). The $1/R_i$ was calculated as:

$$1/R_i = \frac{2\pi}{\lambda} \sqrt{\frac{k'(\sqrt{1 + \tan^2 \delta} - 1)}{2}}$$

where λ is equal to 12.245 cm and $\tan \delta$ is equal to the ratio of k'' to k' (Von Hippel 1954). The dielectric properties were measured at ambient temperature and during heating to 90°C using a conventional oven with a waveguide placed inside. Samples

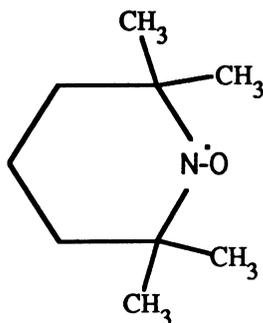


Fig. 1. Chemical structure for nitroxide probe TEMPO (2,2,6,6-Tetramethyl piperidine-1-oxyl).

were placed in capillary tubes without air introduction and centered in the cavity for measurement. Two grams of protein or starch were mixed with water to obtain a final moisture content of 30, 40, 50, 60, or 80% on a wet basis. The dry ingredients were mixed first and the appropriate amount of water was added. The protein-starch ratios in these mixtures were always 1:1 (w/w).

ESR Measurements

A 1:0.001 (w/w) aqueous solvent solution of water and TEMPO was prepared by slurring the probe and water for 24 hr at ambient temperature. An aliquot of the probe solution was added to the protein or wheat starch until the system was 60% water; the mixtures were magnetically stirred for 24 hr. The results could be compared to the dielectric properties experiments or to results of previously reported data. An aliquot of the prepared sample was placed in a 2-mm capillary tube for heating studies; the capillary tubes were heat-sealed and heated in a water bath for 6 min at either 50, 75, or 90°C and then quenched for 5 min in an ice-water bath before ESR measurements.

The 2-mm capillary sample tubes were placed in a 5-mm nuclear magnetic resonance tube and spectra were recorded on an IBM Bruker ER-200D (Billerica, MA) spectrophotometer at about 9.78 GHz at ambient temperature. The center field was 3,450 G and the scan range was 100; the attenuation power was low enough to avoid saturation. Correlation times were calculated (Kivelson 1960) assuming isotropic motion of the nitroxide radical.

Statistical Analysis

The unbalanced data sets of k' , k'' , and $1/R_i$ were each analyzed using the MacAnova program for the main effects of system type and moisture content and their interaction (Oehlert 1989). The correlation times data set was also analyzed. Duncan's multiple

TABLE I
Dielectric Properties as a Function of Moisture Content
at Ambient Temperature

Moisture Content (%)	System	Dielectric Properties ^a		
		k'	k''	$1/R_i$
30	Starch	10.48 b	2.99 a	0.23 a
	Whey protein isolate (WPI)	NA ^b	NA	NA
	Ca-caseinate (Ca-cas)	5.91 a	2.04 a	0.21 a
	WPI and starch	24.36 c	8.23 c	0.42 b
	Ca-cas and WPI	6.42 a	1.68 a	0.17 a
	WPI and Ca-cas and starch	7.25 a	2.20 a	0.21 a
	Ca-cas and starch	13.12 b	4.25 b	0.30 a
50	Starch	40.04 c	8.63 b	0.35 a
	WPI	25.35 a	7.06 a	0.36 a
	Ca-caseinate	31.92 b	7.01 a	0.32 a
	WPI and starch	33.37 b	8.77 b	0.39 a
	Ca-cas and WPI	NA	NA	NA
	WPI and Ca-cas and starch	NA	NA	NA
	CA-cas and starch	32.9 b	8.69 b	0.39 a
60	Starch	51.87 b	8.88 a	0.32 a
	WPI	49.99 b	10.61 b	0.38 b
	Ca-caseinate	36.71 a	10.7 b	0.45 b
	WPI and starch	45.53 b	10.37 b	0.39 b
	Ca-cas and WPI	35.87 a	9.24 a	0.39 b
	WPI and Ca-cas and starch	37.32 a	9.29 a	0.39 b
	Ca-cas and starch	37.42 a	8.57 a	0.36 b
80	Starch	58.52 b	8.26 a	0.28 a
	WPI	59.02 b	9.64 a	0.32 b
	Ca-caseinate	57.92 b	11.06 b	0.37 b
	WPI and starch	61.56 b	9.20 a	0.30 a
	Ca-cas and WPI	63.27 b	11.38 b	0.37 b
	WPI and Ca-cas and starch	63.04 b	11.47 b	0.37 b
	Ca-cas and starch	50.66 a	10.27 ab	0.37 b

^aStandard deviation ($n = 2$) k' :1.53; k'' :0.33; $1/R_i$:0.03. Means with the same letters were not significantly different ($P < 0.05$).

^bNot available.

range test was used for comparisons between means at $P < 0.05$. Two replicates were run.

RESULTS AND DISCUSSION

Dielectric Properties at Ambient Temperature

The dielectric properties of various systems containing WPI, Ca-caseinate, and starch are shown in Table I. Data for WPI at 30% moisture content are not available because WPI systems were solid at this moisture content and measurements could not be made with the cavity perturbation method. The statistical analysis indicated that system type as well as the interaction of system type and moisture content was significant ($P < 0.05$) for k' , k'' , and $1/R_i$. Since the interaction between the main effects was significant, comparisons between the various systems within a moisture content level were evaluated.

Moisture content significantly affected the dielectric properties of all the systems. The k' for all systems increased with moisture content. Small differences were observed among the systems for a given moisture content level, but generally at lower moisture contents, starch had a higher k' than that of WPI or Ca-caseinate. Adding WPI to starch influenced the k' of starch at lower moisture contents. More specifically, the mixture of WPI and starch showed

a considerably higher k' than did starch alone. At 50% moisture content, the k' value for the mixture of WPI and starch was lower than that of starch alone but higher than that of WPI alone. At higher moisture contents, starch, WPI, and the starch-WPI system had similar values for k' . Adding Ca-caseinate did not affect the k' of starch at 30% moisture content. At 50% moisture content, the k' of the Ca-caseinate and starch system was lower than that of starch, and it was very similar to the k' for Ca-caseinate. At higher moisture contents, starch, WPI, and the starch-WPI system had similar k' . The Ca-caseinate and starch system at higher moisture contents had a lower k' than did starch, which indicates that the adding Ca-caseinate might result in less coupling of electromagnetic energy, which could mean that less free water is present, and the macromolecular interactions are greater.

The k'' for starch was similar to the k'' for the WPI at all moisture content levels, except for 60%, where starch had lower k'' . Adding WPI to the starch and water system increased k'' at moisture contents 30 and 60%, which is indicative of greater molecular mobility and, therefore, greater dissipation of energy. The effect was weaker with Ca-caseinate. At 80% water content, k'' for starch was not affected by the addition of either WPI or Ca-caseinate. The combined presence of both proteins though, increased the k'' of starch and therefore its ability to dissipate energy, as evidenced by the higher k'' value for the WPI and Ca-caseinate and starch system when compared to the starch and water system.

The systems showed fewer differences for $1/R_i$ than for k' and k'' . At lower moisture contents, it appears that the WPI and starch system has a higher $1/R_i$ than starch does. At higher moisture contents, this effect is not seen, probably because water has a predominant effect on $1/R_i$. Since $1/R_i$ is affected by k' and k'' , a balance between the two is such that $1/R_i$ is similar. Many believe that k'' affects mostly $1/R_i$, but this is not necessarily true as shown in these data.

Dielectric Properties During Heating

When pure water is heated above ambient temperature, all the dielectric properties decrease with increasing temperature. Figure 2 shows the dielectric properties of the systems in this study at 30% moisture content during heating to 90°C. The k' of starch, Ca-caseinate, and Ca-caseinate and starch systems increased with increasing temperature, whereas the k' of the WPI and starch system remained fairly constant. The k' of the Ca-caseinate and starch system was higher than that of Ca-caseinate or starch alone, at all temperatures. Adding WPI to the starch and water system increased the ability of the starch to couple energy throughout the heating process. The k'' of the WPI and starch system decreased with heating, as did the k'' of starch. Note that the k'' and $1/R_i$ of the WPI and starch system was, at all temperatures, higher than that of starch. It appears that adding WPI during heating not only increases the energy coupling ability of starch but also increases its ability to release energy as frictional heat. The k'' and $1/R_i$ of Ca-caseinate increased with increasing temperature (Fig. 2b and c), whereas the k'' of the Ca-caseinate and starch system remained constant. The effect does not appear to be additive because the k'' and the $1/R_i$ for the mixture were higher than any of the two components alone.

Figure 3 shows the 50% moisture content results. The k' remained constant for starch and WPI during heating, whereas the k' of Ca-caseinate decreased. The WPI and starch system and the Ca-caseinate and starch system showed fairly constant k' , with values between those of the single components. The k'' and $1/R_i$ showed fewer differences among the different systems at this moisture content but a few observations could be made. Starch showed decreasing k'' and $1/R_i$ with increasing temperature, as expected. WPI showed an increasing k'' and $1/R_i$ during heating. A slight increase in k'' and $1/R_i$ can also be seen for Ca-caseinate. The k'' and $1/R_i$ of the WPI and starch system decreased somewhat with heating. The k'' and $1/R_i$ of the Ca-caseinate and starch system was constant up to 65°C and then decreased. None of the system components, when examined alone,

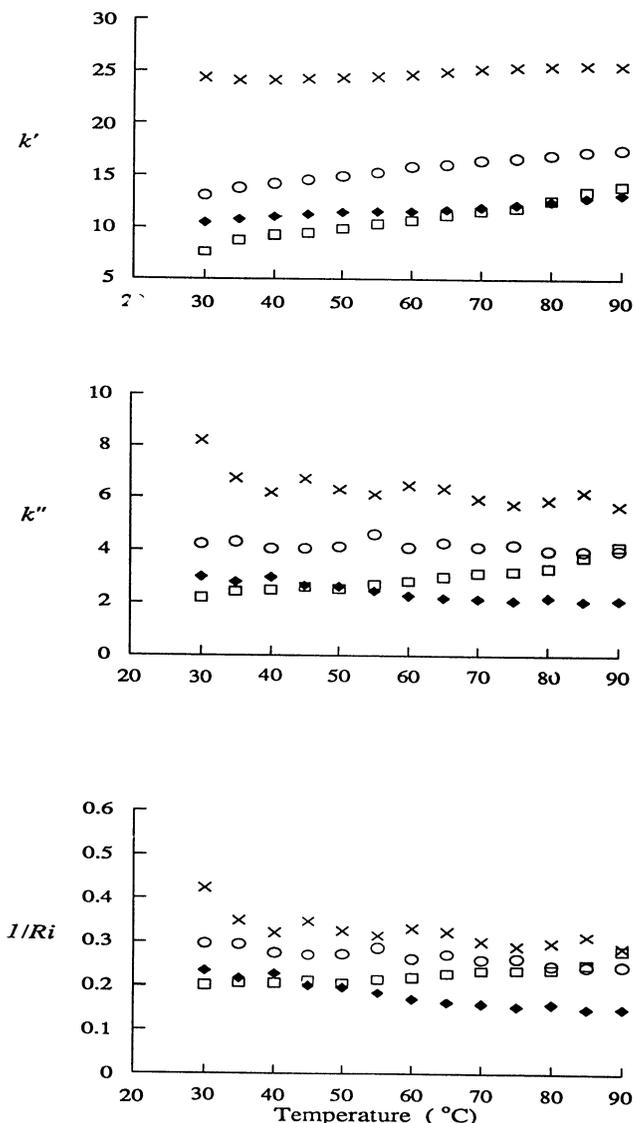


Fig. 2. Dielectric constant (k'), dielectric loss (k''), and absorptivity ($1/R_i$) as a function of temperature for: starch (◆), Ca-caseinate (□); Ca-caseinate and starch (○); and whey protein isolate and starch (×) systems at 30% moisture content.

showed similar behavior, but it is possible that the molecular mobility of the protein decreases as a result of starch gelatinization, resulting in lower k'' values.

At 60% moisture content (Fig. 4), the k' remained practically constant for all the systems, except for Ca-caseinate where it decreased with increasing temperature. The k'' and $1/R_i$ of WPI increased with increasing temperature, which in agreement with the results at 50% moisture content; at 60% moisture content, however, the phenomenon is more evident. Ca-caseinate on the other hand, showed a decreasing $1/R_i$ and k'' with increasing temperature at 60% moisture content. At all temperatures, the $1/R_i$ of Ca-caseinate was higher than that of starch. The WPI and starch system showed a decreasing k'' and $1/R_i$ with increasing temperature. The decrease in k'' for this mixture was less pronounced than the decrease for starch. The effect, though, does not appear to be additive and possibly depends on the interactions taking place between the two components. The mixture of Ca-caseinate with starch exhibited a decreasing absorptivity and k'' with increasing temperature. This behavior is very similar to that of starch, indicating the dominant role of starch in this system. The WPI and Ca-caseinate system (data not shown) showed behavior similar to that of WPI. Also, the behavior of the three-component system is very similar to that of WPI and starch, as if Ca-caseinate does not have any effect (data not shown).

These results suggest that WPI affects the dielectric properties of both starch and Ca-caseinate. This might be important when formulating a product, since the heat transfer properties (and therefore the heating rate) would be affected. The increasing absorptivity of WPI during heating at 50 and 60% moisture contents might be attributed to a stronger hydrophobic environment, which may stabilize the hydrogen bonds of liquid water, shifting the relaxation to a lower wave length and, thus, increasing the absorptivity. WPI is more susceptible to structural changes in the temperature range studied than Ca-caseinate, which is fairly stable. Still, partial unfolding and, possibly, pH changes could affect the k'' and $1/R_i$ of Ca-caseinate. WPI probably has higher molecular mobility due to a stronger interaction with water resulting from charged side chains such as amino groups. Behavior similar to that of WPI has been reported for corn starch substituted with quaternary ammonium (Miller et al 1991). In that study, k'' and $1/R_i$ increased during heating for corn starches substituted by quaternary ammonium. The k'' and $1/R_i$ of those starches were higher than those of unmodified corn starch at all temperatures, which is similar to the results for WPI in the present study. Both WPI and starch appear to be equally effective in coupling dielectric energy during heating at higher moisture contents (as shown by the similar k' values), whereas the opposite is true

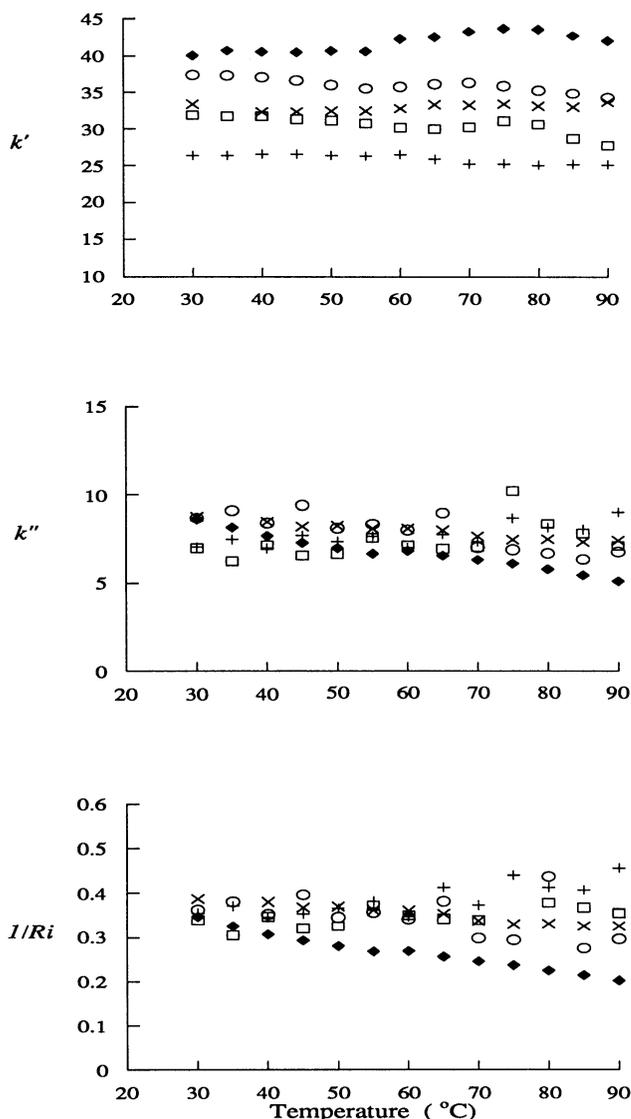


Fig. 3. Dielectric constant (k'), dielectric loss (k''), and absorptivity ($1/R_i$) as a function of temperature for: starch (◆); whey protein isolate (+); Ca-caseinate (□); Ca-caseinate and starch (○); and whey protein isolate and starch (×) during heating at 50% moisture content.

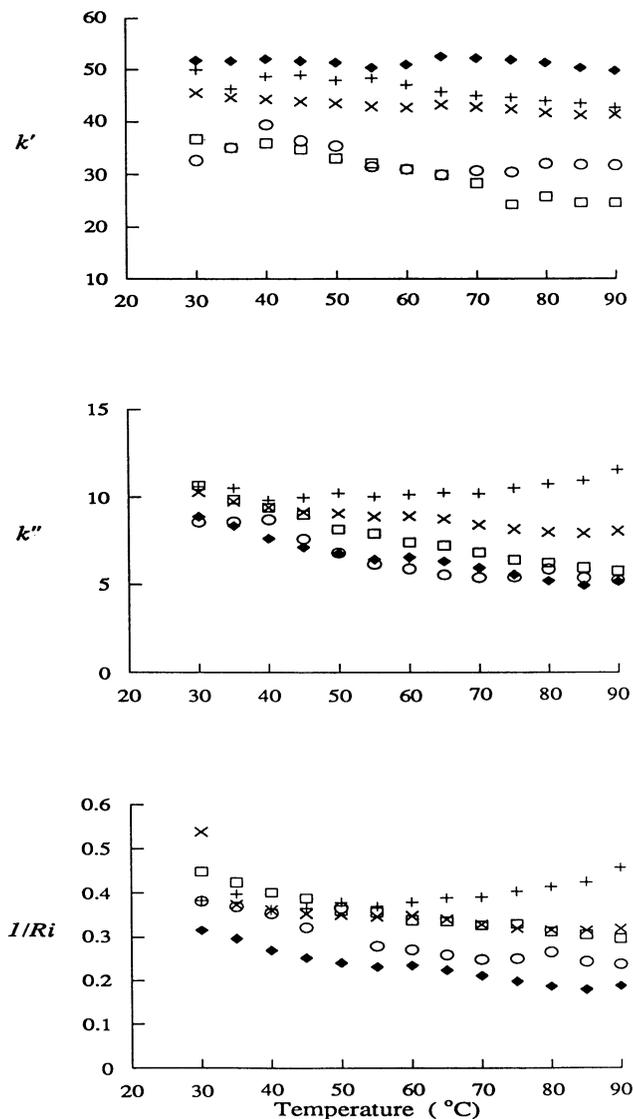


Fig. 4. Dielectric constant (k'), dielectric loss (k''), and absorptivity ($1/R_i$) as a function of temperature for: starch (◆); whey protein isolate (+); Ca-caseinate (□); Ca-caseinate and starch (○); and whey protein isolate and starch (×) during heating at 60% moisture content.

for Ca-caseinate, which had lower k' and k'' than WPI during heating.

ESR Measurements

ESR and dielectrics do not measure the same quantity. ESR relaxation maps the spectral density of a free-electron motion at a specific frequency; the dielectric experiment gives a distribution of correlation times in reality for an average dipole reorientation at maximum absorption. Nevertheless, differences in the mobility of water molecules, as measured by ESR, among the different systems examined this far can give information on the structuring effect that the various macromolecules exhibit on the water. The correlation time of TEMPO in water (1:1,000, w/w) was 0.034 ns, indicating that the majority of the probe is in free motion. Measurements were taken over the temperature range of 50–90°C, and it was found that the correlation time was not changed by heating. This is in agreement with Biliaderis and Vaughan (1987) and Pearce et al (1988).

Correlation times for TEMPO in WPI, starch, and WPI-starch systems as a function of temperature are shown in Table II. Note that as the correlation time (τ) gets larger, the mobility of the probe is restricted. For all of the systems, the correlation times were larger than that for water-probe alone. The correlation times at room temperature and 50°C were significantly lower than the correlation times at 75 and 90°C. These changes indicate that a transition takes place between 50 and 75°C, increasing the correlation times.

The changes in correlation times for WPI, compared with that of the probe alone in solution, are indicative of a structuring effect exhibited by the protein on water. This was also manifested by the dielectric behavior of WPI. Studies using deuterium NMR (Lambelet et al 1989, Padua et al 1991) have suggested that the

state of water in protein-water systems can be described by a rapid exchange between a small number of water molecules tightly bound to the protein and free water molecules. Two populations of water with higher and lower mobility were also observed in gluten-starch systems by Umbach et al (1992) using diffusion NMR. Lambelet et al (1989) showed that a sharp increase in the relaxation rate took place between 40 and 75°C for whey protein samples. This was attributed to a decrease in mobility of water molecules bound to the protein that could be related to conformational changes. The transition identified for the WPI system in the present study is in agreement with those cited above and can be related to the increasing absorptivity and dielectric loss factor exhibited by WPI as a result of stabilization of the liquid structure of water during heating in the presence of the macromolecule.

Figure 5 shows the spectra obtained for WPI at 30, 50, 75, and 90°C. Similar spectra were obtained for starch, WPI and starch, and Ca-caseinate and starch mixtures. At all temperatures, fairly sharp three-line spectra were obtained. Schanen et al (1990) observed a splitting of the high field line for WPC-water-TEMPO samples. Splitting of the high field line of the TEMPO spectrum is attributed to the exposure of the probe to both hydrophilic and hydrophobic environments (McConnell et al 1972). Schanen et al (1990) attributed the splitting for the WPC samples to the denaturation of the protein and the exposure of the probe to hydrophobic/hydrophilic environments. In the present study, no splitting was observed for the WPI-water-TEMPO system as seen in Figure 5. The isolation process for WPI results in a product with less denaturation and lower lipid contents than were present in the WPC. The absence of the splitting of the high field line in the WPI-water-TEMPO spectra suggests that the splitting in the WPC-water-TEMPO spectra was due to a hydrophobic environment associated with lipids as the lipids became more accessible with heating. Splitting of the high field line due to lipids associated with gluten was also suggested by Pearce et al (1988).

Starch-TEMPO-water spectra do not show splitting of the high field line. Only the slowed motion of the probe is seen. The correlation times of the probe for the starch sample and the starch-milk protein mixtures showed behavior similar to that of WPI (Table II). Above 50°C the heat treatment increased the correlation time for all the mixtures. At 50–75°C, starch gelatinization is very likely to take place, resulting in a rise in the correlation time as a consequence of a greater immobilization of the water. During gelatinization, the starch chains become hydrated, leaving less total solution available as solvent for TEMPO, thus increasing the correlation time. This is in agreement with Biliaderis and Vaughan (1987), who addressed starch-water-probe interactions and found that the mobility of the probe decreases between 30

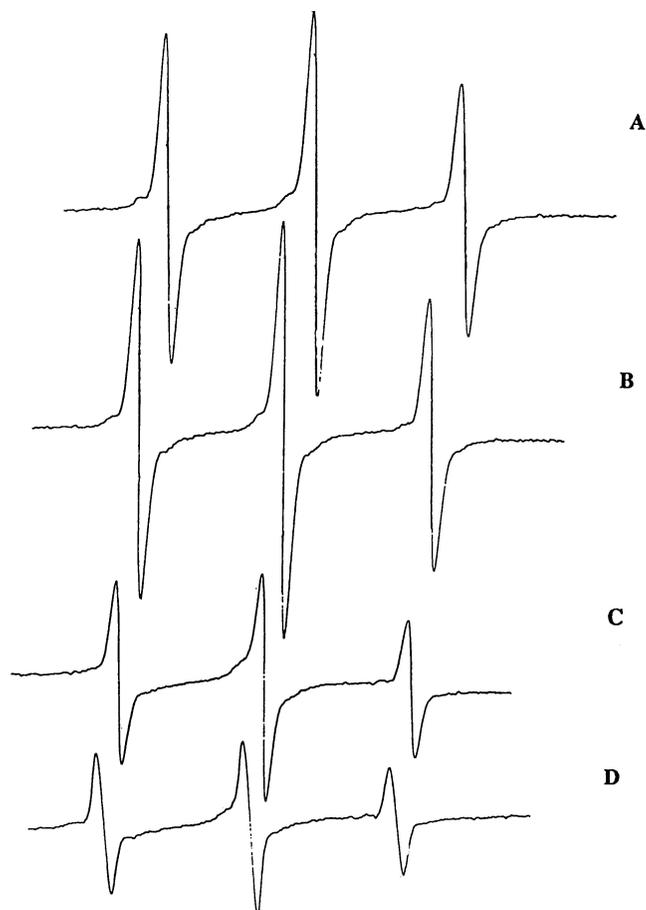


Fig. 5. Electron spin resonance spectra for whey protein isolate and TEMPO at 60% moisture. A, room temperature; B, 50°C; C, 75°C; D, 90°C.

TABLE II
TEMPO Correlation Times (τ_c) for Milk Protein-Starch Mixtures
at 60% Moisture Content at Ambient Temperature and
After Heating to 50, 75, and 90°C

System	Temperature °C	τ_c (ns) ^a
Whey protein isolate (WPI)	RT ^b	0.20
	50	0.18
	75	0.28
	90	0.26
Starch	RT	0.14
	50	0.17
	75	0.27
	90	0.27
WPI/starch	RT	0.16
	50	0.16
	75	0.25
	90	0.25
Ca-caseinate/starch	RT	0.19
	50	0.18
	75	0.23
	90	0.22

^aStandard deviation: 0.02 ($n = 2-3$).

^bRoom temperature.

and 65°C, indicating an increasing microviscosity in the environment of the radical, with a subsequent decrease in the correlation time with further heating. In that study, the measurements were taken at elevated temperatures. In the present study, the correlation time seems to level off with heating above 75°C. This might be due to a progressive increase in water mobility above 65°C after gelatinization. The increase in the correlation time above gelatinization temperatures for starch-sugar-water systems was also noted by Johnson et al (1990), who found that the transition temperature for the correlation time was dependent on the sugar type. In the present study, none of the milk proteins examined affected the transition temperature for the correlation time of starch. This seems to be in agreement with the observation that starch had a predominant effect on the dielectric properties of the WPI-starch and Ca-caseinate and starch systems. From the ESR data, it appears that the hydration properties of the systems were similar in water mobility (manifested by the motion of the probe). However, TEMPO does not selectively measure the mobility of water molecules but measures the effect of the macromolecule on the entire solution mobility, which in turn is affected by changes on the microenvironment of the macromolecule. Therefore, differences in their dielectric behavior could be explained by differences in the interactions between the macromolecules and water molecules. In that context, although the transition occurring in the starch system as well in the WPI system causes a structuring effect (evident from the increased correlation time), the absorptivity and dielectric loss of starch system decrease with increasing temperature, whereas the absorptivity of WPI system increased with increasing temperature. Thus, both the macromolecules and the water content affect the dielectric properties. Differences among the dielectric properties of the various systems were observed, yet the water environments appear to be similar.

CONCLUSIONS

At lower moisture contents, Ca-caseinate has a lower dielectric constant than WPI or starch. The addition of WPI in the starch-water system could increase the ability of starch to dissipate energy, manifested by the higher dielectric loss at almost all moisture contents. On the other hand, addition of Ca-caseinate could result in less coupling of electromagnetic energy. During heating, the dielectric loss and absorptivity of WPI increased with temperature, whereas the dielectric properties of Ca-caseinate decreased during heating. The dominant effect of starch on the protein-starch mixtures was evident, although it was clear that adding WPI did affect the k'' and $1/R_i$ of starch during heating. Such an effect was not seen with Ca-caseinate. Although some correlation was found between the dielectric behavior and water mobility (manifested by changes in the mobility of the probe TEMPO), the differences observed in the dielectric properties could not be solely explained by the ESR results. One reason

for this is that the two methods monitor different aspects of the problem. It was evident that although the water environments were similar among the systems studied, their dielectric properties differed. Thus, the dielectric properties do not solely depend on water but are also influenced significantly by the macromolecule present.

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[Received September 9, 1993. Accepted September 23, 1994.]