The Ability of Phosphates or κ-Carrageenan to Coagulate Whey Proteins and the Possible Uses of Such Coagula in Cheese Manufacture[1]

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ABSTRACT

A method is postulated for manufacturing cheese in which whey proteins are coagulated by food-grade phosphates or anionic polyelectrolytes and the aggregates are incorporated into casein coagula produced from concentrated UF retentates. The ability of monosodium phosphate, tetrasodium pyrophosphate, sodium hexametaphosphate, monobasic calcium phosphate, or κ-carrageenan to coagulate whey proteins to facilitate this manufacturing procedure was evaluated in solutions of whey protein concentrate at various pH and heat treatments. Treatments that were selected to produce whey protein coagulates included 0.20% tetrasodium pyrophosphate, followed by 0.15% calcium chloride after 5 min in whey protein solutions at pH 6.4, 0.05% sodium hexametaphosphate in whey protein solutions at pH 2.5, or 0.05% κ-carrageenan in whey protein solutions at pH 4.6. The treated whey protein solutions (13.3 ml) were combined with UF retentates prepared from whole milk with a concentration factor (by weight) of 4.8× (66.7 ml), the mixtures were set with rennet, and the coagula were analyzed after 20 and 30 min. Addition of solutions of treated whey protein to the UF retentate generally increased syneresis while reducing curd tension, coagulum hardness, and protein recovery (calculated) in the coagula.

(**Key words**: whey protein coagulation, cheese yield, phosphates, κ-carrageenan)

Abbreviation key: **ddd** = deionized and double distilled, **KC** = κ-carrageenan, **MCP** = monobasic calcium phosphate, **MSP** = monosodium phosphate, **R** = reference (used with number), **SHMP** = sodium hexametaphosphate, **TP** = total protein, **TSPP** = tetrasodium pyrophosphate, **TSPP + CC** = the TSPP and calcium chloride, **WPC** = whey protein concentrate.

INTRODUCTION

Despite intensive research (16), the manufacturing procedures that have been developed to increase whey protein recovery in cheese generally fail to produce cheese with significant amounts of retained whey protein (6, 19). The failure of these methods implies the need for an alternative or supplemental approach to enhance the recovery of whey proteins in cheese. Theoretically, the retention of whey proteins in cheese can be increased by the development of procedures for binding whey proteins directly into the casein coagulum. Application of this approach to cheese manufacture requires agents that coagulate whey proteins during casein coagulation, thereby creating an integrated coagulum with both the caseins and whey proteins. Alternatively, the recovery of whey proteins in cheese can be enhanced by developing procedures to aggregate or coagulate whey proteins independently and then entrap the whey protein particles in a subsequently produced casein coagulum.

Many casein properties depend upon the phosphorylation of selected serine residues (9, 30). If phosphorylation of whey proteins (18) creates in these proteins a sensitivity to coagulation by casein coagulants or indigenous milk components, then curd formation would produce an integrated casein-whey protein coagulum. Woo and Richardson (35) and Woo et al. (34) reported that 100 m*M* ionic calcium gelled 6% (wt/vol) solutions of chemically phosphorylated β-LG. The concentration of ionic calcium in milk should coagulate or gel such phosphorylated whey proteins during cheese manufacture. Unfortunately, the reagents used for chemical phosphorylation, phosphorus pentoxide and phosphorus oxychloride, are hazardous compounds (20) that cannot be used for cheese manufacture (3).

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Food-grade phosphates and anionic polyelectrolytes are compounds that have GRAS (generally regarded as safe) status (4) and may be applicable for cheese manufacture (8, 22). Food-grade phosphates and anionic polyelectrolytes that are routinely used in the manufacture of many dairy and food products include monosodium phosphate (**MSP**), tetrasodium pyrophosphate (**TSPP**), monobasic calcium phosphate (**MCP**), sodium hexametaphosphate (**SHMP**), and κ-carrageenan (**KC**) (10, 11, 12, 13, 14, 15, 37). Previous studies show that the addition of either SHMP (7) or KC (24) to skim milk enhanced the yield of cottage cheese, and the addition of MCP to milk increased the yield of Edam (29). Yet, most of the increase in yield observed in these studies seems to be related to a recovery of the added reagent rather than to an enhanced recovery of whey protein.

The phosphates and anionic polyelectrolytes just listed probably will not produce phosphorylated whey proteins that can be coagulated by casein coagulants. Hence, these agents presumably would be used to produce whey protein coagula or aggregates that are retained or entrapped within casein coagula. The addition of such reagents directly to the cheese milk may promote reactions with the caseins, thereby disrupting cheese manufacture and failing to produce whey protein coagula. Isolation and independent treatment of the whey proteins limit the ability of these reagents to instigate undesirable reactions with the caseins. The treated whey proteins can be added to a subsequent supply of cheese milk prior to casein coagulation. Independent whey protein solutions that are suitable for such treatment are readily produced from whey by UF as whey protein concentrate (**WPC**) or as whey protein isolate.

Several benefits are realized by combining a procedure for increasing the recovery of whey proteins in cheese by the creation of whey protein coagula with procedures for producing cheese from concentrated UF retentates. Cheese production from concentrated UF retentates should independently enhance whey protein recovery in the cheese (1, 5, 27, 28). The casein coagulum produced from concentrated UF retentates may be more suited to recovery of coagulated whey protein particles. The production of cheese from concentrated UF retentates limits syneresis, reducing the ability of whey drainage to remove the coagulated whey protein particles. Finally, the ease of producing a highly concentrated, highly purified WPC or whey protein isolate is enhanced by using whey produced from concentrated UF retentates. An outline of a manufacturing procedure for maximizing whey protein recovery in cheese by this approach is shown in Figure 1.

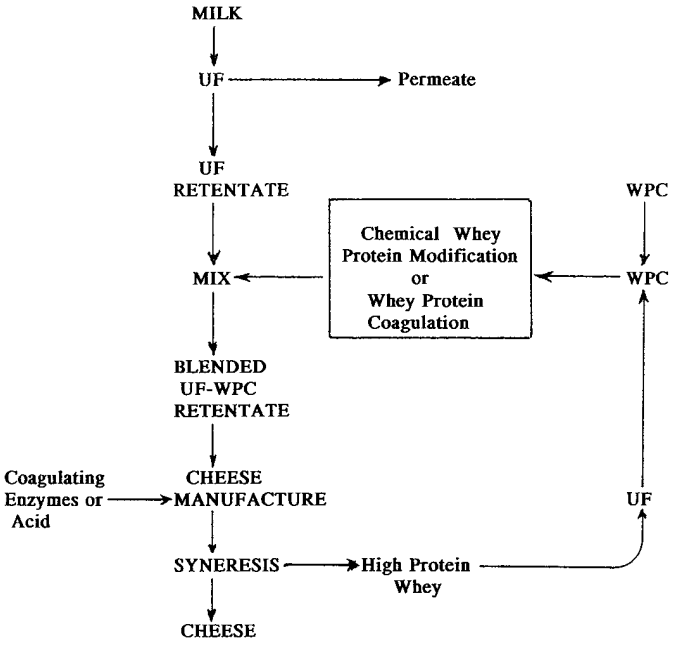

Figure 1. Proposed procedure for incorporating whey proteins into cheese. Simultaneously, milk is fractionated by UF to produce a highly concentrated UF retentate, and whey protein concentrate (WPC) is either chemically modified to produce whey proteins capable of coagulating with the caseins during cheese curd formation or coagulated to produce particles that will be retained by the casein coagulum during cheese manufacture. The concentrated UF retentate and treated WPC are combined in a desired ratio, and the mixture is set with either coagulating enzymes (i.e., rennet) or acid to produce a coagulum for cheese manufacture. High protein WPC can be produced by fractionating the whey created by syneresis during cheese manufacture or obtained from other sources.

The overall objective of this study is to evaluate the potential of using the manufacturing procedure shown in Figure 1 to increase the recovery of whey proteins in cheese. Emphasis is focused on producing a whey protein coagulum that can be recovered in a rennet-induced casein coagulum produced from concentrated UF retentates. Specific objectives include the following:

1. To determine the influence of MSP, TSPP, MCP, SHMP, and KC upon whey protein solutions with variation in the solution pH, heat treatment, and calcium concentration.
2. To select treatments that produce whey protein coagula that can be used to retain the whey proteins in cheese.
3. To determine the curd tension, coagulum hardness, percentage syneresis, and protein recovery of rennet induced, casein coagula produced from 4.8× whole milk UF retentate and the treated WPC solutions by the manufacturing procedure shown in Figure 1.

MATERIALS AND METHODS

Whey Protein Treatments with the Phosphates and KC

A whey protein solution was prepared with 5% (wt/vol) TS and 1.75% (wt/vol) total protein (**TP**), pH 6.75. The solution was prepared from 35% protein WPC powder (Davisco International, Inc., St. Peter, MN) and deionized, double distilled (**ddd**) water. The solution was divided into four equal portions, and the pH of two of these portions was adjusted to either 2.5 or 4.6 with 1 and 0.1*N* phosphoric acid. The pH of another portion was adjusted to 12.0 with 40% sodium hydroxide. The pH of the remaining portion was maintained at 6.75. The solutions at each pH were held at 5°C for 12 h following pH adjustment to facilitate protein hydration.

The whey protein solutions were heated to 23°C. Each of the whey protein solutions was used to prepare separate, individual 0.20% (wt/vol) solutions for each one of the following reagents: MSP ($NaHPO_4$), TSPP ($Na_4P_2O_7$), SHMP [$(NaPO_3)_{13}Na_2O$], MCP [$Ca(HPO_4)_2$] (all obtained from Fisher Scientific Co., Fair Lawn, NJ), or KC (Sigma Chemical Co., St. Louis, MO). Three individual 5-ml samples of each of the whey protein solutions were prepared, and the individual samples were held at 23°C or heated to 65°C or 85°C. The samples were visually analyzed for coagulum formation, gel formation, and syneresis after 30 min. The samples were centrifuged at $2000 \times g$ for 5 min at 23°C and visually analyzed for coagulum formation and syneresis. The criteria for evaluating treatments included no coagulation, samples that remained clear with the formation of no visible precipitate; very slight coagulation, formation of a few visible particles that could be concentrated by centrifugation; slight coagulation, the formation of sufficient particles to give the sample a milky appearance; moderate coagulation, sufficient coagulation to produce a uniform but weak coagulum; firm coagulation, complete coagulation throughout the sample; very firm coagulation, formation of a dense coagulum that produced a rubber-like curd upon centrifugation; weak suspended complex formation, production of curd-like particles suspended throughout the sample; gelation, formation of a translucent gel showing no to limited syneresis after centrifugation; and insoluble precipitate, formation of an encrusted pellet of crystalline material on the bottom of the sample tube following the heat treatment.

Treatments that induced whey protein coagulation were repeated after the concentration of the reagent was adjusted to 0.05, 0.10, 0.15, or 0.20% (wt/vol). These solutions were then supplemented with 0, 0.05, 0.10, 0.15, or 0.20% (wt/vol) of either calcium chloride (Sigma Chemical Co.) or calcium acetate (Fisher Scientific Co.). The samples were subjected to the specified heat treatment for 30 min and visually analyzed for coagulum formation and syneresis.

Selection of WPC Treatments

A WPC solution was prepared with 20% (wt/vol) TS and 14% TP (70% protein on a dry basis) from whey protein isolate powder (Davisco International, Inc., Le Sueur, MN) and ddd water. This solution was divided into three portions. The pH of two of the WPC solutions was adjusted to 4.6 or 2.5 with 1 or 0.1*N* phosphoric acid. The pH of the remaining solution was maintained at 6.4. The solutions were held at 5°C for 12 h after pH adjustment to promote protein hydration. The solutions were heated to 23°C and received the following treatments. First, the WPC solution at pH 6.4 was used to produce a solution with 0.20% TSPP (wt/vol). Portions of this solution were held at 23°C for 5, 60, or 90 min and then supplemented with 0.15% (wt/vol) calcium chloride. Second, the WPC solution at pH 2.5 was used to prepare a solution with 0.05% (wt/vol) SHMP. Third, the WPC solution at pH 4.6 was used to prepare a solution with 0.05% (wt/vol) KC. Each solution was held at 23°C, and the viscosity was measured at 5, 30, 60, 90, 180, and 210 min with a Haake Rotovisco RVII viscometer (Haake Buehler Instruments, Inc., Saddle Brook, NJ).

Preparation and Analysis of Casein Coagula

The coagulation study was conducted by separately preparing a UF retentate with a concentration factor by weight of 4.8× and three independently treated WPC solutions. Combining each of the treated WPC solutions with separate portions of UF retentate followed the cheese manufacturing procedure outlined in Figure 1.

The production of the UF retentate began with the pasteurization of raw whole milk at 73°C for 16 s with a plate heat exchanger (Super Plate, Cherry-Burrell, Chicago, IL). The milk was discharged at 5°C, and the pH was adjusted to 6.0 with 88% lactic acid (Purac, Inc., Arlington Heights, IL). The milk was held at 5°C for 12 h. The milk was heated to 50°C and fractionated by UF using a spiral wound membrane with a molecular mass cutoff of 20,000 (Osmonics, Inc., Minnetonka, MN). The inlet and outlet pres-

sures of the UF unit were maintained at 0.55 and 0.45 mPa, respectively, throughout operation. Deionized water was added to the retentate when the concentration factor reached 3.0×. The amount of water used for diafiltration was 5.4 kg of water/453.6 kg of initial milk. The UF processing continued until the diafiltration water was completely removed and until the retentate concentration factor reached 4.8×. The retentate was collected and stored for 12 h at 5°C.

A WPC solution was prepared with 20% (wt/vol) TS and 14% (wt/vol) TP (70% protein on a dry basis) from whey protein isolate and ddd water. The solution was divided into three portions, and the pH of the individual portions was adjusted to 2.5, 4.6, or 6.4 as required with 1 or 0.1*N* phosphoric acid. Each solution was held at 5°C for 12 h, heated to 23°C, and then used for one of the following treatments: treatment 1, the WPC solution at pH 6.4 was supplemented to produce a 0.20% TSPP (wt/vol) solution, held at 23°C for 5 min, and further supplemented with 0.15% (wt/vol) calcium chloride; treatment 2, the WPC solution at pH 2.5 was supplemented to produce a 0.05% (wt/vol) SHMP solution; and treatment 3, the WPC solution at pH 4.6 was supplemented to produce a 0.05% (wt/vol) KC. Each solution was held at 20°C for 90 min in a water bath.

The temperature of the UF retentate was adjusted to 20°C, and then 66.7 ml of UF retentate were combined with 13.3 ml of one of the treated WPC solutions in 100-ml beakers. Samples consisting of UF retentate and WPC treated with TSPP and calcium chloride were identified as the TSPP + CC treatment; samples containing UF retentate and WPC treated with SHMP were identified as the SHMP treatment; and samples consisting of UF retentate and WPC treated with KC were identified as the KC treatment. Three types of reference samples were also prepared: the reference (**R**) 1 treatment consisted of 80 ml of the 4.8× UF retentate, the R2 treatment consisted of 13.3 ml of untreated WPC (pH 6.4) and 66.7 ml of the 4.8× UF whole milk retentate, and the R3 treatment consisted of 80 ml of pasteurized whole milk. Four individual samples were prepared for each treatment. All samples were placed in a water bath at 20°C.

Calf rennet (Chr. Hansen's Laboratory, Inc., Milwaukee, WI) was diluted 1:30 with ddd water, added to each sample (90 ml of rennet/453.6 kg of sample), and dispersed by vigorous stirring for 3 min with a hand-held spatula. Duplicate samples for each treatment were cut after 20 or 30 min with the device described by Mohamed and Morris (21) for measuring curd tension and coagulum hardness. This device was connected to an Instron Universal Testing Machine (model 1122; Instron Corp., Canton, OH) equipped with load cell number 2511–102 using a full-scale load of either 0 to 10 or 0 to 20 g, a crosshead speed of 20 mm/min, and a chart speed of 50 mm/min. The force-distance curves produced upon cutting the coagula were interpreted as described by Mohamed and Morris (21) and Kebary and Morris (17). The percentage syneresis of the coagula (PSC) was measured by the method of Mohamed and Morris (21) using the following equation:

$$PSC = \frac{WC}{WS} \times 100$$

where WC = the whey collected (milliliters), and WS = the amount of water within the sample (milliliters).

Compositional Analysis

Procedures of the AOAC (33) were used to determine the TS and TP content in the pasteurized milk, 4.8× retentate, prepared WPC, and whey. The vacuum oven method was used to show the TS content. The percentage of nitrogen in each sample was determined by the Kjeldahl procedure, and the TP content was shown by multiplying the percentage of nitrogen by 6.38.

Statistical Analysis

Treatments were evaluated by ANOVA for a simple randomized design containing six treatments with two replication of each treatment. Differences between treatments were determined by means comparison using the Tukey procedure (26). Differences among comparisons had to achieve $P < 0.05$ to show significance.

RESULTS AND DISCUSSION

Effect of Phosphates or KC on Whey Proteins

Table 1 shows the extent of protein coagulation in whey protein solutions containing the various reagents after being held for 30 min at 23°C. At pH 12.0, the whey protein solution with MSP formed the firmest coagulum. Firm coagula also occurred in whey protein solutions at pH 2.5 containing SHMP and in whey protein solutions at pH 4.6 containing KC. Weaker coagula occurred in the whey protein solu-

TABLE 1. The extent of protein coagulation in whey protein solutions held at 23°C for 30 min.

	Whey protein solution			
Treatment[1]	pH 2.5	pH 4.6	pH 6.75	pH 12.0
Control	–[2]	M	–	–
MSP	–	S	–	VF
TSPP	–	S	–	–
SHMP	F	M	–	–
MCP	–	S	–	–
KC	–	F	–	–

[1]Treatments = Whey protein solutions with 5% (wt/vol) TS, 1.75% (wt/vol) protein, and 0.20% (wt/vol) of the specified reagent. Control is whey protein solution containing no added reagent. Abbreviations of specified reagents: MSP = monosodium phosphate, TSPP = tetrasodium pyrophosphate, SHMP = sodium hexametaphosphate, MCP = monobasic calcium phosphate, and KC = κ-carrageenan.

[2]– = No coagulation, S = slight coagulation, M = moderately firm coagulation, F = firm coagulation, and VF = very firm coagulation. The criteria for evaluating coagula are stated in the text.

TABLE 2. The extent of protein coagulation in whey protein solutions held at 65°C for 30 min.

	Whey protein solution			
Treatment[1]	pH 2.5	pH 4.6	pH 6.75	pH 12.0
Control	S[2]	M	SC	–
MSP	S	S	SC	VF
TSPP	S	S	SC	VS
SHMP	F	M	–	VS
MCP	S	S	PT	PT
KC	S	F	G	G

[1]Treatments = Whey protein solutions with 5% (wt/vol) TS, 1.75% (wt/vol) protein, and 0.20% (wt/vol) of the specified reagent. Control is whey protein solution containing no added reagent. Abbreviation of specified reagents: MSP = monosodium phosphate, TSPP = tetrasodium pyrophosphate, SHMP = sodium hexametaphosphate, MCP = monobasic calcium phosphate, and KC = κ-carrageenan.

[2]– = No coagulation, VS = very slight coagulation, S = slight coagulation, M = moderately firm coagulation, F = firm coagulation, VF = very firm coagulation, SC = weak suspended complex formation, G = gelation, and PT = insoluble precipitate. The criteria for evaluation coagula are stated in the text.

tions at pH 2.5 containing 0.05% SHMP and in the whey protein solutions at pH 4.6 containing 0.05% KC. All treatments produced weak coagula in the whey protein solutions at pH 4.6, and the remaining treatments did not induce whey protein coagulation.

The addition of calcium chloride mainly enhanced the coagulation of whey proteins by TSPP. Supplementation of the whey protein solution containing 0.2% TSPP at pH 6.75 with 0.15% calcium chloride produced a firm coagulum. The ability of ionic calcium to enhance the ability of TSPP to produce a milk protein coagulum reflects the use of TSPP and calcium acetate to produce a coagulum with cold milk in instant puddings (8). Supplementation of WPC solutions containing either SHMP or KC at pH 4.6 with 0.2% calcium chloride slightly enhanced protein coagulation. The addition of calcium chloride or calcium acetate to any other treatment that induced protein coagulation did not further enhance whey protein coagulation.

The extent of whey protein coagulation occurring in the treated WPC solutions heated to 65°C for 30 min is shown in Table 2. The firmest coagulum occurred at pH 12.0 in the whey protein solution containing MSP. Firm coagula formed in the whey protein solution at pH 2.5 containing SHMP and in the whey protein solution at pH 4.6 containing KC. The solution at pH 6.75 containing SHMP and the whey protein solution at pH 12.0 did not noticeably affect the whey protein solutions. All other solutions produced coagula, suspended complexes, precipitates, or gels. Insoluble calcium phosphate presumably formed the white precipitates observed in the whey protein solutions containing MCP at pH 6.75 and 12.0. Heating the whey protein solutions containing KC at pH 6.75 and 12.0 produced gels. Maillard browning (32) occurred in all whey protein solutions that were heated to 65°C at pH 12.0. The various reagents produced the identical type of coagula, precipitates, or gels in the whey protein solutions heated at 85°C for 30 min as observed in the whey protein solutions heated at 65°C for 30 min.

Selection of Whey Protein Treatments

Viscosity measurements showed that the various reagents had essentially maximized whey protein coagulation after reacting with the whey protein solutions for 90 min (data not shown). Treatments selected for further study included 0.2% TSPP and 0.15 calcium chloride in whey protein solutions at pH 6.75, 0.05% SHMP in whey protein solutions at pH 2.5, and 0.05% KC in whey protein solutions at pH 4.6. All of the WPC treatments selected for further study occur at 23°C, thereby eliminating the need to heat the solutions to higher temperatures during processing. The TSPP + CC at pH 6.75 eliminated pH adjustments, and use of 0.05% SHMP and KC reduced the amount of reagent needed to promote coagulation from 0.2 to 0.05%.

Curd Properties and Protein Recoveries in Treated Coagula

The TS, TP, and pH of the UF retentate, UF retentate and WPC mixtures, whole milk, and untreated

WPC are shown with the estimated casein and whey protein contents in Table 3. The TS and TP contents of the R3 sample are representative of raw milk produced in Minnesota (H. A. Morris, S. T. Coulter, and C. Gates, University of Minnesota, St. Paul, 1964, personal communication) and the immediate area (2, 36). The TS and TP contents of the WPC were formulated to match the expected composition of WPC created by the procedure outlined in Figure 1. As formulated, the WPC has a higher TP content but lower amounts of TS and casein than does the retentate. Addition of the WPC to the retentate, therefore, produced a product with more TP but less TS and casein than the initial UF retentate.

Mixing retentate with WPC at pH 6.4 increased the pH of the combined sample, and mixing the retentates with WPC at pH 2.5 (the SHMP treatment) or at pH 4.6 (the KC treatment) proportionally reduced the pH of the combined sample. The pH reduction in the UF retentate receiving the SHMP treatment coagulated the UF retentate as an acid curd. Mixing rennet into this coagulum produced a semiliquid product that subsequently formed a weak enzymatic coagulum.

The greatest curd tension and coagulum hardness ($P < 0.05$) occurred in R1 coagula, and the lowest ($P < 0.05$) curd tension and coagulum hardness was observed in coagula produced by R3 (Table 4). Coagula produced in samples receiving the KC treatment usually had higher ($P < 0.05$) curd tension and coagulum hardness than coagula produced by the R2 and TSPP + CC. The ability of the KC treatment to increase curd firmness may be due in part to the ability of the low pH of the mixture of treated WPC and retentate to enhance rennet activity (23). Curd tension and coagulum hardness of the coagula containing the SHMP treatment were usually lower ($P < 0.05$) than in coagula from R2 and TSPP + CC treatments. Increasing the set from 20 to 30 min did not affect curd tension. However, the coagulum hardness of samples receiving the TSPP + CC treatment increased ($P < 0.05$) when the set was extended to 30 min.

The greatest percentage syneresis of the curd ($P < 0.05$) occurred in the coagula produced from R3 (Table 4). Greater syneresis ($P < 0.05$) occurred in samples receiving KCT than in samples receiving TSPP + CC treatment. Syneresis in cheese is inversely proportional to the ability of the coagulum to hold moisture and limit shrinkage (31), which is usually inversely proportional to the amount of casein incorporated into the curd. Incorporation of a whey protein coagulum into a rennet-induced casein coagulum

TABLE 3. Total solids (TS), total protein (TP), estimated casein (CN), estimated whey protein (WP), and pH of the treated samples and prepared whey protein concentrate (WPC).

Treatment[1]	Component				
	TS	TP[2]	CN[3]	WP	pH
	(%)				
R1	38.52	13.95	11.16	2.79	6.04
R2	36.50	14.77	9.30	5.47	6.10
R3	12.08	3.40	2.58	0.64	6.67
TSPP + CC	36.50	14.77	9.30	5.47	6.10
SHMP	36.50	14.77	9.30	5.47	5.23
KC	36.50	14.77	9.30	5.47	5.78
WPC	26.40	18.86	0.00	18.86	6.40

[1]Treatment abbreviations: R1 = reference treatment 1, 80 ml 4.8× UF retentate; R2 = reference treatment 2, 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 6.4); R3 = reference treatment 3, 80 ml of pasteurized whole milk; TSPP + CC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 6.4), with the WPC containing 0.20% (wt/vol) tetrasodium pyrophosphate and 0.15% (wt/vol) calcium chloride; SHMP = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 2.5), with the WPC containing 0.05% (wt/vol) sodium hexametaphosphate; and KC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 4.6), with the WPC containing 0.05% (wt/vol) κ-carrageenan.

[2]TP = Percentage of nitrogen × 6.38.

[3]CN = 0.80 × TP for the 4.8× retentate and 0.76 × TP for the pasteurized milk; WP = 0.20 × TP for the 4.8× retentate, and 0.19 × TP for the pasteurized milk, and TP for the WPC.

TABLE 4. Curd tension (fracturability), coagulum hardness (firmness), and percentage of syneresis of the coagulum (PSC) of the treated samples at 20 and 30 min following rennet addition.

Treatment[1]	Curd tension		Coagulum hardness		Syneresis	
	20 min	30 min	20 min	30 min	20 min	30 min
	(g of force)				(%)	
R1	8.40[a]	10.65[a]	13.48[a]	14.43[a]	41.68[bc]	38.43[bc]
R2	2.80[bc]	4.35[c]	5.23[c]	7.03[c]	36.91[bc]	38.43[bc]
R3	0.05[c]	0.25[e]	0.35[e]	0.93[e]	81.75[a]	75.36[a]
TSPP + CC	2.70[bc]	4.09[c]	4.64[c,B]	6.78[c,A]	30.52[c]	30.52[c]
SHMP	1.65[bc]	1.78[d]	2.93[d]	3.57[d]	43.80[bc]	39.37[bc]
KC	4.45[b]	5.90[b]	7.40[b]	8.35[b]	48.72[b]	48.72[b]

[a,b,c,d]Means within a column followed by no common superscript letter differ ($P < 0.05$).

[A,B]Means within a row followed by no common superscript letter differ ($P < 0.05$).

[1]Treatment abbreviations: R1 = reference treatment 1, 80 ml of 4.8× UF retentate; R2 = reference treatment 2, 66.7 ml of 4.8× UF retentate and 13.3 ml of whey protein concentrate (WPC; pH 6.4), R3 = reference treatment 3, 80 ml of pasteurized whole milk; TSPP + CC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 6.4) that contains 0.20% (wt/vol) tetrasodium pyrophosphate and 0.15% (wt/vol) calcium chloride; SHMP = 66.7 ml 4.8× UF retentate and 13.3 ml of WPC (pH 2.5) that contains 0.05% (wt/vol) sodium hexametaphosphate; and KC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 4.6) that contains 0.05% (wt/vol) κ-carrageenan.

TABLE 5. Percentage of TS and total protein (TP) recovered in the whey.

Treatment[1]	TS in Whey		TP[2] in Whey	
	20 min	30 min	20 min	30 min
	(%)			
R1	14.79[b]	14.89[c]	5.25[c]	5.46[d]
R2	18.31[ab]	18.52[b]	8.91[ab]	9.16[ab]
R3	7.97[c]	8.09[d]	1.17[d]	1.18[e]
TSPP + CC	18.92[ab]	19.36[a]	9.44[a]	9.73[a]
SHMP	19.64[a]	19.37[a]	8.60[b]	8.26[c]
KC	18.07[ab]	18.14[b]	8.75[b]	8.74[bc]

[a,b,c]Means within a column with no common superscript letter differ ($P < 0.05$).

[1]Treatment abbreviations: R1 = reference treatment 1, 80 ml of 4.8× UF retentate; R2 = reference treatment 2, 66.7 ml of 4.8× UF retentate and 13.3 ml of whey protein concentrate (WPC; pH 6.4); R3 = reference treatment 3, 80 ml of pasteurized whole milk; TSPP + CC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 6.4), with the WPC containing 0.20% (wt/vol) tetrasodium pyrophosphate and 0.15% (wt/vol) calcium chloride; SHMP = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 2.5), with the WPC containing 0.05% (wt/vol) sodium hexametaphosphate; and KC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 4.6), with the WPC containing 0.5% (wt/vol) κ-carrageenan.

[2]TP = Percentage of nitrogen × 6.38.

TABLE 6. Calculated recovery of percentage TS and total protein (TP) in the coagula at either 20 or 30 min after rennet addition.

Treatment[1]	TS in Curd		TP in Curd	
	20 min	30 min	20 min	30 min
	(%)			
R1	90.16[a]	90.87[a]	90.03[a]	90.77[a]
R2	88.01[a]	87.48[ab]	85.84[abc]	84.70[bc]
R3	52.57[b]	55.63[c]	72.25[d]	76.97[d]
TSPP + CC	89.96[a]	89.73[a]	87.62[ab]	87.25[ab]
SHMP	85.03[a]	86.74[ab]	83.78[bc]	86.00[bc]
KC	89.69[a]	84.63[b]	81.68[c]	81.70[c]

[a,b,c]Means within a column with no common superscript letter differ ($P < 0.05$).

[1]Treatment abbreviations: R1 = reference treatment 1, 80 ml of 4.8× UF retentate; R2 = reference treatment 2, 66.7 ml of 4.8× UF retentate and 13.3 ml of whey protein concentrate (WPC; pH 6.4); R3 = reference treatment 3, 80 ml of pasteurized whole milk; TSPP + CC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 6.4), with the WPC containing 0.20% (wt/vol) tetrasodium pyrophosphate and 0.15% (wt/vol) calcium chloride; SHMP = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 2.5), with the WPC containing 0.05% (wt/vol) sodium hexametaphosphate; and KC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 4.6), with the WPC containing 0.05% (wt/vol) κ-carrageenan.

should increase the amount of total protein present in the final coagulum, thereby reducing the amount of whey available for removal by syneresis. The high syneresis in KC treatment samples may be caused by a reaction of KC with the casein (25).

The TS and TP in whey from the retentates containing added or treated WPC usually exceeded ($P < 0.05$) the TS and TP contents of whey produced by R1 and R3 (Table 5). Samples containing WPC receiving the SHMP and TSPP + CC treatments produced whey with the highest ($P < 0.05$) TS content. The TSPP + CC treatment produced whey with a greater ($P < 0.05$) amount of TP than all treatments except R2. Whey produced by R3 consistently contained the lowest ($P < 0.05$) amounts of TS and TP.

Recovery of calculated TS and TP was usually greatest ($P < 0.05$) in the R1 and TSPP + CC treatment samples, particularly in coagula produced at 30 min (Table 6). The lowest ($P < 0.05$) recovery of calculated TS and TP occurred in R3. The calculated recovery of whey protein by all treatments resembled the recovery of indigenous whey proteins in R1 (Table 7). The calculated recovery of whey protein by TSPP + CC treatment marginally exceeded whey protein recovery in R2, which exceeded the calculated whey protein recovery in samples receiving SHMP and KC treatments. The inability of the WPC treatments to enhance greatly protein recovery in the curd shows a failure of these treatments to promote whey protein recovery in cheese. Although about 60% of the added whey proteins remained with the coagula immediately after cutting, subsequent syneresis would limit whey protein recovery to 30 to 33% for Cheddar type cheeses (6, 19).

TABLE 7. Calculated recovery of total protein (TP) and added whey protein (AWP) in the treatment coagulums.

Treatment[1,2]	TP in Curd		AWP in Curd	
	20 min	30 min	20 min	30 min
	(g)		(%)	
R1	10.05	10.13	57.02[2]	60.21[2]
R2	10.14	10.01	64.22	61.34
R3	1.97	2.09	0[2]	17.05[2]
TSPP + CC	10.35	10.31	68.72	67.78
SHMP	9.90	10.16	59.01	64.64
KC	9.65	9.65	57.02	60.21

[1]Treatment abbreviations: R1 = reference treatment 1, 80 ml of 4.8× UF retentate; R2 = reference treatment 2, 66.7 ml of 4.8× UF retentate and 13.3 ml of whey protein concentrate (WPC; pH 6.4); R3 = reference treatment 3, 80 ml of pasteurized whole milk; TSPP + CC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 6.4), with the WPC containing 0.20% (wt/vol) tetrasodium pyrophosphate and 0.15% (wt/vol) calcium chloride; SHMP = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 2.5), with the WPC containing 0.05% (wt/vol) sodium hexametaphosphate; and KC = 66.7 ml of 4.8× UF retentate and 13.3 ml of WPC (pH 4.6), with the WPC containing 0.05% (wt/vol) κ-carrageenan.

[2]Estimated recovery of indigenous whey proteins.

Although none of the selected treatments successfully increased whey protein recovery in the casein coagula, the failure of the TSPP + CC treatment contrasts sharply with the ability of this treatment to coagulate whey proteins in the initial screening studies. The WPC solutions used in the screening study were prepared from commercial WPC with a higher milk salt content, and the milk salt concentration in the WPC added to the UF retentates (produced from whey protein isolate) was lower. If greater amounts of milk salt promote whey protein coagulation by the TSPP + CC treatment procedure, then this procedure could be improved by increasing the mineral content of the WPC.

CONCLUSIONS

A method is postulated for manufacturing cheese in which whey proteins are aggregated by either food-grade phosphates or anionic polyelectrolytes, and the aggregates are incorporated into the casein coagula produced from concentrated UF retentates. However, the whey protein treatments used in this study did not produce whey protein coagula that were recovered as cheese. Therefore, the promotion of whey protein recovery within cheese coagula requires different whey protein treatments or alternative manufacturing approaches.

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