Multiple Regression and Response Surface Analysis of the Effects of Calcium Chloride and Cysteine on Heat-Induced Whey Protein Gelation

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Multiple regression analysis was used to obtain prediction equations to measure individual and combination effects of $CaCl_2$ and cysteine on gel texture parameters (hardness, cohesiveness, and springiness) and on compressible water of dialyzed whey protein concentrate (WPC) systems. Predicted maximum gel hardness occurred at 11.1 mM $CaCl_2$ or 9.7 mM cysteine. Cysteine levels at 30 mM or above drastically reduced gel hardness. Cohesiveness tended to decrease with addition of either reagent. Reagent addition generally decreased gel springiness while compressible water increased. Response surface contour predictions suggested that increases in $CaCl_2$ or cysteine in reagent combination systems decreased hardness and increased compressible water. Predicted cohesiveness and springiness maxima were at 13.9 mM cysteine and 18.4 mM $CaCl_2$ and at 10.3 mM cysteine and 10.0 mM $CaCl_2$, respectively. Significant $x_1{\cdot}x_2$ interaction terms in the mathematical model for combined reagent effects were observed on hardness, cohesiveness, and compressible water in the WPC gel systems.

The formation of gel matrices capable of water and ingredient retention is an essential functional property of cheese whey proteins in food systems (Hermansson and Akesson, 1975; McDonough et al., 1974). This heat-induced gel phenomenon has been investigated with whey protein concentrates (WPC) under a variety of conditions (Haggett, 1976; Sternberg et al., 1976; Hermansson, 1972a,b). Recent reports indicate improved gelling ability of a WPC after dialysis treatment which minimizes lactose and ash content (Schmidt et al., 1978b.) The dialyzed WPC gels were characterized as stronger, more cohesive, less springy, more gummy, and more chewy than were nondialyzed WPC gels. The dialyzed WPC gel systems were also more responsive with respect to effect of salt addition on textural characteristics.

Multiple regression and response surface plots have been successfully employed to investigate the factors affecting foaming and denaturation of whey protein (Nielson et al., 1973; Richert et al., 1974). The objective of the present investigation was to use this reserch method to investigate effects of $CaCl_2$ and cysteine combinations on the textural and water-holding characteristics of dialyzed WPC gel systems.

EXPERIMENTAL SECTION

Protein Preparation. Ten percent dispersions of commerical WPC (Enrpro 50, Stauffer Chemical Co., Rochester, MN) were prepared and dialyzed in deionized water at pH 7.0 as previously discussed (Schmidt et al., 1978b). Cysteine was added at levels of 0, 10, 20, 30, and 40 mM; $CaCl_2$ was added at levels of 0, 5, 10, 15, 20, 25, 30, and 40 mM for experiments studying individual reagent effects. Levels used in the experiment investigating combined $CaCl_2$ and cysteine effects are presented in Table I. The dispersions were equilibrated with stirring at room temperature for 30 min following reagent addition.

Gel Preparation and Characterization. Gels were prepared by heating 10% protein dispersions (pH 7.0) at 100 °C for 15 min in screw-capped centrifuge tubes as previously discussed (Schmidt et al., 1978a). Instrumental texture profile analysis on gel sections (1.5 × 1.0 cm) was done according to Bourne et al. (1966). Compression was measured with a 5.5-cm diameter probe on an Instron operating at 2.0 cm/min to a 5.0-mm displacement.

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Table I. Definitions and Levels of Independent Variables

independent variable	symbol	levels, mM −1.414	−1	0	1	1.414
cysteine	x_1	2.6	8.0	21.0	34.0	39.4
$CaCl_2$	x_2	2.5	6.5	16.2	25.9	29.9

Texture parameters of hardness, cohesiveness, and springiness were compared. The water-holding capacity of the gel sections was measured during compression on the Instron. The "compressible water" was determined by increased weight of filter paper (Whatman No. 3) due to water uptake during compression.

Experimental Design. Mathematical analyses were performed using the Statistical Analysis System (SAS) programs at the University of Florida computing center. Regression equations of the form: $\hat{y} = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + e$ were set up to measure effects of individual independent variables (x), $CaCl_2$ or cysteine, on dependent variables (y) which were the texture parameters or compressible water. Plotted data are from five replicate trials.

A central composite experimental design, described by Cochran and Cox (1957), was used to estimate regression coefficients (β_i) for the combined effects of $CaCl_2$ and cysteine according to the model: $\hat{y} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1^2 + \beta_4 x_2^2 + \beta_5 x_1 x_2$. The means of five replicate trials were fitted to the model (Table II). Statistical significance of the coefficients was determined by the "t test".

RESULTS AND DISCUSSION

Effect of Individual Reagent Addition on Whey Protein Gelation. The gel parameter effects of individually added $CaCl_2$ or cysteine to whey protein dispersions are summarized in Figures 1 and 2. The coefficient of determination (R^2) provides a measure of the correlation between predicted and observed response values or is a measure of degree of fit of the data by the mathematical model. The range of R^2 values was from 0.838 for hardness vs. $CaCl_2$ to 0.982 for compressible water vs. cysteine, and it was felt that the regression equations explained a significant amount of variation ($p < 0.01$) for all systems studied.

As discussed previously (Schmidt et al., 1978b), ionic bonding plays a complex role in protein gelation. While primary involvement may be in protein–solvent interactions (Anglemier and Montgomery, 1976), divalent cations such as calcium may improve protein gel strength or

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Table II. Effect of Independent Variable Combination on Four Dependent Variables

independent variables[a]			dependent variables							
			hardness[b] (y_1)		cohesiveness[b] (y_2)		springiness[b] (y_3)		compressible H_2O[c] (y_4)	
trial	cysteine (x_1)	$CaCl_2$ (x_2)	obsd[d]	pred.[e]	obsd[d]	pred.[e]	obsd[d]	pred.[e]	obsd[d]	pred.[e]
1	−1	−1	2.48	2.67	0.55	0.54	1.95	1.88	0.22	0.23
2	1	−1	0.91	0.90	0.52	0.49	1.37	1.36	0.67	0.66
3	−1	1	0.71	0.99	0.67	0.66	1.74	1.70	0.57	0.54
4	1	1	0.41	0.48	0.36	0.33	1.20	1.22	0.69	0.64
5	−1.414	0	2.28	2.00	0.59	0.60	1.75	1.81	0.33	0.33
6	1.414	0	0.35	0.37	0.31	0.34	1.13	1.11	0.67	0.71
7	0	−1.414	2.14	2.07	0.54	0.56	1.68	1.73	0.42	0.41
8	0	1.414	0.78	0.59	0.51	0.53	1.51	1.51	0.57	0.62
9	0	0	1.50	1.53	0.66	0.67	1.80	1.78	0.44	0.47
10	0	0	1.66	1.53	0.66	0.67	1.79	1.78	0.50	0.47
11	0	0	1.48	1.53	0.66	0.67	1.79	1.78	0.50	0.47
12	0	0	1.41	1.53	0.66	0.67	1.77	1.78	0.43	0.47
13	0	0	1.58	1.53	0.66	0.67	1.73	1.78	0.47	0.47
	cum. SD		±0.14		±0.02		±0.06		±0.04	

[a] Levels defined in Table I. [b] Texture profile parameters (Bourne et al., 1966); hardness (kg), cohesiveness, springiness (mm); gel plug (1.5 × 1.0 cm) compressed 5 mm at 2.0 cm/min. [c] Compressible water (g); water expressed under compression of 5 mm at 2.0 cm/min. [d] Observed value. Mean of five replicates. [e] Predicted value from model.

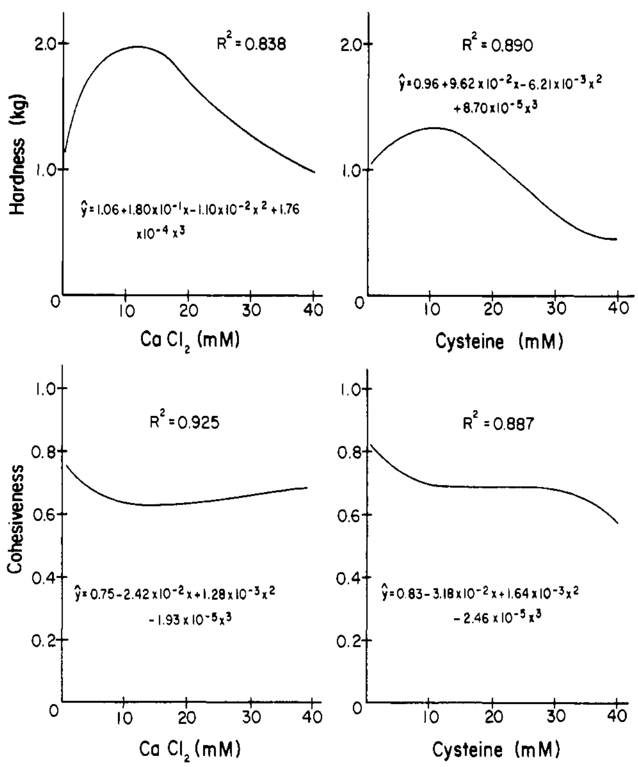

Figure 1. Effect of $CaCl_2$ or cysteine addition to dialyzed 10% whey protein dispersions at pH 7.0 on hardness and cohesiveness of gels prepared by heating at 100 °C for 15 min. Prediction equation: $\hat{y} = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$. All coefficients were significant ($p < 0.01$).

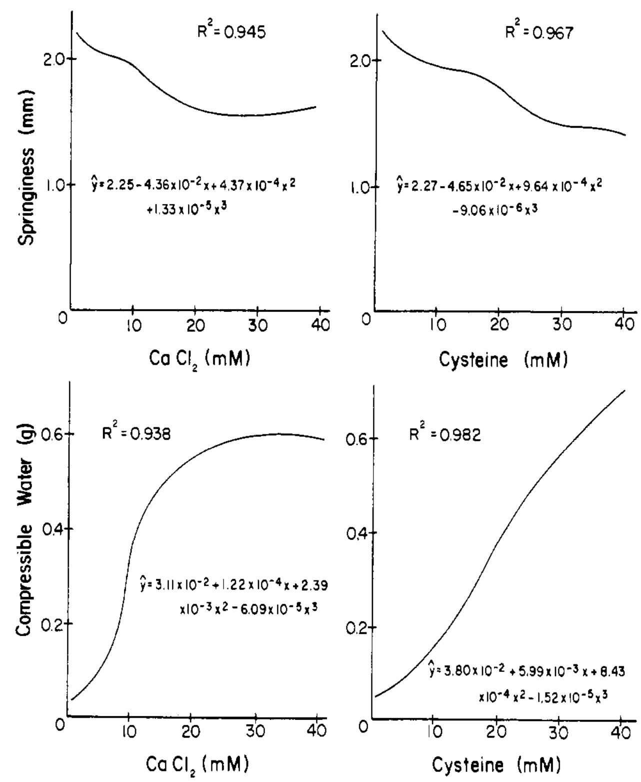

Figure 2. Effect of $CaCl_2$ or cysteine addition to dialyzed 10% whey protein dispersions at pH 7.0 on springiness and compressible water of gels prepared by heating at 100 °C for 15 min. Prediction equation: $\hat{y} = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$. Significant coefficients ($p < 0.01$): $CaCl_2$ effect on springiness (β_0 and β_1); cysteine effect on springiness (β_0 and β_1); all compressible water coefficients.

provide gel stability. However, interpretation of ion–protein interactions would be difficult in a protein gel system. For the WPC investigated (Figure 1), gel hardness apparently maximized at 11.1 mM added $CaCl_2$, while cohesiveness decreased to a minimum at 13.8 mM $CaCl_2$ addition.

Sulfhydryl reagents such as cysteine can be used to increase protein gel strength through mercaptan interchange reactions (Huggins et al., 1951). Their action, however, is extremely concentration dependent (Schmidt et al., 1978a). Beyond a very specific cysteine concentration, disulfide cleavage and reduction may occur, totally destroying gelation. Gel hardness apparently maximized in the WPC system at 9.7 mM added cysteine. Higher cysteine levels dramatically reduced gel hardness. Gels formed in the presence of cysteine at 30 mM or greater were extremely pasty in appearance. In previous experiments with nondialyzed WPC (Schmidt et al., 1978a), gel strength (measured by penetration) maximized at a higher level (25mM) than was observed here for the dialyzed system. Cysteine is apparently more effective in the less complex dialyzed environment.

Sulfhydryl reagents would be expected to more directly effect protein gelation than would ionic reagents because of a direct reaction with covalent disulfide bonding. However, gels formed in the presence of added $CaCl_2$ exhibited much higher maximum hardness than those formed with added cysteine. Extremely low hardness was observed with higher cysteine concentrations. Cohesiveness decreased and leveled off with cysteine addition. At cysteine concentrations of 30 mM or greater (where gelation was virtually destroyed), a further decrease in gel cohesiveness was observed.

Since quadratic and cubic coefficients (β_2 and β_3) were not statistically significant, it can only be stated that springiness data followed a general decreasing trend with respect to addition of either reagent. While low gel springiness data may indicate a high degree of interchain gel cross-linking (Huggins, 1946), interpretation of slight differences in this parameter with reagent addition can by very complex (Schmidt et al., 1978b). For example, a high degree of cross-linking would not be expected at high cysteine levels where springiness was minimal. It is apparent that in the WPC gel system, conditions (with respect to either reagent system) which maximize hardness, minimize cohesiveness with springiness following a decreasing trend..

The effects of $CaCl_2$ or cysteine addition on compressible water in the WPC gels have been summarized in Figure 2. This parameter, as measured, can probably only be interpreted as a measure of loosely held solvent in the gel structure. Since mechanical compression was employed, interpretation should not be made as to potential water loss (or syneresis) under milder conditions (shaking, centrifugation, storage, etc.). We have designated this parameter merely as compressible water. Relatively high water-holding capacity (slightly less than that of egg white gels) and water retention (by mild centrifugation) have been reported previously for WPC gels (Sternberg et al., 1976).

$CaCl_2$ addition dramatically increased compressible water in the dialyzed WPC gels. Compressible water data leveled off at 26.2 mM and higher $CaCl_2$ concentrations. Wheying off or syneresis was also noticeable in the gel tube at these higher $CaCl_2$ concentrations. In comparing $CaCl_2$ effects on compressible water with its effect on texture parameters, it is apparent that at $CaCl_2$ levels where hardness is maximal and cohesiveness is minimal, compressible water dramatically increases.

Increased compressible water was also observed with cysteine addition. However, increases noted at lower cysteine levels were not as dramatic as those observed with $CaCl_2$ addition and a leveling off did not occur at high cysteine levels. Gels formed at 30 mM cysteine or above, were visually pasty and exhibited considerable syneresis in the tube. Despite expected water loss during handling of these gels, high compressible water data were observed. Perhaps compressible water is not indicative of water-holding ability of gels formed at high cysteine levels. At reagent levels which maximized gel hardness, more compressible water was apparent with $CaCl_2$ than with cysteine.

Effect of Reagent Combinations on Whey Protein Gelation. Data observed experimentally and predicted from the model for the 13 combinations of independent variables are tabulated in Table II.

The maximum observed hardness occurred at the combination of 8.0 mM cysteine, 6.5 mM $CaCl_2$. This maxima value was slightly higher than that observed at 9.7 mM $CaCl_2$ individually (Figure 1). Gels formed with combined reagents had slightly lower cohesiveness than was observed when the reagents were added at similar levels individually. As would be expected, the lowest gel hardness was observed in systems which contained higher cysteine levels.

Table III. Definitions and Analysis of Variance (ANOVA) Summaries of Four Dependent Variables[a]

dependent variable	coeff. of determ. (R^2)	mean sq. regress.	mean sq. error	$F_{5,7}$
y_1	0.952	1.098	0.0399	27.48[b]
y_2	0.978	0.034	0.0006	61.53[b]
y_3	0.977	0.148	0.0024	59.72[b]
y_4	0.949	0.042	0.0017	26.01[b]

[a] Dependent variables defined in Table II. [b] Significant ($p < 0.01$).

Table IV. Regression Analysis of Four Dependent Variables[a]

model term	regression coefficient (β_i)			
	y_1	y_2	y_3	y_4
constant	1.526[b]	0.666[b]	1.776[b]	0.468[b]
x_1	−0.575[b]	−0.092[b]	−0.250[b]	0.131[b]
x_2	−0.524[b]	−0.010	−0.078[b]	0.073[b]
$x_1 \cdot x_1$	−0.171[c]	−0.099[b]	−0.156[b]	0.026
$x_2 \cdot x_2$	−0.098	−0.061[b]	−0.079[b]	0.023
$x_1 \cdot x_2$	0.318[c]	−0.070[b]	0.010	−0.083[b]

[a] Dependent variable defined in Table II. [b] Significant ($p < 0.01$). [c] Significant ($p < 0.05$).

Analysis of variance (ANOVA) summaries are presented in Table III. Higher R^2 and significant F values ($p < 0.01$) indicate that the separate models explained much of the variation with respect to each dependent variable.

Regression coefficient (β_1) estimates are presented in Table IV. The coefficients are in terms of coded levels of x (see Table I). Tests performed on the magnitudes of the coefficients relative to their standard errors are indicated as significant when the value is followed by one or two asterisks.

A significant interaction term for cysteine–$CaCl_2$ ($x_1 \cdot x_2$) was observed for all dependent variables with the exception of springiness (y_3). This suggests that cysteine and $CaCl_2$ have an altered effect on hardness (y_1), cohesiveness (y_2), and compressible water (y_4). For example, the positive $x_1 \cdot x_2$ term for hardness (y_1) suggests that the reduction in hardness effect from increasing cysteine depends on the particular level of $CaCl_2$. In fact, perusal of the observed hardness values (Table II) informs us that increasing cysteine from 8.0 to 34.0 mM (or x_1 from −1 to 1) at the 6.5-mM level of $CaCl_2$ (or $x_2 = -1$) reduced the hardness by 2.48 − 0.91 = 1.57 kg. At 25.9 mM $CaCl_2$ (or $x_2 = 1$), the same increase in cysteine reduced hardness only 0.71 − 0.41 = 0.30 kg.

Response surface contour plots were generated by computer to aid in visualizing the combined effects of the two reagents on the dependent responses. The contour plots are presented in Figure 3. These plots indicate a decreasing trend in hardness (y_1) and an increasing trend in compressible water (y_4) with increasing cysteine or $CaCl_2$. These trends more closely parallel those observed for individual cysteine effects than those of $CaCl_2$ (Figure 1 and 2). Dramatic reductions in hardness were observed with reagent increases, and compressible water was low at reagent levels where hardness maximized. The predicted values for y_1 and y_4, in fact, suggest that gels of maximal hardness but of minimal compressible water may be possible at lower combined reagent levels than were used in this experiment (2.6 mM cysteine and 2.5 mM $CaCl_2$).

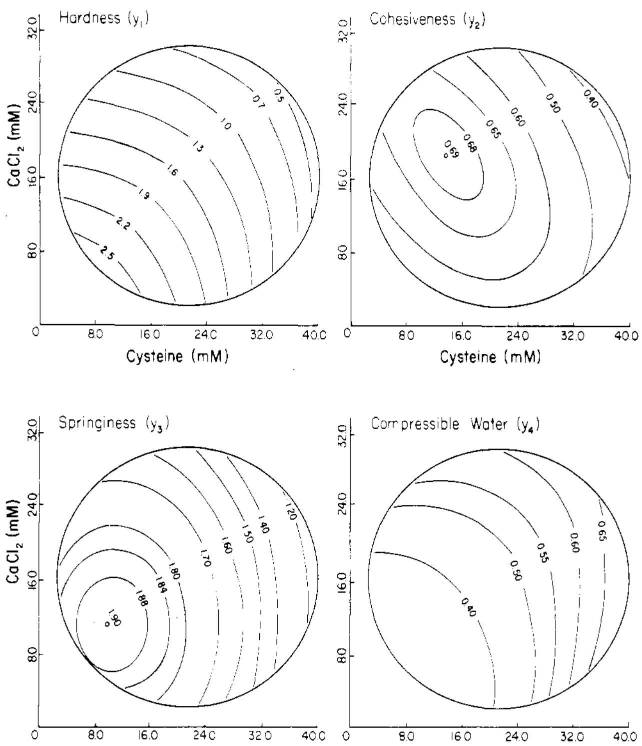

Figure 3. Response surface contour plots for cysteine (x_1) and $CaCl_2$ (x_2) addition to 10% whey protein dispersions at pH 7.0 on hardness (y_1), cohesiveness (y_2), springiness (y_3), and compressible water (y_4) of gels prepared by heating at 100 °C for 15 min.

Predicted gel cohesiveness (y_2) was apparently maximum at a combination of 13.9 mM cysteine and 18.4 mM $CaCl_2$ and gel springiness (y_3) was maximum at 10.3 mM cysteine and 10.9 mM $CaCl_2$. Upon close examination, however, it can be observed that at slightly higher or lower reagent concentrations (i.e., an increase to 24.0 mM or a decrease to 8.0 mM in either reagent), only a slight decrease in cohesiveness results. A greater decrease is apparent at higher levels of the reagents which suggests a trend similar to that of the cysteine effect on this parameter individually. The combined effects of the reagents on springiness also more closely paralleled the individual effect of cysteine on this parameter.

Correlation of Dependent Variables. Correlation coefficients for the dependent variables used in the experiment are given in Table V. Significant correlation was observed between hardness (y_1) and springiness (y_3) or compressible water (y_4), between cohesiveness (y_2) and (y_3), and between y_3 and y_4. Apparently, in the WPC gel system studied, as hardness decreased, springiness would also be expected to decrease (positive correlation) and compressible water would increase (negative correlation). This again more closely parallels what would be expected with cysteine addition individually at levels beyond the hardness maximum.

Table V. Correlation of Dependent Variables[a]

	y_1	y_2	y_3	y_4
y_1	1.000			
y_2	0.502	1.000		
y_3	0.792[b]	0.867[b]	1.000	
y_4	−0.932[b]	−0.510	−0.843[b]	1.000

[a] Dependent variables defined in Table II. [b] Significant ($p < 0.01$).

As desirable protein gelation parameters for specific food applications become better defined through further research in this area, it may be possible that judicious reagent addition may be used to optimize these parameters. However, because of the complex nature of heat-induced protein gelation, extrapolation of data presented here to other protein or whey protein gel systems would not be advisable. Data trends do suggest combined effects of cysteine and $CaCl_2$ on the gelation phenomenon in the WPC system. Significant interactions were apparent between reagents with respect to their effects on gel hardness, cohesiveness, and compressible water. Further research using more definitive techniques is necessary to more clearly interpret the nature of these reagent and protein interactions at the molecular level.

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