

Functional Properties of Chemically Phosphorylated Whole Casein

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ABSTRACT

The functional properties of unmodified whole casein were compared with those of casein that had been chemically superphosphorylated using phosphorus oxychloride at three different pH values. Casein modified at pH 5, 7, and 9 had an additional 3.30, 4.78, and 6.75 mol of P bound/mol of casein, respectively, either as monophosphates when modified at pH 5 and 7 or as mono-, di-, and polyphosphates when modified at pH 9. Solubility studies indicated that the isoelectric point of the modified caseins shifted to the acid side of pH 4.6 as the amount of bound P increased. None of the modified caseins was as soluble in water at pH 7 as the unmodified control, and caseins modified at pH 5 and 7 had lower solubility than casein modified at pH 9. Compared with control casein, casein modified at pH 9 was more soluble in the presence of Ca^{2+} , and caseins modified at pH 5 and 7 were less soluble. Superphosphorylated caseins had improved foam stability and poorer emulsion stability than the controls. Superphosphorylation of whole casein altered the ability of the casein to interact with its environment, as demonstrated by the changes in its functional properties, which could be of value in creating or improving food products.

(**Key words:** calcium, casein, functionality, phosphorylation)

Abbreviation key: **EAI** = emulsion activity index, **EC** = emulsion capacity, **ES** = emulsion stability, **NMR** = nuclear magnetic resonance, **Sup** = superphosphorylated casein (used with numbers 5, 7, and 9 to indicate pH of modification reaction).

INTRODUCTION

Casein, the major phosphoprotein of bovine milk, is valued in food for its properties of emulsification, water-binding, and texturization (16). The phosphate groups are essential for many of the interactions in-

volving casein in food systems (9, 15, 25). Modification of the phosphate content of casein alters their functional properties (8, 11, 12, 15, 20, 21). Girerd et al. (8) studied the foaming and emulsification of superphosphorylated caseins, but their modified proteins were not well characterized. Both Matheis et al. (11) and Medina et al. (12) found lower emulsification properties and higher viscosities for phosphorylated casein, but Matheis et al. (11) reported on only two of the four modified caseins that they prepared, and Medina et al. (12) reported on only two of the three different superphosphorylated caseins that they had prepared. Schmidt and Poll (15) and Yoshikawa et al. (25) showed that increased Ca^{2+} binding of superphosphorylated casein did not affect micelle formation but did adversely affect micelle stability. There are no reports on a systematic study of the effect of superphosphorylation of casein on its properties of solubility, emulsification, and foaming.

Enzymatic phosphorylation procedures are preferred when the modified proteins are intended for food use; such procedures have been used to increase the P content of κ -CN and β -CN B (2). Unfortunately, the prohibitive costs of enzyme and cofactors required to produce adequate quantities of modified proteins for food use, as well as other factors, make this procedure impractical at this time. Chemical phosphorylation offers a cheaper way to modify casein in sufficient quantities to determine whether the functionality of the protein is improved in ways that would warrant its use as a value-added food ingredient. Chemical and enzymatic superphosphorylation of casein might not result in caseins that are modified in the same way. The basic trends for the functional properties of chemically superphosphorylated casein should give valuable insight into the importance of the phosphate groups in casein interactions and should help researchers determine how alterations in P content can change the properties of the casein.

Our study compared Ca^{2+} sensitivity, solubility, and the surface-active properties of foaming and emulsification of unmodified and superphosphorylated caseins. Caseins were reacted with POCl_3 at three different pH to achieve different degrees of phosphorylation. Some structural information on the

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superphosphorylated casein was obtained through ^{31}P nuclear magnetic resonance (NMR).

MATERIALS AND METHODS

Modification of Casein

Raw skim milk, purchased locally, was used to prepare sodium caseinate according to the isoelectric precipitation procedure described by Van Hekken and Strange (20). This sodium caseinate, referred to as whole casein in this study, was used for all subsequent procedures and had an estimated molecular mass of 23000 Da.

Superphosphorylated caseins were prepared using the procedures of Medina et al. (12) with slight modifications. We treated a 2% whole casein solution with POCl_3 to a final molar ratio of 1:2000 casein: POCl_3 . The solution was maintained at pH 5, 7, or 9 (± 1) with 5N NaOH and below 25°C. One hour after the last of the POCl_3 was added and the pH was stabilized, the solution was centrifuged. The aqueous layer containing the modified casein was removed, was placed in dialysis tubing with a 12,000 molecular mass cut-off (Spectrapor; Spectrum Medical Industries, Inc., Los Angeles, CA), was dialyzed against two changes of 0.15 M NaCl and three changes of deionized water, and was lyophilized.

Batches of superphosphorylated casein, modified at the same pH with similar protein (1, 10) and P (18) contents, were pooled (Table 1), dialyzed extensively against deionized water, lyophilized, and reassayed for protein and P content (Table 1). The combined caseins are identified as Sup5, Sup7, and Sup9 according to pH of modification. Control casein was treated with CCl_4 only.

Bound P was determined using the following method. A 0.1% (wt/wt) casein solution in 0.15 M NaCl was stirred at 4°C for 96 h; duplicate aliquots were removed immediately after preparation and every 24 h for 4 d. Aliquots were treated with trichloroacetic acid to a final concentration of 10% and centrifuged at $12,000 \times g$ for 15 min at 22°C. The P content of the supernatant was measured and subtracted from total P content to determine bound P.

Evaluation of Modification

Electrophoresis. Isoelectric focusing, urea-PAGE, and SDS-PAGE separation used the Phast System[®] (Pharmacia LKB Biotechnology, Uppsala, Sweden). Samples for isoelectric focusing were prepared and separated on modified 5% homogeneous gels accord-

ing to the procedure of Vegarud et al. (23). Samples for urea-PAGE were prepared and separated on modified 8 to 25% gradient gels according to the method of Van Hekken and Thompson (22). Samples for SDS-PAGE were prepared and separated on 20% homogeneous gels according to the procedure of Woychik et al. (24).

NMR. Solutions of 1% casein (wt/vol) were prepared in D_2O (Cambridge Isotope Laboratories, Woburn, MA) without pH adjustment, and ^{31}P NMR spectra were obtained using a JEOL GX-400 NMR Spectrometer (Peabody, MA) operating at 161.8 MHz. A 45° pulse width with a 2.5-s recycle time was used to collect the data. Chemical shifts were determined using hexamethyl phosphoramide (warning: hexamethyl phosphoramide is a known carcinogen) in a reference capillary as an internal standard (chemical shift of 30.73 ppm relative to 85% H_3PO_4). Data were analyzed using the NUTS-2D program (Acorn NMR; Fremont, CA).

Functional Properties

Ca^{2+} sensitivity. The Ca^{2+} sensitivity of superphosphorylated casein was determined according to the procedure of Van Hekken and Strange (20) with slight modifications. We prepared 0.1% casein solutions in imidazole buffer at pH 8; 0.75 M CaCl_2 in imidazole buffer was used to adjust the Ca^{2+} concentration, and the solutions were mixed overnight at 22°C before centrifugation. Sensitivity of casein was determined on two separate series for control and Sup9 and on a single series for Sup5 and Sup7.

Solubility. Stock solutions of 0.12% casein (wt/wt) in water or 0.15 M NaCl were prepared at pH 8. Duplicate samples (2.5 g) of stock solutions were adjusted to the desired pH and diluted with water or NaCl to 0.1% casein. The solutions were mixed for 1 h before centrifugation at $8800 \times g$ for 15 min at 22°C (Eppendorf centrifuge 5413; Brinkmann Inc., Westbury, NY). Protein concentrations of the supernatants were determined in duplicate using the bicinchoninic acid assay (Pierce, Rockford, IL) on 0.1-ml aliquots. Standard curves were made using dilutions of control in water.

Foaming. Foaming properties of casein were evaluated using a modified sparging procedure described by Van Hekken and Strange (20). Both foam stability and foam capacity were determined in triplicate.

Emulsions. Emulsion properties were determined in duplicate on stock solutions (0.1% casein and 0.15 M NaCl, pH 7) according to the procedure of Van Hekken and Strange (20). Duplicate emulsions were made between pH 2 and 8, and the turbidity was

TABLE 1. The P content and protein percentage of control casein and whole casein superphosphorylated at pH 5 (Sup5), 7 (Sup7), and 9 (Sup9). Batches of casein, modified at the same pH and with similar protein and P contents, were combined and further dialyzed to obtain a homogeneous pooled sample.

Sample	Batch					Pooled							
	Batches	P Content		Protein		Total		Free		Bound		Protein	
		(no.)	(mol of P/mol of casein)		— (%) —		(mol of P/mol of casein)		— (%) —		— (%) —		— (%) —
		\bar{X}^1	SD	\bar{X}^2	SD	\bar{X}^3	SD	\bar{X}^3	SD	\bar{X}^3	SD	\bar{X}^4	SD
Control	1	5.79	0.1	88.8	0.4	5.8	0.1	0	0	5.79	0.1	88.8	0.4
Sup5	4	9.43	1.3	80.3	4.3	11	0.42	1.84	0.21	9.09	0.47	75.7	1.3
Sup7	6	11.3	1.3	80.1	6.5	13	1.9	2.81	0.39	10.6	2.1	72.5	1.4
Sup9	5	17.1	2.1	74.9	4.8	18	1.4	5.23	0.27	12.5	1.6	77.5	0.7

¹Calculated with three replicates per batch (i.e., mean and SD for Sup5 calculated from 12 assays).

²Calculated with two replicates per batch (i.e., mean and SD for Sup5 calculated from 8 assays).

³Calculated with three replicates per pooled sample (i.e., mean and SD for Sup5 calculated from 3 assays).

⁴Calculated with two replicates per pooled sample (i.e., mean and SD for Sup5 calculated from 2 assays).

measured in duplicate. The emulsion activity index (**EAI**) was calculated for samples using the protein concentrations determined in the pH solubility study and only for samples that had at least 0.00045 g of soluble protein/ml. TableCurve software (TableCurve Version 2.1; Jandel Scientific, Corte Madera, CA) was used to evaluate the time versus absorbance data and to determine emulsion stability (**ES**) for each run.

Emulsion capacity (**EC**) of the caseins was determined in duplicate using the procedure described by Van Hekken and Strange (20).

Statistical Analysis

Data were analyzed with ANOVA, and means were compared using Student-Neuman-Keuls *t*-test ($P = 0.05$) (14).

RESULTS AND DISCUSSION

Degree of Modification

P content. The degree of modification achieved by the phosphorylation reaction is shown in Table 1. Our control casein had 5.8 mol of P/mol of casein, which was slightly lower than the 6.4 mol of P/mol of casein estimated from the primary structure of α_{s1} -CN B, α_{s2} -CN A, β -CN A, and κ -CN B in a 4:1:4:1 ratio in which 64 out of 158 serine residues are phosphorylated naturally (6).

Phosphorylation of casein increased as the pH of the reaction increased (Table 1). Even after extensive dialysis, the modified casein preparations still contained free P. The bound P contents were similar to those reported by Matheis et al. (11) for casein

modified with POCl_3 at pH 7 and by Schmidt and Poll (15) for caseins modified using H_3PO_4 and P_2O_5 . Other researchers (8, 12) reported higher phosphorylation. Medina et al. (12) reported 12, 9, and 20 mol of P/mol of casein when the casein was treated with POCl_3 at pH 5, 7, and 9, respectively. Girerd et al. (8) measured extremely high phosphorylation using POCl_3 at pH 8.5 but did not state the amount of free and bound P in their samples.

Medina et al. (12) suggested that the increase in phosphorylation occurring at high pH was due to the increased availability of lysine as a potential phosphorylation site. However, Matheis et al. (11) found no evidence for the phosphorylation of basic amino acids and presented evidence that superphosphorylated casein contained only oxygen-linked phosphate. Polyphosphates form in the presence of higher concentrations of OH^- and POCl_3 (3), a condition that occurred when the reaction was at pH 9. Ferrel et al. (7) noted that dialysis of phosphorylated proteins against a salt solution removed the phosphate that was associated with basic groups and peptide bonds. Because our caseins were extensively dialyzed, the dialysis may have removed P that was bound initially to the casein or hydrolyzed the polyphosphates present. We examined the amount of free P in casein solutions stored for 4 d and found no significant changes after 24 h, suggesting that most P hydrolysis occurred within the initial day.

NMR. The ^{31}P NMR spectra of the modified and unmodified whole caseins are shown in Figure 1. The spectrum for control casein (Figure 1d) had overlapping broad-based multiple peaks between 2.8 and 4.2 ppm, which were typical for the serine mono-phosphates in unmodified whole casein (9). The spec-

tra for Sup5 and Sup7 (Figure 1, b and c) showed single peaks at chemical shifts of 1.7 and 2.9 ppm, respectively. Those values were within the range for monophosphates (4) and were identified as serine monophosphates. The spectrum of Sup9 (Figure 1a) showed a broad-based peak between 3.5 and 4.5 ppm, a sharp peak at 3.0 ppm, a broad-based doublet between 0.1 and 0.7 ppm, three broad-based peaks between -5.8 and -6.8 ppm, a broad doublet between -9.8 and -11.0 ppm, and a broad-based peak at -20.7 ppm. The peaks were identified as serine monophosphate, inorganic monophosphate, diphosphate, either diphosphate or terminal polyphosphate, diphosphate, and nonterminal polyphosphate, respectively (4, 11). These results were consistent with the theory that, at high pH, polyphosphates are formed, contributing to higher phosphorylation (3). Matheis et al. (11) reported that their caseins bound phos-

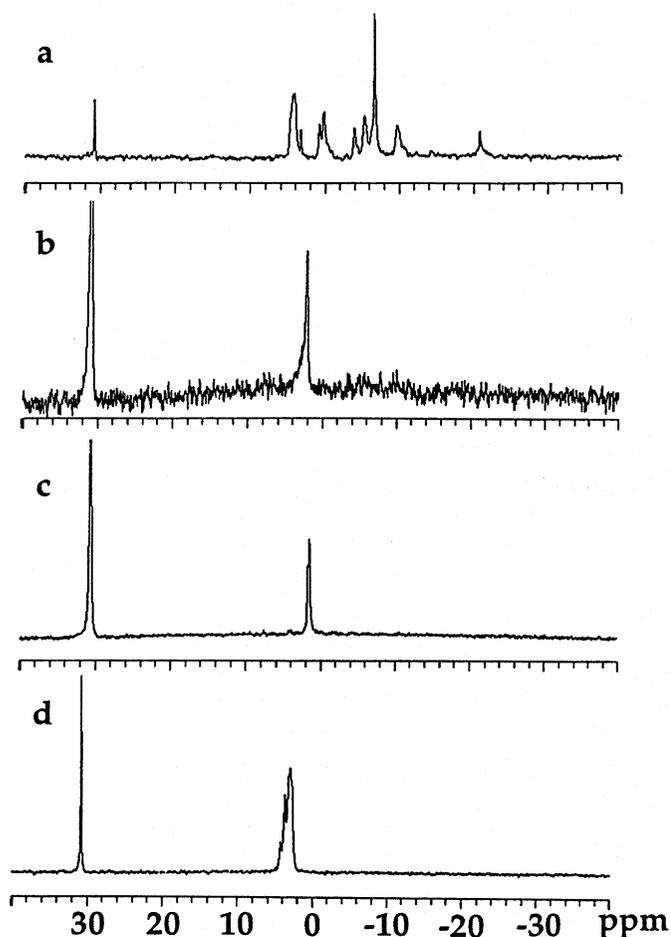


Figure 1. The ^{31}P nuclear magnetic resonance spectra of casein phosphorylated at a) pH 9, b) pH 7, and c) pH 5 and d) control casein.

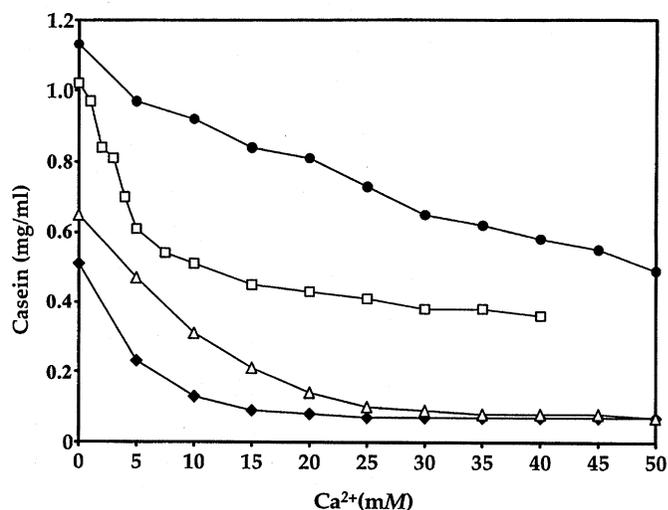


Figure 2. Solubility of control casein and chemically phosphorylated casein (0.1% casein in 0.01 M imidazole at pH 8) in the presence of Ca^{2+} . Control casein (\square) and casein modified at pH 5 (\blacklozenge), pH 7 (\triangle), and pH 9 (\bullet). Sensitivity of casein Ca^{2+} was determined on two separate series for control and superphosphorylated casein modified at pH 9 and on a single series for superphosphorylated casein modified at pH 5 and 7.

phates strictly through hydroxyl groups and contained 86.6% mono-phosphates and 14.6% diphosphates.

Electrophoresis. Electrophoretic studies of chemically phosphorylated caseins supplied limited information. For all three PAGE experiments, the modified casein remained at the origin and did not enter the 5% homogeneous gel used for isoelectric focusing, the 8 to 25% gradient gel used for urea-PAGE, or the 20% homogeneous gel used for SDS-PAGE (profiles not shown). There was no indication of the modified casein moving from the origin, which suggested that the modified casein had not dissociated in the presence of mercaptoethanol and SDS or urea. Others (11, 12) have reported difficulties getting modified casein to enter polyacrylamide gels and suggested that the protein formed intermolecular crosslinks.

Functional Properties

Ca^{2+} sensitivity. The solubility of control, Sup5, Sup7, and Sup9 as a function of Ca^{2+} concentration is shown in Figure 2. The control sample behaved as expected; solubility decreased 50% between 0 to 7.5 mM Ca^{2+} and leveled out at 40% solubility beyond 10 mM Ca^{2+} . The solubility decreased rapidly as the Ca^{2+} concentration increased from 0 to 10 mM for Sup5 and from 0 to 20 mM for Sup7. At higher Ca^{2+} concentrations, only about 14% of the original Sup5

and Sup7 remained in solution, which was much lower than in the control sample. As the Ca^{2+} concentrations increased from 0 to 30 mM, the solubility of Sup9 decreased more gradually than did that of control, Sup5, and Sup7 and always was more soluble in the presence of Ca^{2+} than any of the other caseins studied.

The addition of extra phosphates to casein should have caused the casein to bind more Ca^{2+} before initial precipitation, which was the case with Sup7 and Sup9 but not with Sup5, which showed the same sensitivity to Ca^{2+} as did the control sample. The amount of the initial soluble casein that precipitated at 5 mM Ca^{2+} was 40% for control, 55% for Sup5, 28% for Sup7, and 14% for Sup9. The behavior of Sup5 to Ca^{2+} was confounded by the lower solubility of Sup5. If Ca^{2+} binding to the protein was the cause of the initial stability to precipitation, as suggested by Yoshikawa et al. (25), Sup5 solutions would have had fewer Ca^{2+} binding sites than did the other caseins. If the assumption was that all of the phosphates bound Ca^{2+} , and, if values of phosphates were multiplied by the molarity of casein in solution, then it was calculated that control had 2.4×10^{-5} , Sup5 had 1.9×10^{-5} , Sup7 had 2.9×10^{-5} , and Sup9 had 6.0×10^{-5} mM of Ca^{2+} -binding sites.

Solubility as a function of pH. Figure 3a shows solubilities of control, Sup5, Sup7, and Sup9 in water as a function of pH. The control sample behaved similarly to the sodium caseinate sample described by Strange et al. (17), except that the control was more soluble at pH 4. The Sup5 and Sup7 samples were less soluble than the control at pH >6 ($P < 0.01$) but were more soluble between pH 5 and 5.5 ($P < 0.01$), which suggested a shift in the isoelectric point. This trend of additional acid groups (negative charges) shifting the isoelectric point of a protein to a lower pH, occurred with dephosphorylated caseins (20); the isoelectric point was lower as the P content increased. At pH <3.5, Sup5 regained 95% of its original solubility, and, at pH <3, Sup7 regained 86%. This behavior was consistent with the increasing number of bound phosphates from the control to Sup5 to Sup7. The Sup9 was equivalent to the control in solubility at pH ≥ 6 if corrected for percentage of protein. The solubility of Sup9 decreased at pH <4 and did not redissolve at lower pH.

The solubilities of control, Sup5, Sup7, and Sup9 in 0.15 M NaCl, as a function of pH, are shown in Figure 3b. Again, the control sample behaved similarly to sodium caseinate (17), except for lower solubility at pH 2. The point of isoelectric precipitation continued to decrease as the phosphate content increased, although the differences in the initial pH of precipitation were less pronounced among the samples. None

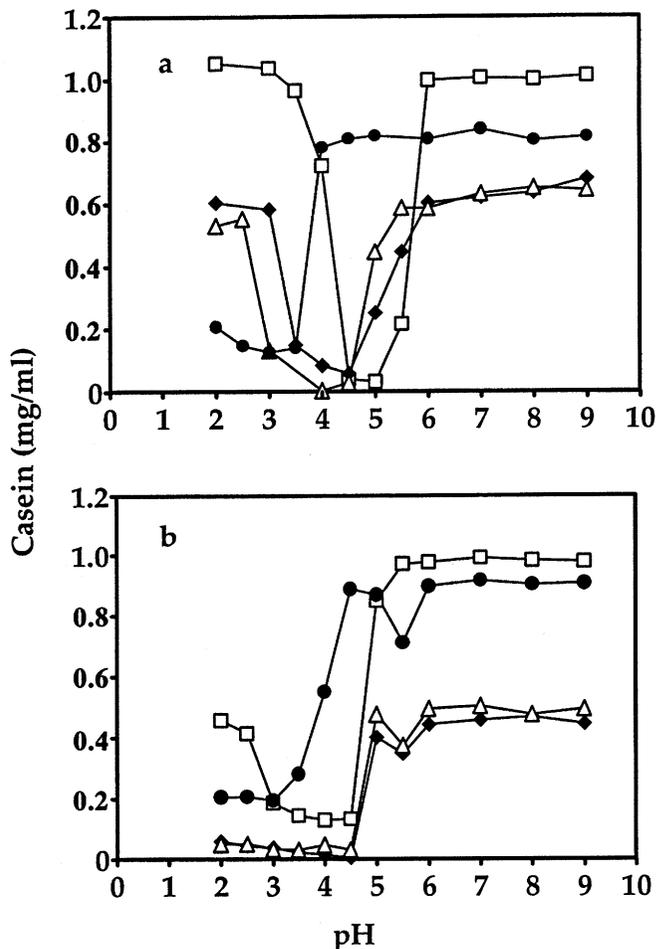


Figure 3. Solubility of control casein and chemically phosphorylated casein (0.1%) in a) water or b) 0.15 M NaCl at pH 2 to 9. Control casein (\square) and casein modified at pH 5 (\blacklozenge), pH 7 (\triangle), and pH 9 (\bullet). Casein solubility was determined on duplicate samples.

of the superphosphorylated casein samples returned to solution in 0.15 M NaCl at low pH, in contrast to results with dephosphorylated casein (17, 20).

The Sup5 and Sup7 samples were rather insoluble at all pH values. Even at pH 8 and 9, they were only 70% soluble in water, 50% soluble in 0.15 M NaCl, and 50% (Sup5) and 60% (Sup7) soluble in 0.01 M imidazole buffer. The rest of the protein was present as a transparent suspension. Gelation occurred when solutions >0.5% were attempted. The solubility of Sup9 was similar to the control at higher pH, and no difficulty was encountered when solutions were made.

The addition of phosphate groups to a protein increases the negative net charge and the hydrophilic nature of the casein. Matheis et al. (11) reported an increase in water binding and a decrease in water

solubility for casein modified at pH 7 and suggested that these results were due to the crosslinking of the casein. Others (8, 12) have reported reduced solubility for phosphorylated casein. Medina et al. (12) examined the solubility of 2 superphosphorylated caseins in 0 and 0.1 M NaCl from pH 2 to 11 and reported that modified caseins were less soluble than control casein at pH 2, 8, 9, and 11 and that the casein modified at pH 7 was more soluble at pH 4 to 5. Girerd et al. (8) reported that all their extremely superphosphorylated caseins were equally soluble at pH >5.5.

Foaming properties. Foaming properties (Figure 4) were evaluated using a sparging method because of limited amounts of modified casein available. Although the foaming capacities of modified casein were significantly different from that of the control ($P < 0.05$), the actual differences were small. Phosphorylation significantly improved foam stability ($P < 0.01$). The foam stabilities of Sup5 and Sup9 were threefold higher, and that of Sup7 was sevenfold higher, than the stability for the control. Compared with foam made using control casein, the modified casein foams coalesced into larger bubbles before collapsing. Improved foam stability was in agreement with observations of Girerd et al. (8).

The ability of the chemically phosphorylated casein in the liquid phase to flex and interact with the gaseous phase was not significantly altered, but the ability to maintain the interaction and association was significantly improved. The net charge of the protein influenced the adsorption of the protein at air-water interfaces (5). Townsend and Nakai (19) reported that the charge density, hydrophobicity, and viscosity of the proteins have significant impacts on foam stability. Several researchers (11, 12) have suggested that chemical phosphorylation resulted in crosslinked casein. Therefore, the addition of negatively charged phosphate groups to the four individual caseins that constitute whole casein and the possible crosslinking of the proteins might contribute to the increased stability of the protein foam. The fact that the Sup7 foams had significantly higher stabilities than those of the other modified caseins may be caused by the type and distribution of the phosphates added, and a configuration unique to that modified casein that might be particularly conducive to foam stability. Research is underway to study the structural differences of the superphosphorylated caseins and investigate this phenomenon.

Emulsion properties. The emulsion properties (EAI, ES, and EC) of control and modified caseins are in Table 2. The EAI was highly dependent upon the soluble protein concentration in the aqueous

phase of the emulsion. Our samples containing <0.045% soluble protein had initial absorbances <0.08 and showed indications of immediate emulsion collapse. Because of the low solubility of all the caseins near their isoelectric points and the general low solubility of Sup5 and Sup7, we calculated EAI only for solutions containing $\geq 0.045\%$ protein. A minimum of 0.1% protein is required to obtain repeatable turbidity values (13). The EAI for Sup5 was highest at pH 5.5 and was significantly higher than all other samples at pH 6 to 8. At pH ≥ 5 , the EAI for the control and Sup9 had small, but significant, differences between samples and between pH and were lower than all other samples. The EAI for Sup7 was highest at pH 5.5 and 8.

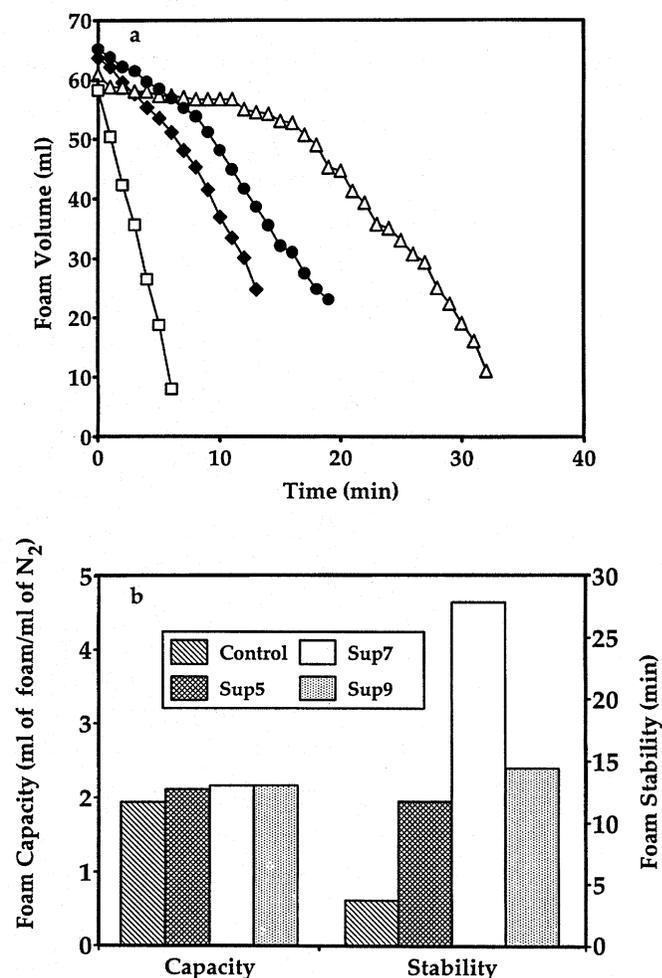


Figure 4. Foaming properties: a) foam volume and b) foam capacity and stability of control casein and chemically phosphorylated casein (0.1% casein in 0.15 M NaCl at pH 7). Control casein (□) and casein modified at pH 5 (◆), pH 7 (△), and pH 9 (●). Casein foam stability and capacity were determined on triplicate samples.

TABLE 2. The emulsion activity index (EAI), emulsion stability (ES), and emulsion capacity (EC) of control casein and chemically phosphorylated casein (0.1% casein in 0.15 M NaCl) at pH 2 to 8; casein was modified at pH 5 (Sup5), pH 7 (Sup7), and pH 9 (Sup9).

pH	EAI				ES				EC			
	Control	Sup5	Sup7	Sup9	Control	Sup5	Sup7	Sup9	Control	Sup5	Sup7	Sup9
	(m ² /g)				(min)				(g of oil/mg of casein)			
2.0	65 ^f	... ¹	31 ^f	7 ^g	8 ^g	4 ^g	4 ^{ijklm}	16 ^h	23 ^f	6 ^{ik}
2.5	1 ^g	6 ^g	7 ^g	3 ^g	4 ^{ijklm}	27 ^e	21 ^g	5 ^{jk}
3.0	7 ^g	4 ^g	5 ^g	3 ^g	9 ⁱ	26 ^e	36 ^d	6 ⁱ
3.5	2 ^g	6 ^g	3 ^g	2 ^g	10 ⁱ	47 ^b	21 ^{fg}	4 ^{ijklm}
4.0	57 ^g	5 ^g	0 ^g	5 ^g	4 ^g	8 ⁱ	82 ^a	4 ^{ijklm}	3 ^{lm}
4.5	37 ^k	2 ^g	6 ^g	2 ^g	0 ^g	9 ⁱ	40 ^c	4 ^{ijklm}	2 ^m
5.0	53 ^{ghi}	...	52 ^{hi}	46 ^j	158 ^e	7 ^g	0 ^g	5 ^g	3 ^{lm}	3 ^{lm}	5 ^{ijkl}	2 ^m
5.5	49 ^{ij}	184 ^a	81 ^d	64 ^f	177 ^d	33 ^f	2 ^g	8 ^g	3 ^{lm}	5 ^{jk}	3 ^{lm}	3 ^{lm}
6.0	50 ^{ij}	163 ^c	68 ^e	55 ^{gh}	192 ^c	181 ^d	7 ^g	14 ^g	3 ^{lm}	4 ^{ijklm}	3 ^{lm}	3 ^{lm}
7.0	56 ^g	168 ^b	69 ^e	51 ^{hi}	223 ^a	198 ^c	1 ^g	23 ^f	4 ^{ijklm}	4 ^{ijklm}	4 ^{ijklm}	3 ^{lm}
8.0	56 ^g	166 ^b	82 ^d	62 ^f	221 ^a	209 ^b	5 ^g	26 ^f	4 ^{ijklm}	3 ^{lm}	3 ^{lm}	2 ^m
SEM (df)	3.0 (330)				4.0 (128)				1.0 (132)			

a,b,c,d,e,f,g,h,i,j,k,l,m Means for a given response (e.g., EAI, ES, or EC) with no letter in common differ ($P < 0.05$) by Student-Neumann-Keuls t -test.

¹The EAI was not calculated for samples with the protein concentration < 0.45 mg of casein/ml.

The EAI compares emulsion formation in terms of the area of interface stabilized per unit weight of protein. The casein modified by the addition of monophosphates, Sup 5 and Sup7, had higher EAI than did control and Sup9, which indicated that the ability of these modified caseins to form emulsions was improved, although their solubilities were lower. The caseins with the best solubility (control and Sup9) had similar EAI and indicated that modification with mono- and polyphosphates did not alter the ability of the casein to form an emulsion.

The ES was evaluated by heat stressing the emulsion and was expressed as the length of time for the absorbance to decrease by 50%. Most of the emulsions made with solutions at pH < 5 collapsed during the first 5 min of boiling (except for control at pH 2). The stability of control casein emulsions was high above its isoelectric point (from 2 min at pH 4.5 to 158 min at pH 5), increased steadily to pH 7, and remained high at pH 8. Emulsions made with modified caseins were less stable than that of the control. Emulsions of Sup5 were most stable between pH 6 and 8 and were the only modified samples that approached the stability of the control casein. The ES of Sup7 and Sup9 were significantly lower than those of control and Sup5.

The ES study suggested that the addition of phosphate groups to whole casein was not conducive to forming heat-stable emulsions. Changes in the protein conformation would be expected if crosslinking occurred, as suggested (11, 12). Alterations in amphiphilic nature of the casein would occur with the addition of acid groups. Both of these changes could

alter the protein structure or orientation of the whole casein in ways that influence how the protein interacts with itself and the different phases around it.

Because the EC measures the maximum quantity of oil held in an emulsion per milligram of soluble protein, the lowest EC were obtained for solutions in which the proteins were most soluble (i.e., above their isoelectric points). At pH > 5 , the EC was not significantly different for any of the caseins. This result suggested that, at the pH range most common in food systems, superphosphorylation did not alter the ability of caseins to hold oil in an emulsion. At pH < 4 , the EC for Sup5 and Sup7 were significantly greater than those for the control and Sup9. This result disagrees with the lower EC values reported by Matheis et al. (11) for their superphosphorylated caseins.

Research is being conducted on the structure of the superphosphorylated caseins to aid in understanding why the functional properties differed so greatly among the modified caseins.

CONCLUSIONS

Modification of bovine whole casein using phosphorus oxychloride at three different pH resulted in adding up to 6.75 mol of P/mol of casein and in altering functional properties. Modification reactions maintained at pH 5 or 7 resulted in binding additional monophosphates, and, at pH 9, mono-, di-, and polyphosphates were added. Modified casein could not be dissociated in the presence of mercaptoethanol and SDS or urea. The Sup9 remained soluble at higher

Ca²⁺ concentrations and did not precipitate out of solution as rapidly or to the same degree as the control, Sup5, and Sup7; Sup5 and Sup7 precipitated more completely from solution than did the control casein. Addition of phosphates reduced the pH range of minimum solubility in both water and NaCl compared with that of the control. The Sup5 and Sup7 were less soluble in water and NaCl above their isoelectric points but returned to solution in water at pH <3.

Superphosphorylation improved foam stability but did not alter foaming capacity. The modification significantly improved the EAI for Sup5 and Sup7. The superphosphorylated caseins had lower ES than did the control. Superphosphorylated whole casein, with its altered functionality, might have potential utilization in creating or improving value-added dairy foods. This study provided insight into the role of phosphate groups in casein interactions and how modifications can alter the functional properties that are of concern in food systems.

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