

## Functional Properties of Dephosphorylated Bovine Whole Casein<sup>1</sup>

### ABSTRACT

Native whole casein and dephosphorylated (40 and 93%) whole caseins were evaluated for solubility between pH 3 and 8, Ca<sup>2+</sup> sensitivity, emulsion capacity, emulsion stability, foaming capacity, and foam stability. The isoelectric point of the modified caseins shifted upward (.5 pH units for 93% dephosphorylated casein) as the degree of dephosphorylation increased. Caseins in NaCl solutions were equally soluble above their isoelectric points. Dephosphorylated caseins were more soluble in NaCl at pH 3 and in the presence of Ca at pH 7. Emulsifying properties were dependent on the amount of casein in solution and were greatest at the pH at which caseins were most soluble. Emulsions made with modified caseins were similar to native casein emulsions in initial turbidity and emulsion activity index values but had lower oil capacities and tended to be less stable. Dephosphorylated caseins formed less foam volume during sparging, and the foam was very unstable. The removal of the phosphate groups from whole casein improved its solubility and reduced emulsion and foaming properties in ways that may be of value to the food industry.

(Key words: dephosphorylated casein, calcium sensitivity, emulsion, foam)

Abbreviation key: DP = dephosphorylated, EAI = emulsion activity index, EC = emulsion capacity.

### INTRODUCTION

Casein has many desirable functional properties and is used extensively in the food industry. For example, because of their amphipathic nature and flexible structure, caseins have excellent solubility and the capacity to form films; the protein interacts with other components in the solution either to remain soluble or to form protein films at phase interfaces to create an emulsion or foam. Enzymatic removal of bound P from caseins may produce proteins that have unique functional properties of value to the food industry.

Bovine whole casein is a mixture of four phosphoproteins,  $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ -, and  $\kappa$ -caseins, which are present in a ratio of 3:1:3:1 and have 8 to 9, 10 to 14, 5, and 1 phosphate group per molecule, respectively (8). The negatively charged phosphate groups bound to the caseins can be removed enzymatically with acid phosphatase (4, 9, 16), alkaline phosphatase (9, 13), and phosphoprotein phosphatase (2, 24, 25).

The removal of the phosphate groups from casein alters some of the physical and functional properties of the protein, thereby changing the way in which the caseins associate and form micelles (2, 3, 14, 16). Calcium-binding capacities of dephosphorylated (DP) whole and  $\alpha_s$ -caseins are significantly lower than those of the native caseins (22). The DP  $\beta$ -casein becomes independent of Ca<sup>2+</sup> and is able to stabilize native  $\alpha_s$ -casein in the presence of Ca<sup>2+</sup> (24). The DP whole casein is more readily hydrolyzed by pepsin than native casein, and the modified casein acid curd microstructure resembles human casein more closely than it does bovine casein (9). A few studies exist that describe the investigation of DP caseins in terms of their cheese-making qualities [i.e., rennet coagulation time (13, 23), curd strength (25), and syneresis (13)]. Functional properties related to other food uses for DP caseins have not been reported.

The goal of our study was to investigate the effect of removal of phosphate groups from

whole casein on such functional properties as solubility, emulsification, foam formation, and foam stability.

## MATERIALS AND METHODS

### Materials

Potato acid phosphatase was obtained from Calbiochem (59 U/ml at 25°C, pH 4.8; La Jolla, CA). Food-grade corn oil (100%) was purchased locally. All other chemicals were analytical grade.

### DP Casein

Casein was precipitated (1N HCl) from raw skim milk obtained from a commercial herd. Acid casein was rinsed with deionized water and stored frozen. Sodium caseinate was obtained by dissolving thawed acid casein in deionized water at pH 7 to 7.5 (1N NaOH) followed by lyophilization.

Batches (500 ml each) of aqueous sodium caseinate (2.5 mg casein/ml) were adjusted to pH 6.5 and warmed to 37°C in a shaking water bath (100 rpm). Potato acid phosphatase was used to remove the phosphate groups from the casein (4). Maximally DP casein was obtained by addition of .13 U of enzyme/ml of casein solution and incubation for 2 h at 37°C. The solution was dialyzed 3 d at 4°C to remove free phosphates and then lyophilized. Partially DP casein was obtained by addition of .065 U of enzyme/ml of casein solution and incubation for 1 h at 37°C. The casein solution was held at 80°C for 5 min to inactivate the enzyme. The solution was dialyzed for 36 h at 4°C and lyophilized. The P content was assayed as described by Sumner (18). Compared with native casein (230 ± 11 nmol of P/mg of casein), partially and maximally DP casein had 40 and 93% of the phosphate groups removed (139 ± 3 and 16 ± 2 nmol of P/mg of casein, respectively). Urea-PAGE of the partially DP casein (not shown) displayed multiple bands migrating more slowly than the fully phosphorylated  $\alpha_s$ - and  $\beta$ -casein bands, indicating a heterogeneous mixture of 0 to 100% DP caseins.

### Solubility

*NaCl*. Stock solutions (.1% casein in .15 M NaCl, pH 7) of 0, 40 and 93% DP caseins were

stored at -20°C. Aliquots of thawed stock solution were adjusted to the appropriate pH (3 to 8) with either .1N HCl or .1N NaOH and then centrifuged (Sorvall RC5C; DuPont, Wilmington, DE) at 12,000 × g for 15 min at 25°C. Protein concentration of the resulting supernate was determined by measuring the absorbance at 280 nm (Shimadzu UV-240; Shimadzu, Kyoto, Japan) and calculating concentrations from standard curves made from diluted protein stock solution. Solubility was expressed as the percentage of protein in solution. Means were calculated from a minimum of three runs. The supernate was then used in the emulsion study.

*Ca<sup>2+</sup> Sensitivity*. Stock solutions of .2% casein in .01 M imidazole, pH 7, were prepared for 0, 40, and 93% DP caseins. A 3-ml aliquot of stock casein solution was adjusted to a final Ca<sup>2+</sup> concentration of between 0 and .03 M with 1 M CaCl<sub>2</sub> and centrifuged (Beckman L-8-70 and SW-60 rotor; Beckman Instruments, Inc., Palo Alto, CA) at a minimum of 100,000 × g for 36 min at 37°C. Protein concentration was determined as already described. Cuvettes of both 1.0- and .5-cm path lengths were used to prepare casein standards and to collect data. The Ca<sup>2+</sup> solubility was expressed as a percentage of soluble casein at each Ca<sup>2+</sup> concentration.

### Emulsions

Emulsion capacity (EC) of native and modified caseins was determined using an apparatus described by Webb et al. (21). A 5-ml aliquot of casein stock solution (.1% casein on .15 M NaCl), adjusted to the desired pH, was placed in a glass container. The Polytron homogenizer (Brinkman Polytron, Westbury, NY) with a PTA 10 shaft and set at 6 was used to mix the solution as corn oil was pumped (peristaltic pump, P-1; Pharmacia, Piscataway, NJ) into the solution at a rate of 4.5 ml/min. A volt-ohm meter (Multimeter; Sears and Roebuck Co., Downers Grove, IL) was used to measure resistance of the solution during emulsion formation and collapse. The point of emulsion collapse or phase inversion, identified by sight and confirmed by a sudden increase in solution resistance, was used to calculate EC (expressed in milliliters of oil per milligram of casein). The EC experiments were conducted in triplicate.

Casein emulsions were made according to the procedure of Chobert et al. (7) and used a 3:1 ratio (6 ml total) of casein solution (pH-adjusted supernate described in NaCl solubility section):corn oil (oil volume fraction = .25). Emulsions were formed at room temperature using the Polytron homogenizer (described earlier and with time control) for 30 s. A capped test tube containing the emulsion was placed in boiling water, maintained at  $100 \pm 2^\circ\text{C}$ , and stirred magnetically. Turbidity of the emulsion was determined spectrophotometrically at 500 nm according to the procedure of Pearce and Kinsella (12). Aliquots (.1 ml) were withdrawn from the emulsion immediately after formation, after 5 min in the water bath, and at either 5-, 15-, or 30-min intervals until the turbidity readings were less than one-half of the initial value. Emulsion aliquots were diluted 500-fold in .1% SDS and .1 M NaCl, pH 7. Matched 1-cm path length quartz cuvettes were rinsed and dried with acetone between each reading. The emulsion activity index (EAI), which measures the area of interface stabilized per unit weight of protein (square meter per gram), was calculated using the following corrected equation by Cameron et al. (5) and only for samples that had at least .6 mg of soluble protein/ml:

$$\text{EAI} = \frac{(2.303)(2)(A_{500})(\text{dilution factor})}{(c)(1 - \text{oil volume})(10,000)}$$

where  $A_{500}$  = absorbance at 500 nm, and  $c$  = grams of protein per milliliter of aqueous solution before emulsion. Emulsion stability was the time in minutes for the turbidity of the emulsion to decrease 50%. Each emulsion was made in duplicate and repeated three times.

### Foaming

Foaming properties of caseins were studied using a modified version of Waniska and Kinsella's (20) gas sparging method. Fifteen milliliters of 0, 40, and 93% DP casein stock solution (.1% casein on .15 M NaCl, pH 7) were placed in a calibrated column ( $2.2 \times 40$  cm) with a sintered glass disc at the bottom. The  $\text{N}_2$ , at a flow rate of 30 ml/min, was sparged from the bottom of the column for 2 min. Foam height was measured immediately

after the gas flow was stopped and at every min until the foam volume collapsed 50%. Foam capacity was calculated as maximum foam volume divided by  $\text{N}_2$  flow rate. Foam stability was the time in minutes for the initial foam volume to collapse 50%. Four observations were made of each sample.

### Statistics

Data were analyzed with ANOVA, and means were compared using Bonferroni  $t$  test ( $P = .05$ ) (15).

## RESULTS

### Solubility

**NaCl.** The solubility of 0, 40, and 93% DP casein in .15 M NaCl was determined over a pH range of 3 to 8 (Figure 1). Native casein was completely soluble at  $\text{pH} \geq 5.5$  but almost insoluble (over 90% of the protein precipitated) below its isoelectric point ( $\text{pH} 4.6$ ). The DP caseins behaved similarly, but their isoelectric points shifted to a higher pH (up to .5 pH units for 93% DP casein). The DP caseins were as soluble as native casein at pH 6 to 8. At pH 3, maximally, partially, and native caseins were 48, 16, and 8% soluble, respectively.

**$\text{Ca}^{2+}$  Sensitivity.** The  $\text{Ca}^{2+}$  solubility curves for native and DP caseins showed the percentage of protein soluble in solution (Figure 2)

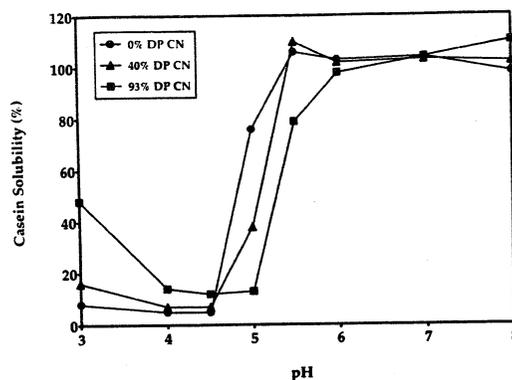


Figure 1. Effect of pH on the solubility of .1% native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins (CN) in .15 M NaCl.

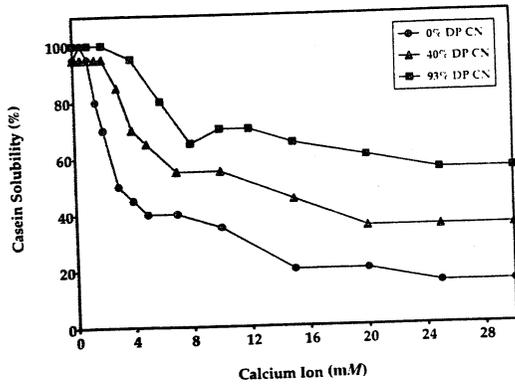


Figure 2. Effect of Ca<sup>2+</sup> concentration on the solubility of .2% native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins (CN) in .01 M imidazole, pH 7.0.

rather than the percentage of protein in suspension that is typically reported. The solubility of native whole casein decreased significantly between .001 and .005 M Ca<sup>2+</sup> and decreased gradually until it stabilized around .015 M Ca<sup>2+</sup>. This calcium sensitivity threshold is lower than the .004 M Ca<sup>2+</sup> reported in suspension solubility studies (22). Compared with the native whole casein, DP caseins were not as sensitive to the presence of Ca<sup>2+</sup>; more soluble protein remained in the solution as the percentage of dephosphorylation increased.

**Emulsion**

EC. The ability of the proteins to support oil in an emulsion or the EC was determined at pH 3 to 8 (Table 1). Native casein EC increased from pH 6 to 8 and were significantly higher than the EC of modified caseins at the same pH. At pH 5, whole casein was only 60% soluble, yet the EC value was not significantly different from those obtained at pH 5.5 and 6, at which pH casein was completely soluble. For all caseins, the EC were significantly lower when obtained below the isoelectric point of the caseins. Values for native casein EC were not significantly different from values for 93% DP casein at pH 3 to 4.5, but they were significantly higher than EC for 40% DP casein at pH 3 and 4. The EC for the modified caseins were similar at pH 4 to 8. At pH 3, EC for the 93% DP casein was higher than that for the 40% DP casein.

TABLE 1. Effect of pH on the emulsion properties of native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins in .15 M NaCl.

pH	Emulsion capacity (ml of oil/rng of casein)			Initial turbidity (500 nm)			Emulsion activity index (m <sup>2</sup> /g)			Emulsion stability (min)		
	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP
3.0	1.77 <sup>gh</sup>	1.08 <sup>klm</sup>	1.85 <sup>gh</sup>	.018 <sup>ef</sup>	.051 <sup>de</sup>	.094 <sup>cd</sup>	. . .	. . .	. . .	3 <sup>f</sup>	4 <sup>f</sup>	4 <sup>f</sup>
4.0	1.44 <sup>hij</sup>	.74 <sup>m</sup>	1.10 <sup>klm</sup>	.007 <sup>f</sup>	.007 <sup>f</sup>	.006 <sup>f</sup>	. . .	. . .	. . .	3 <sup>f</sup>	3 <sup>f</sup>	3 <sup>f</sup>
4.5	1.30 <sup>kl</sup>	.88 <sup>lm</sup>	.98 <sup>klm</sup>	.008 <sup>ef</sup>	.007 <sup>f</sup>	.021 <sup>ef</sup>	. . .	. . .	. . .	6 <sup>f</sup>	5 <sup>f</sup>	3 <sup>f</sup>
5.0	3.08 <sup>c</sup>	1.81 <sup>ghi</sup>	1.40 <sup>ijk</sup>	.110 <sup>bc</sup>	.038 <sup>ef</sup>	.043 <sup>ef</sup>	. . .	. . .	. . .	68 <sup>ef</sup>	6 <sup>f</sup>	3 <sup>f</sup>
5.5	3.39 <sup>bc</sup>	2.28 <sup>de</sup>	2.08 <sup>efg</sup>	.161 <sup>a</sup>	.156 <sup>a</sup>	.155 <sup>a</sup>	47.9 <sup>def</sup>	34.6 <sup>f</sup>	59.8 <sup>a</sup>	209 <sup>bc</sup>	127 <sup>de</sup>	62 <sup>ef</sup>
6.0	3.27 <sup>bc</sup>	2.26 <sup>def</sup>	2.38 <sup>de</sup>	.149 <sup>ab</sup>	.168 <sup>a</sup>	.167 <sup>a</sup>	44.0 <sup>f</sup>	44.0 <sup>f</sup>	51.9 <sup>bcd</sup>	212 <sup>cd</sup>	152 <sup>cd</sup>	188 <sup>bcd</sup>
7.0	3.60 <sup>ab</sup>	2.48 <sup>de</sup>	2.50 <sup>de</sup>	.184 <sup>a</sup>	.172 <sup>a</sup>	.182 <sup>a</sup>	56.4 <sup>bc</sup>	56.4 <sup>bc</sup>	51.1 <sup>cde</sup>	294 <sup>a</sup>	205 <sup>bc</sup>	235 <sup>ab</sup>
8.0	3.94 <sup>a</sup>	2.48 <sup>de</sup>	2.60 <sup>d</sup>	.181 <sup>a</sup>	.186 <sup>a</sup>	.156 <sup>a</sup>	57.0 <sup>ab</sup>	57.0 <sup>ab</sup>	46.3 <sup>ef</sup>	298 <sup>a</sup>	248 <sup>ab</sup>	187 <sup>bcd</sup>
SEM			.15		.014		4.1	4.1			25	

a,b,c,d,e,f,g,h,i,j,k,l,m Data analyzed using ANOVA and Bonferroni t test. Within each category (turbidity, activity index, stability, or capacity), means with no superscript letter in common are significantly different (P < .05).  
 †Emulsion activity index values were not calculated because sample had less than .06% soluble protein/ml.

**Formation.** The formation of casein emulsions, as evidenced by the turbidity values for freshly prepared emulsions (Table 1), reflected the amount of soluble protein in the aqueous phase. At pH 3, 93% DP casein samples had significantly higher turbidity and more solubility than native casein. The lowest turbidity occurred between pH 4 and the isoelectric point of the caseins (pH 4.6 for native caseins; pH 5.0 for DP caseins). At pH 5.5 and above, turbidity was maximal, and no significant differences were noted among the casein emulsions. Because Pearce and Kinsella (12) reported that a minimum of .1% protein was essential to obtain reliable data, we calculated the EAI only for emulsions made with a minimum of .06% of soluble protein/ml. Below this, emulsions were very unstable and replicates varied widely. Above their isoelectric points, the EAI for native and 40% DP casein emulsions increased as pH rose, but EAI for 93% DP casein emulsions decreased above pH 5.5. At pH 7, differences in the EAI were not significant ( $P > .05$ ) for their casein emulsions.

**Stability.** The stability of emulsions, when heated and gently stirred, was determined at pH 3 to 8 (Table 1). Casein emulsions made at pH at or below the isoelectric point of the caseins either did not form or were so weak that they collapsed within 6 min. The stability of native casein emulsions increased from pH 5 to 7 and remained high at pH 8. Stable emulsions of 40% DP casein were formed at pH 5.5, and the stability of the emulsions continued to improve as pH rose. The stability of 93% DP casein emulsions increased from pH 5 to 6 and then remained constant. Native casein emulsions had the highest stability at pH 7 and 8. The modified casein emulsions tended to be less stable, yet the stability of the best of the emulsions (40% DP casein at pH 8 and 93% DP casein at pH 7) was not significantly different from the native casein emulsions at the same pH.

#### Foam

Compared with native casein, sparging of 40 and 93% DP casein solutions produced less foam and a foam that collapsed faster (Figure 3). Native casein foam had a capacity of 1.7, but the DP casein foams had significantly lower capacities of 1.3 and 1.2 (40 and 93%

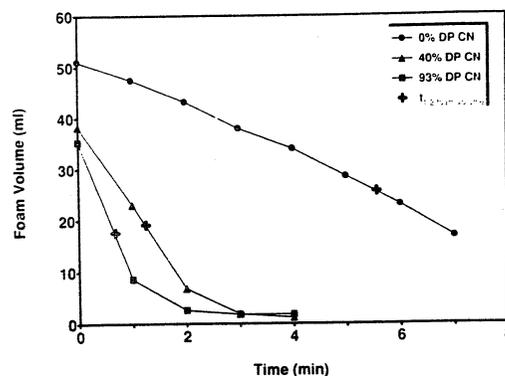


Figure 3. Foaming properties of .1% native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins (CN) in .15 M NaCl, pH 7.

DP, respectively). Foams made with DP caseins collapsed 50% of their volume within 1.5 min, which was significantly faster than that of native casein foams (5.5 min). Foam prepared with maximally (93%) DP casein tended to collapse faster than the 40% DP casein foam.

## DISCUSSION

### Solubility

The ability of a protein to be dispersed in an aqueous phase is an essential functional property. The solubility of a protein indicates how well the protein can interact with the other components present and still remain in solution.

**NaCl.** The food processing industry uses NaCl extensively as a food ingredient. Salt is added to many solutions to improve solubility and stability, which improves emulsion (12) and foaming (20) properties. However, Strange et al. (17) reported that native and DP caseins were less soluble if dispersed in .1 M NaCl before pH adjustment below their isoelectric point than when NaCl was added after pH adjustment. The solubility curve (Figure 1) for native (0% DP) whole casein in .15 M NaCl in the range of pH 3 to 8 was similar to the curve published by Murphy and Fox (11). The isoelectric shift for modified whole caseins in NaCl was similar to the .5 pH unit increase

reported for DP  $\alpha_s$ -casein complex in .2 M  $\text{CaCl}_2$  (14) and the increase of 1 pH unit reported for DP  $\alpha_{s1}$ -casein in .02 M imidazole buffer (3). A protein modification that improves, or at least maintains, the solubility of the protein is of value to the food industry when good solubility is an essential property for proteins.

**$\text{Ca}^{2+}$  Sensitivity.** The DP caseins consistently were more soluble than the native protein; however, results of suspension studies (22, 24) showed that only above the .007 M  $\text{Ca}^{2+}$  level were DP caseins more soluble than native caseins. In both types of studies, samples were ultracentrifuged to remove any large aggregates that may have been present. Protein assays are used in suspension studies to determine the amount of protein in the supernate and include any small aggregates still in suspension. Our spectrophotometric solubility method measured only the soluble proteins in the supernate. Removal of the negative phosphate groups, which are the primary binding sites for  $\text{Ca}^{2+}$ , reduces the amount of  $\text{Ca}^{2+}$  bound to the casein and decreases the association of proteins in solution (3). Dephosphorylated caseins are less likely to form micelles (2, 3, 14, 16), and more are found free in solution (2, 3, 16). The DP  $\beta$ -casein is no longer a  $\text{Ca}^{2+}$ -dependent protein and is capable of keeping  $\alpha_{s1}$ -casein in solution in the presence of  $\text{Ca}^{2+}$  (24). Our study shows that the removal of phosphate groups enhances the ability of whole casein to remain soluble in the presence of  $\text{Ca}^{2+}$ .

#### Interfacial Properties

Caseins readily form stable protein films and are used extensively in the food industry, particularly in frozen desserts, baked products, and meat emulsions. Caseins, because of their flexible structure and many hydrophobic and hydrophilic regions, spread out at the phase interface and interact with all phases to form stable film. Removal of some or most of the negatively charged phosphate groups from casein alters its net protein charge and changes the flexibility of the modified protein structure. Therefore, the ability of a modified casein to form stable protein films in an emulsion or foam is a very important functional consideration.

**EC.** Casein is a good emulsifier, and its EC is widely used in the food industry to determine the maximum amount of oil a protein can support under a certain set of conditions. Our study of the EC of native and modified caseins showed that the DP caseins supported less oil in our emulsion system than did the native casein. We also found that the pH of the solution and, subsequently, the solubility of the proteins were major factors in the EC of the protein. This conclusion agrees with that of Mulvihill and Fox (10) that EC of a casein is influenced by the protein's solubility. In our study, the similarity in the EC for native and 93% DP caseins at pH 3 was probably because 93% DP caseins were more soluble than the native casein.

**Emulsion Formation.** The surfactant in the emulsion, casein, has many hydrophobic and hydrophilic regions that interact with the aqueous and lipid phases to form an emulsion. Turbidity data and EAI values indicate that the removal of the phosphate groups in whole casein did not alter the ability of the casein to form the initial emulsion. A combination of solubility (amount of protein dissolved in solution and available to interact) and hydrophobicity (the combination of charges on the protein that affects how the protein will associate or interact with other components in the solution) determines, in part, the emulsifying properties of casein (1, 11, 12). At pH 3, the degree of dephosphorylation appeared to improve the ability of casein to form an emulsion, probably because of the increased solubility of the DP caseins. The EAI of native and DP 40% casein emulsions, which relate the ability of a protein to coat an interface, were similar ( $P > .05$ ) and improved as pH increased; the EAI of 93% DP casein was highest at pH 5.5. Therefore, in an oil-water emulsion, the presence or partial absence of phosphate groups is not a significant factor in the initial formation of the emulsion.

**Emulsion Stability.** For an emulsion to remain stable, the repulsive forces in the emulsion must be greater than the attractive forces. Over time, the emulsion will separate into oil and water phases, and emulsion stability is a measure of that creaming effect. Casein has many hydrophobic and hydrophilic residues that can interact with the aqueous and lipid phases to maintain dispersion integrity. Overall, the removal of the negative phosphate

groups from whole casein tends to reduce the ability of the modified protein to maintain the integrity of the emulsions, and they collapse faster than unmodified casein emulsions.

*Foam.* Formation of a foam (liquid protein phase surrounding gas bubble phase) is dependent on the protein's surface activity and film-forming properties in a manner similar to that discussed for emulsions. Cherry and McWaters (6) report that net protein charge influences the adsorption of protein at the air-water interface, and Townsend and Nakai (19) found that charge density, hydrophobicity, and viscosity are major factors in foam stability. Caseins have good foaming properties because their flexible structure and hydrophobic regions facilitate protein interaction at the liquid-gas interface (19). Mulvihill and Fox (10) reported that DP increased the surface tension of the casein. In our study, removal of the negatively charged phosphate groups from the caseins resulted in poorer foaming properties. The proteins were less polar and less likely to spread and adsorb at the interface. A lower negative surface charge reduced the electrostatic repulsive force, and proteins aggregated and ruptured the air bubbles.

#### CONCLUSIONS

Removal of the negatively charged phosphate groups from whole casein altered its functional properties in several ways. Dephosphorylation of whole casein raised the isoelectric point of the protein. Compared with the native casein, DP caseins were more soluble in the presence of low concentrations of  $\text{Ca}^{2+}$ . Emulsions made with modified caseins were similar to native casein emulsions in initial turbidity and EAI but had lower oil capacities and tended to be less stable. The DP caseins also had reduced ability to form and to stabilize a foam.

Establishment of the general functional properties of a protein, such as solubility and the ability to form protein films surrounding either oil or air phases, is the first step in determining applications of modified caseins in the food industry. The DP caseins may be of interest as a food ingredient because they are more soluble in low concentrations of  $\text{Ca}^{2+}$  at pH 7 and in NaCl solutions at pH 3, form a good initial emulsion, and are not conducive to foaming.

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