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Effect of Proteolytic Activity of Streptococcus cremoris on Cottage Cheese Yield

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EFFECT OF PROTEOLYTIC ACTIVITY OF *STREPTOCOCCUS CREMORIS* ON COTTAGE CHEESE YIELD

by

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Gary William Stoddard

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ABSTRACT

Effect of Proteolytic Activity of Streptococcus cremoris on Cottage Cheese Yield

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Using proteinase negative variants of Streptococcus cremoris UC310 or UC320 to manufacture cottage cheese, theoretical yields were increased 1.97% and 1.56% respectively when compared to the theoretical yields of the proteinase positive parents. Yield differences were strain dependant and differences between positive and negative variants were not manifest with strains of UC73 and UC97. It was necessary to produce bulk culture using pH control and to add sufficient nitrogenous stimulant to provide carry-over stimulant into the cheese milk. All cultures examined developed normally even when the bulk medium contained a blend of 5% yeast extract and casein hydrolysate. It was possible to use the culture to complete the required acidification after direct acidification to pH 5.2 with phosphoric acid. Careful selection of lactic

culture strains is necessary to achieve maximum product yields.

(62 pages)

INTRODUCTION

Direct acidification methods for cottage cheese manufacture have become popular because they reduce process times and increase yield. Geilman (13) recovered 8.8% more product (16.1 vs. 14.8 kg / 100 kg milk) with direct acidification than with acid development by cultures. When cultures are used, better yields are obtained with whey-based medium under pH control than with conventional milk-based media (26). Losses attributed to use of milk-based culture could be associated with the increase of soluble protein fractions produced by media heat treatment and/or use of proteinase positive (Prt+) lactic cultures (9). Mild heat treatment increases yield through incorporation of whey proteins (1,5,6) but high temperature treatment produces curd defects (5,44). This study examines proteolytic activities of six selected lactic cultures and their effects on cottage cheese yields.

LITERATURE REVIEW

Cultures

Prt+ cultures are wild type cells of each respective strain. These are 'fast' milk-coagulating cultures (17,19) that are predominantly selected for use in the cheese industry. Prt- cells are variants of wild type parent cells which have been selectively removed from cheese starter cultures as 'slow' milk-coagulating cultures (17,19). These Prt- variants are cells which have lost the plasmid-carried genetic codons for some or all of the cell wall-bound proteinase enzymes (20). This effectively reduces or eliminates their ability to break down casein as a source of nitrogen for culture growth. Casein is the major component of cottage cheese and determines yield. Yield, therefore, can be increased by utilizaing Prt- cells, which do not break down as much casein as do wild type Prt+ parent cells.

Cheddar Cheese Yield

Researchers indicate that use of proteinase negative variants (Prt-) of S. cremoris increase Cheddar cheese yield (28,29,35) over use of proteinase positive (Prt+) wild type parent strains. Further, researchers now question whether the Prt- cells are Prt- or peptidase negative (Pep-) (11,30).

Whey-Base Starter Media

Use of whey-based starter media in conjunction with pH control (47) reduces starter cost (31) and increases yield (13,16,26). This starter system (18,31) is now one of the most predominant methods used by the United States cheese industry.

Lactic Starter Acid Production

Prt- cultures produce acid even after cell growth ceases (38) or when cells are inhibited by various substances (33) in the media. Cell mass measurements further demonstrate the growth of Prt+ cells and the limited or minimal growth of Prt- cells in either pure or mixed cultures (46).

Culture Stimulation

Several researchers have proposed the addition of various organic (2,22,39,40,47) and mineral (42) stimulants to cheese milk to increase activity of lactic cultures used in cheese manufacture. Stimulant additions do not cause organoleptic defects in the final product (39).

Direct Acidification

Research has been initiated to determine effects of direct acidification and culture manufacturing methods on cottage cheese yield (36,37). Further studies have shown that cottage cheese from direct acidification produces higher yield but required acidulants increase costs (13).

High Manufacturing Temperature

Prt- cultures may facilitate reduced manufacturing times by increasing incubation temperatures (38). Cheddar cheese manufacturing temperatures can be increased with Prt- cultures (24,25). Cottage cheese is not manufactured at high temperatures because of resulting decreases in acid production (38).

MATERIALS AND METHODS

Culture Maintenance

Isolated cultures classified by previous researchers (12) were from the culture bank in the Department of Nutrition and Food Sciences, Utah State University. Strains of S. cremoris and S. lactis were chosen for this study based on their acid-producing capabilities (46) and abilities to demonstrate product yield differences between proteinase positive (Prt+) wild type and proteinase negative (Prt-) variants (28,35). These strains were tested for activity to determine their suitability for manufacture of cottage cheese. The Prt+ strains were inoculated into vials of sterile, 10% reconstituted nonfat dry milk (RNDM), incubated at 30°C for 3 h and frozen at -40°C until used. The Prt- variant strains were inoculated into vials of sterile, 10% stimulated reconstituted nonfat dry milk (NDMS), then incubated and stored as above. Sufficient seed cultures were frozen and stored at -40°C to prevent proteinase activity changes during the study.

Media

Low heat nonfat dry milk (San Joaquin Valley Dairymen, Los Banos, CA 93635) was reconstituted (RNDM) at 10% (10 g nonfat dry milk in 90 ml de-ionized water), and pasteurized (72°C for 15 s) or sterilized (121°C for 15 min), according to the requirements of the individual experiment. It was

then cooled to 4oC for 18-24 h before use to allow equilibration of the colloidal/bound calcium phosphate system. Stimulated nonfat dry milk was reconstituted (NDMS) as above except that it was fortified with .1% AYE-Light yeast extract (Busch Industrial Products, Inc., St. Louis, MO 63127).

Whey-based pH-control medium for both Prt+ and Prt- cultures consisted of 6.5 L of 5% (w/v) dried whey (Gossner's Foods, Inc., Logan, UT 84321), .4% AYE-Light, and .1% N Z Amine Type E casein hydrolyzate (Humko-Sheffield, Oneonja, NY 13820) (47).

pH Control

Bulk starters were prepared using the Utah State University Lactic Culture System (31). Whey-based media were subjected to continuous external pH control using 30% ammonium hydroxide.

Culture Activity

Culture activity was determined by measuring pH reduction over time of inoculated RNDM or RNDM enriched with .2% yeast extract (SNDM). Change in pH was monitored with a Ross Combination electrode (Orion Research, Inc., Cambridge, MA 02139) and a Beckman model 60 pH Meter (Beckman Instruments, Inc., Fullerton, CA 92634). Acceptable activity was defined as achieving pH 4.7 within 5 h after inoculation (7).

Temperature

Activities of Prt- cultures were compared at manufacturing temperatures of 32, 35, 38, and 41°C (38) to determine which provides maximum activity. Culture activity at each other temperature was compared with the standard manufacturing temperature of 32°C (7).

Inoculum Level

Inoculum volumes (v/v) of 2, 4, 6, 8, and 10% were evaluated.

Stimulant Addition

Organic (47) and mineral (42) stimulants were added to RNDM to confirm the activity increase of Prt- variants as shown by Speck and Ledford (39). Stimulant enriched milk substrate was prepared by adding one of the following to RNDM: .4% yeast extract and .1% casein hydrolysate; .4, .2 or .1% yeast extract; .1% casein hydrolysate; .1% magnesium chloride and .1% potassium chloride; or .1% magnesium chloride, .1% potassium chloride and .01% zinc chloride.

Direct Acidification

Direct acidification procedures (7,13) were followed for maximum product yield information. Partial acidification and partial culture methods were tested and involved acidification of 4°C RNDM or SNDM with phosphoric acid to pH 5.2 and inoculation with various levels of lactic culture.

Stimulant Carry-over

Highly enriched whey-based medium for use in the pH-control system was developed to provide carry-over enrichment with 4% inoculum. This medium contained an additional .05 g/ml AYE-Light (.2% carry-over) or .025 g/ml AYE-Light (.1% carry-over) added to the standard whey-based pH-control medium formulation.

Culture Isolation

Cultures were re-isolated in two steps from previously isolated and characterized strains of S. cremoris and S. lactis (12) to assure their purity. Cultures were first plated onto Fast-Slow Differentiation Agar (FSDA) (17), grown under anaerobic conditions (BBL Gas Pack System, Bectin Dickinson and Co., Cockeysville, MD 21030) and representative colonies picked and cultured for further characterization. The second step consisted of evaluating lactose utilization and proteinase activity. Lactose utilization was determined by plating isolates from the first step onto BCP-indicator agar (21). All colonies gave a lactose positive response (Lac+) and were subsequently tested for proteinase activity. Proteinase activity was based upon the culture's ability to coagulate milk in 24 h (Prt+) or inability to coagulate milk in 48 h (Prt-) (10,32).

Curd Firmness

Curd firmness measurements from a Vatimer (34) curd tension monitor, and a penetrometer in conjunction with a Mettler PC440 balance (Mettler Instrument Corp., Hightstown, NJ 08520) were correlated with cottage cheese yield. The penetrometer is a motor-driven device which pushes a multiple cut, vertical blade into coagulated milk. A 250 ml wide-mouth bottle, used for incubation and cooking, is placed upon the Mettler balance that provides a digital reading of the grams of curd firmness as the curd resists the cutting action of the blade.

Cottage Cheese Manufacture

Bulk culture was weighed (4±.01 g) into 100±.01 g of pasteurized SNDM in a 250 ml wide-mouth bottle that was sealed with a rubber stopper to prevent evaporation. Inoculated substrates were incubated in a constant temperature water bath at 32 or 35°C. The change in pH was continuously monitored with a Ross Combination electrode (Orion, op cit.) and a Beckman model 60 pH Meter (Beckman, op cit.). The curd was cut (at either pH 4.8, 4.7, or 4.6) with a spatula and the rubber stopper replaced to minimize evaporation during cooking, processing, etc. The curd was allowed to stand undisturbed for 15 min after cutting to allow curd annealing (7). The sealed bottle was placed upon a Sommer-Matson test apparatus (43) and the bottle(s) rolled continuously (8 rpm) for 15 min at the incubation

temperature. Cooking temperature was increased slowly to 40°C over 45 min, and then raised to 50°C over 60 min (6). The whey was decanted, filtered through Whatman #4 filter paper, and frozen at -40°C until analyzed.

Cell Mass

Cell mass (45) was determined using a DB-G spectrophotometer (Beckman, op cit.) at 480 nm on the freshly inoculated milk substrate and on the coagulated milk at cutting time, and reported as T_{480}.

Whey Analysis

Whey samples were analyzed for protein and non-protein nitrogen on a Kjeltec Autoanalyzer 1030 and Digestion System (Tecator AB, Hoganas, Sweden). Total nitrogen (TN) was determined by sampling the filtered whey obtained from simulated cheesemaking, digesting the sample at 410°C in 5 ml concentrated sulfuric acid, 1 g sodium sulfate, and 1 ml mercuric sulfate (45). Non-protein nitrogen (NPN) content was determined by precipitating soluble protein (SP) in the whey with 12% trichloroacetic acid (TCA), centrifuging the precipitated sample and sampling the supernatant. The fluid supernatant was tested for nitrogen content by the TN procedure. TN and NPN results were converted to protein content by multiplying by the 6.38 Kjeldahl factor for milk and milk products. The dilution in the NPN/TCA procedure was compensated for prior to calculating percent protein.

Data from protein analyses were compared to maximum theoretical and direct acidification yields (1,13). Casein protein (CP) was calculated by analyzing SNDM substrate for TN, NPN, and SP (from the Direct Acidification whey) using the equation CP = TN(milk) - TN(whey). Theoretical yields were calculated using the formula: Yield = .45 + 5.7 X % Casein Protein (CP) (1).

Statistical Analysis

Product yield resulting from use of Prt+ and Prt- variants were calculated from whey analysis data and the results evaluated statistically using factorial analyses of variance.

RESULTS AND DISCUSSION

Culture Maintenance

Several Prt- variants of *Streptococcus cremoris* and *S. lactis* were selected and tested for their usefulness in cottage cheese manufacture. *S. lactis* strains UL21A and UL7A, and *S. cremoris* strains UC73, UC97, UC310 and UC320 were tested.

Vials of cultures were stored and carried at -40°C to prevent mutation or proteinase activity change during this study. This was to eliminate error between experiments due to culture variability.

Media

Culture acitivity was tested in sterile 10% RNDM or SNDM to eliminate potential competition from contaminants. Simulated cheese manufacture used pasteurized milk substrate (10% RNDM or SNDM) to follow the normal cottage cheese manufacturing procedure and to eliminate yield loss due to curd defects (5). Prt- cultures were carried in the 10% NDMS to provide sufficient nitrogenous matter for growth. Prt+ cultures were carried in 10% RNDM since stimulant enrichment is not required for growth. Whey-based pH-control medium was used since yield increases have been demonstrated over standard milk-based bulk starter media (4,13,15,16,26,47).

Culture Isolation

Cultures were re-isolated from existing cultures in the USU culture bank before final cheesemaking. This was done to assure measurement of "real" differences in yield between Prt+ and Prt- cultures. Lactic cultures are well known for developing slow-coagulating cells (17,19) which increase in number with each successive transfer. Re-isolation was conducted to assure that the Prt+ and Prt- cultures were pure. These cultures should provide highest attainable yield differences and advantages. Lactose utilization was also of concern since it is known that strains of S. lactis simultaneously lose their proteinase producing and lactose utilizing capabilities (20). Lac- variants are undesirable since they are unable to metabolize lactose. Strains of UC310 and UC320 were Prt- and Lac+ when plated on differentating media (17,21). These cultures were propagated and used in final cheesemaking experiments.

pH Control

The USU Lactic Culture System (31) is a widely used and accepted method for preparing bulk starter cultures because of its low cost and efficiency. The use of the whey-based medium in conjunction with pH control provides state-of-the-art culture propagation with minimal starter cost. Ammonium hydroxide was used as the neutralizer since it also provides a source of nitrogen for cell growth.

Culture Activity and Temperature

Previous research (38) indicated that Prt- lactic strains continue to produce acid after growth ceased or in the presence of inhibitory substances (33). Therefore, there may be several advantages in using these strains instead of Prt+ strains. The ability of Prt- variants to decrease the pH of skim milk enough to make cottage cheese in 5 h or less was evaluated at different temperatures (Figure 1 - Appendix A Tables 2-4). During 5-6 h incubation at 32, 35, 38, and 41°C, none of the strains was able to decrease the pH below 4.9. The fastest pH change occured at 35°C. A maximum pH reduction of about 1.5 units was consistent with the upper limits measured in 5 h culture activity tests based upon pH change (8). Activity of the S. lactis strains was considerably lower than activity of the S. cremoris strains and were, therefore, not used subsequently.

Inoculum Level

During 5-6 h incubation, none of the strains inoculated at 2, 4, or 6% (w/v) was able to decrease the pH below 4.9. Subsequent experiments showed that raising the inoculum levels to 6, 8, and 10% (w/v) also proved insufficient to reduce the pH enough to enable cutting of cottage cheese curd in 6 h (Figure 2 - Appendix A Tables 2-6). The pH decrease must occur more rapidly to compete with direct acidification, in which 1 h after lactone addition is

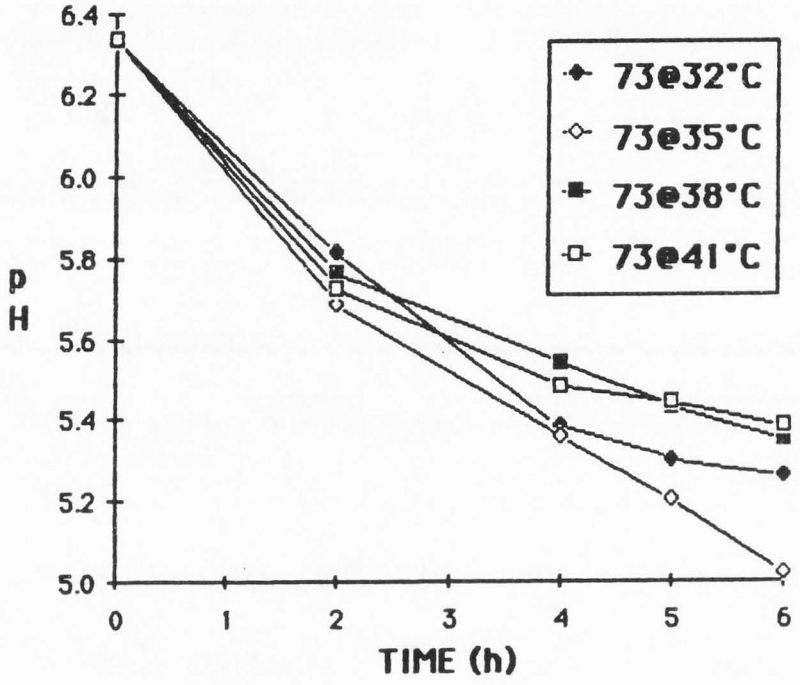

Figure 1. Effect of incubation temperature upon ability of lactic strain UC73 Prt- (4% inoculum) to reduce the pH in reconstituted nonfat dry milk for cottage cheese (max. standard deviation = .028).

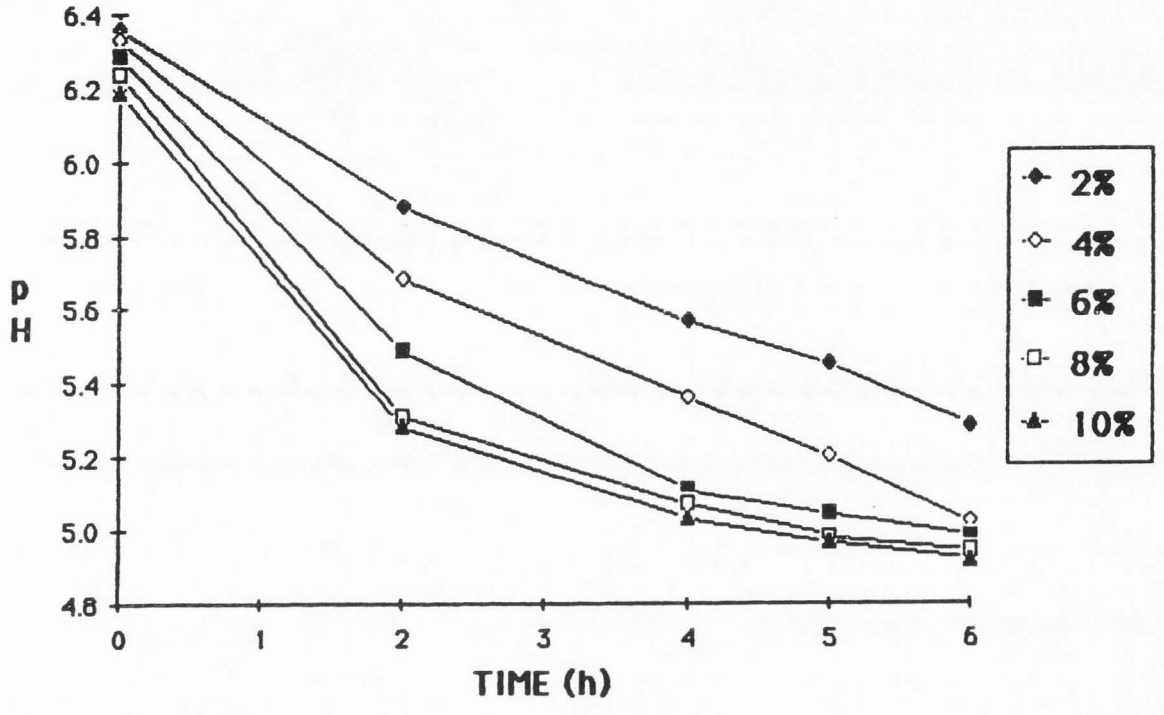

Figure 2. Effect of inoculum levels on ability of lactic strain UC73 Prt- to reduce the pH in reconstituted nonfat dry milk for cottage cheese at 35°C (max. standard deviation = .028).

required to reduce the pH from 5.2 to 4.7 (13). Addition of stimulants (38,39,40,42) allows sufficient reduction in pH and were considered to facilitate use of higher cooking temperatures (24,25).

Stimulant Addition

Organic (47) and mineral (42) stimulants were added to RNDM substrate to accelerate reduction in pH. Culture activity was measured on each stimulant-enriched substrate to measure effectiveness of each in increasing acid production and subsequently reducing pH (Figure 3 - Appendix A Tables 9-10). AYE-Light yeast extract enrichment at .1 to .2% accelerated acid prduction in RNDM suficiently to assure normal acid development in 3.5 to 4 h when using 4% inoculum. Speck and Ledford (39) shortened cheese make times by 17 to 41% when .015 to 1% pancreas extract stimulant was added to the cheese milk. Findings of this study support previous findings that these stimulants can effectively reduce the time required to manufacture cultured cottage cheese.

Direct Acidification

Direct acidification and Prt- culture acidification were combined (Figure 4 - Appendix A Table 7) in a further attempt to reduce incubation time required to achieve sufficient pH reduction. Even 10% inoculum (27) did not reduce the milk pH from 5.3 to cutting levels in 6 h. When

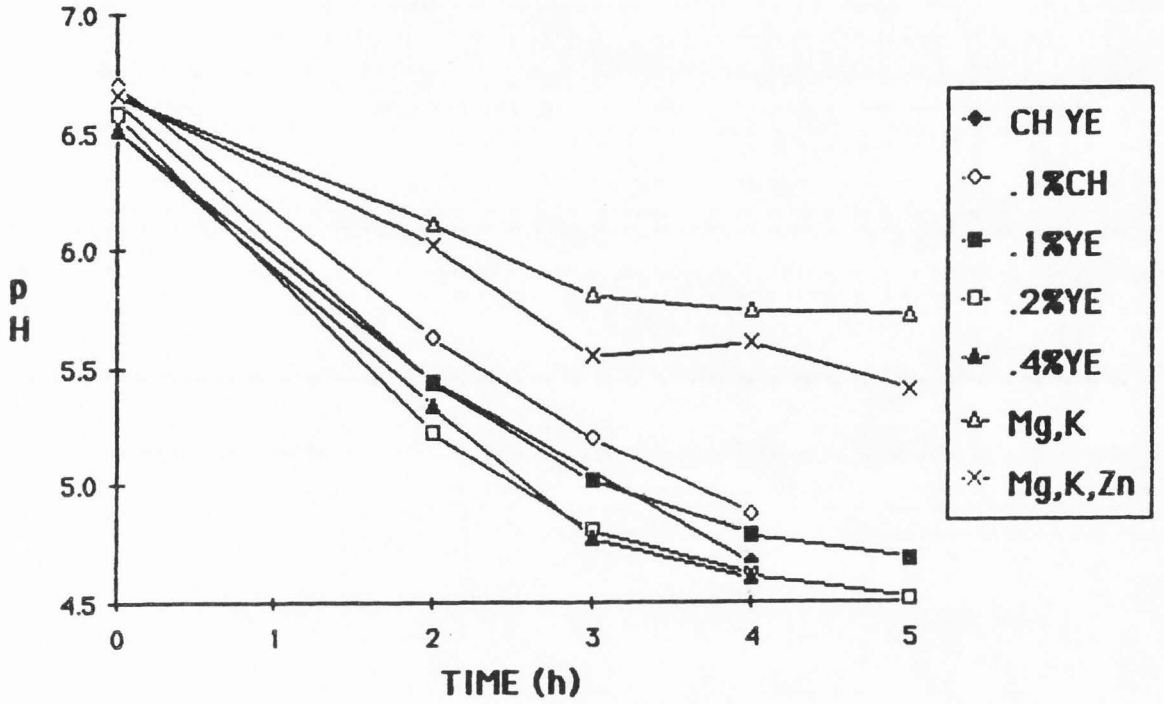

Figure 3. Effect of various stimulants upon the ability of lactic strain UC73 Prt- to reduce the pH in reconstituted nonfat dry milk for cottage cheese at 35°C. (CH+YE = combination of .1% CH and .4% YE (47)) (max. standard deviation = .028).

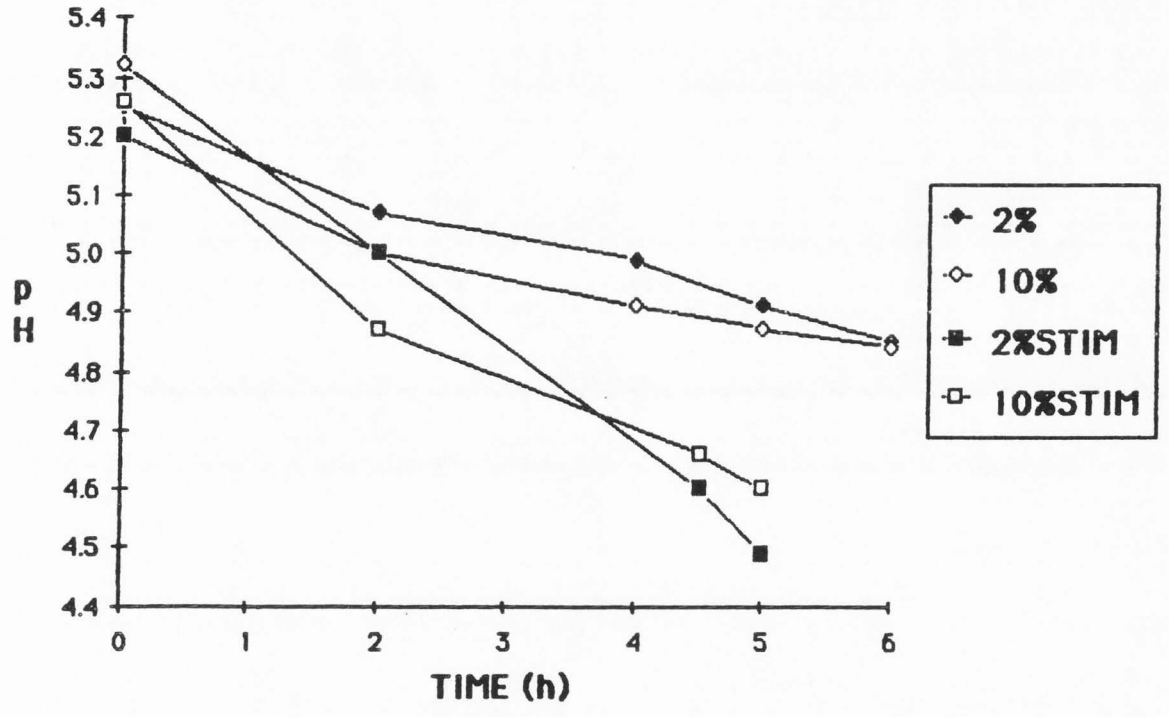

Figure 4. Effect of .4% AYE-Light yeast extract with .1% casein hydrolysate upon the ability of 4% inoculum of lactic strain UC310 Prt- to reduce the pH in directly-acidificed reconstituted nonfat dry milk for cottage cheese at 35°C (max. standard deviation = .028).

SNDM was used as substrate, however, pH below 4.7 was obtained in less than 3.5 h at all inoculum levels: 2, 4, 6, 8, and 10% (Figure 4 - Appendix A Table 8). There was no advantage to using higher inoculum levels because of the already high cell numbers in pH-controlled cultures (14,31). Feasability of using inoculum levels below the lowest examined in this study (2%) should be investigated.

Stimulant Carry-over

Enough yeast extract (.025 and .05 g/ml) was added to pH-control starter medium to provide .1% and .2% final concentrations carried over into the RNDM with 4% inoculum. Such excess concentrations did not inhibit culture performance during bulk starter propagation. Starter culture performance was identical to that observed when bulk culture contained only .4% yeast extract. Carry-over from bulk culture with 4% inoculum stimulated the Prt- culture to reach a cutting pH in 3.5 h (Figure 5) and did not cause noticeable flavor change in the finished product (2,39). There was not a significant difference between the .1 and .2% levels. Thus, it is possible to make cottage cheese with Prt- cultures without adding stimulants directly to the cheese milk.

Stimulant carry-over discovered herein additionally enables sufficient acid production by lactic cultures in 16% solids retentate to reduce its pH to 4.7 within 4-5 h.

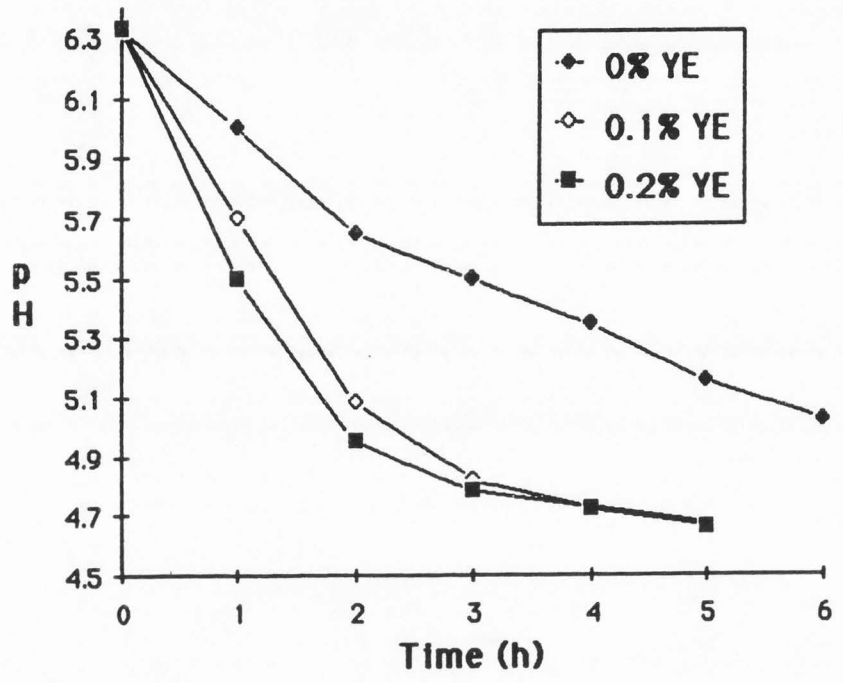

Figure 5. Effect of stimulants carried over from the bulk culture into milk for cottage cheese upon the ability of lactic strain UC310 Prt- to reduce the pH in milk for cottage cheese at 35°C (max. standard deviation = .028).

Studies currently being conducted (Oberg, C. J. unpublished data) indicate .7% inoculum of UC310 Prt- cultures grown in a .1% carry-over starter culture medium is able to reduce the pH in Cheddar cheese manufacture at the same rate as Prt+ cultures at .7% inoculumm.

Curd Firmness

The Vatimer (34) curd tension monitor was initially used to determine correlation between curd firmness and yield (3). This instrument was designed for use on a production scale in commercial vats and not on a laboratory scale. In laboratory trials the instrument shattered the curd regardless of the probe size (1, 2, 4, or 6 inch). The adhesion of curd to the probe (34), the proximity of container walls to the probe edge and the delicate nature of the curd (23), caused shattering of the curd matrix. Such problems prevented use of the Vatimer in measuring curd firmness on small volumes of cottage cheese coagulum.

Curd firmness was measured using a penetrometer device and a Mettler PC440 balance to determine grams of curd firmness at cutting (34). Firmness measurements of curd formed with Prt+ and Prt- cultures were compared at each cutting pH. There were no significant differences in firmness of cottage cheese curd made in wide-mouth bottles with either Prt- or Prt+ cultures, as long as the same pH was reached at cutting, even when yield differences were measured.

Cottage Cheese Manufacture

The manufacturing procedure was designed to simulate standard manufacturing procedures (7) on a small, laboratory scale. Wide-mouth 250 ml bottles were sealed with rubber stoppers to prevent evaporation, contamination and the resulting errors involved. Initially, three cutting pH levels (4.8, 4.7, & 4.6) were tested to determine yield differences. In preliminary studies there were not significant differences ($P>.31$, duplicate trials) between yields at the three cutting pH levels tested. Therefore, curd was cut at pH 4.7 in subsequent trials.

Cell Mass

Cell mass measurements (46) were made to determine if stimulants facilitated growth of the Prt- cultures at a rate comparable to the Prt+ cells. Prt+ and Prt- cell mass measurements are shown in Table 1. Cell mass increases were essentially the same in the Prt+ and Prt- cultures.

Yield

Preliminary experiments were carried out on three strains of S. cremoris; UC73, UC97 and UC310. There were no significant yield differences between the Prt+ and Prt- cultures of UC73 and UC97 (Figure 6). The variants of these strains may in fact be peptidase negative (Pep-) instead of Prt- (30). These two strains were dropped from further study. Strain UC310, however, had significantly more

TABLE 1. Cell Mass on freshly inoculated milk at pH 6.7 (Initial) and on coagulated milk curd at pH 4.7 (Final) measured spectrophotometrically

Strain	Initial[1] LOG CFU[3]	Initial[1] CFU	Final[2] LOG CFU	Final[2] CFU
UC310 Prt+	8.59	3.89×10^8	9.26	1.83×10^9
UC310 Prt-	8.64	4.36×10^8	9.25	1.79×10^9
UC320 Prt+	8.83	6.82×10^8	9.35	2.25×10^9
UC320 Prt-	8.81	6.41×10^8	9.17	1.49×10^9

[1]Readings taken at time of inoculation
[2]Readings taken at time of cutting
[3]Colony Forming Units/ml

nitrogenous matter (P<.001, duplicate trials) lost in the whey with Prt+ cultures compared to the Prt- variant (Figure 6). S. cremoris strain UC320 Prt- improved casein yields over the Prt+ parent culture (15) and was, therefore, used in final cheesemaking runs.

Theoretical CP yield was calculated (Figure 7) (3.597 - .949 = 2.648% for UC310 Prt+, 3.597 - .895 = 2.702% for UC310 Prt-, 3.597 - .957 = 2.640% for UC320 Prt+ and 3.597 - .915 = 2.682% for UC320 Prt-). Theoretical yields for UC310 and UC320 Prt- cultures were .45 + 5.7 X 2.702 = 15.857 kg/100 kg and .45 + 5.7 X 2.682 = 15.743 kg/100 kg respectively (P<.001, Appendix B Table 14). UC310 and UC320 Prt+ strains similarly produced .45 + 5.7 X 2.648 = 15.544 kg/100 kg and .45 + 5.7 X 2.640 = 15.498 kg/100 kg respectively (P<.001, Appendix B Table 14). The difference

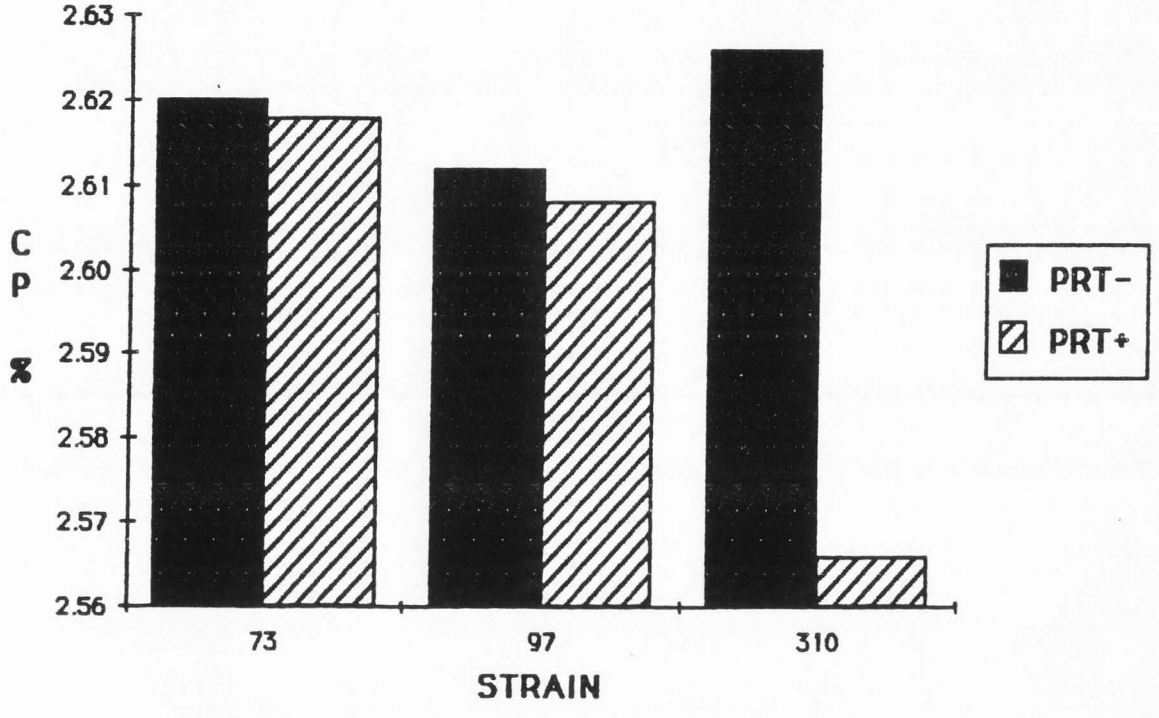

Figure 6. Yield of casein protein (CP) resulting from the activity of Prt+ and Prt- strains of UC73, 97 and 310 in enriched, reconstituted nonfat dry milk for cottage cheese (standard deviation of residuals = .0062).

between Prt+ and Prt- theoretical yields was 15.857 - 15.544 / 15.857 X 100 = 1.974% for UC310 and 15.743 - 15.498 / 15.743 X 100 = 1.556% for UC320. Previous experiments indicated up to 2.26% yield increase with strain UC310 (41). Heap and Richardson (15) found similar results in casein manufacture with UC310 Prt- providing 5.4% higher yield than UC310 Prt+.

Subsequent plasmid mapping has shown the UC320 Prt+ reported herein to have a different profile than the parent strain from the culture bank. It should therefore be designated UC320A Prt+ in further studies.

Another researcher (Tad Butterfield, unpublished data) compared cottage cheese yields with Prt- cultures, Prt+ cultures and direct acidification methods for pH reduction in small vats holding 6 kg of milk. His data show trends that verify results of the study reported herein. Yield differences were not significant due to the large standard deviations incurred (Appendix D - Tables 17-18). Improved accuracy is achieved with the rolling bottle method.

Strains selected for cottage cheese manufacture, as with Cheddar cheese production, can significantly affect total cheese yields (28,35) and casein production (5). Losses increase when cultures in cottage cheese and casein manufacture are used instead of using direct acidification (13,15). Certain culture strains, however, minimize or eliminate such losses (15) and, coupled with the added

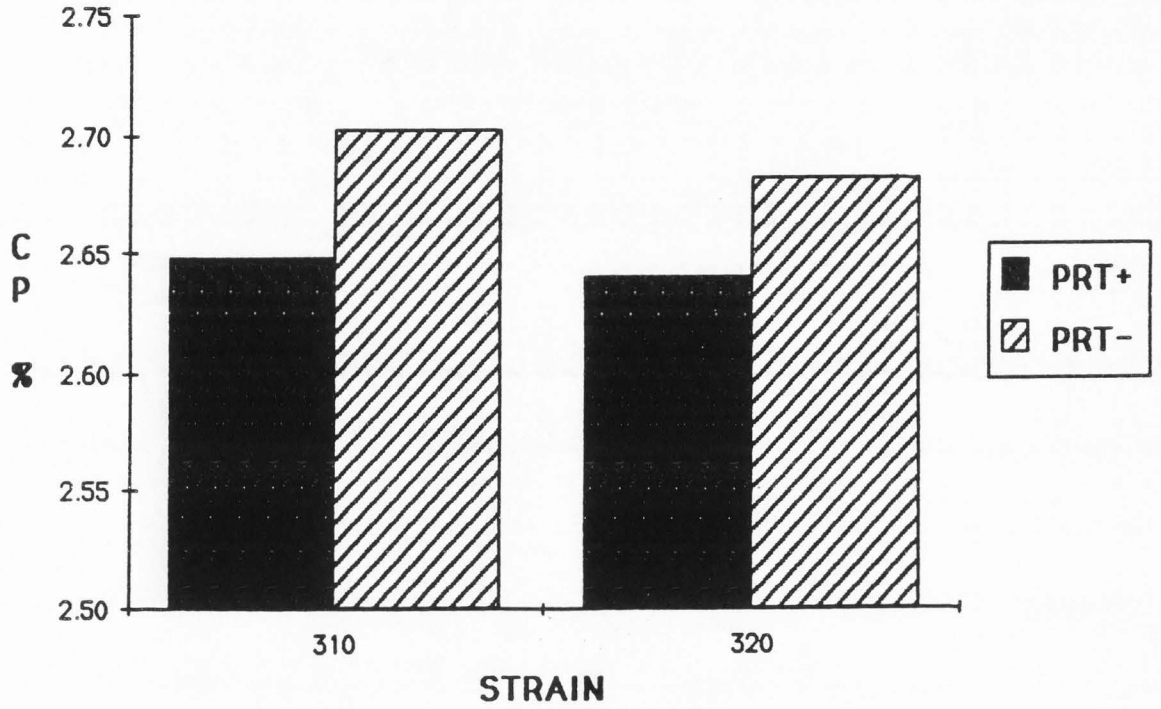

Figure 7. Yield of casein protein (CP) resulting from the activity of Prt+ and Prt- strains of UC310 and 320 in enriched, reconstituted nonfat dry milk for cottage cheese (standard deviation of residuals = .0058).

advantages associated with use of Prt- isolates, make it possible to use cultures in cottage cheese production.

Economic estimates based upon yield increases obtained with use of Prt- culutres indicate that Prt- cultures are economically beneficial. Yield advantages provide up to a $6.22/10,000 lbs ($1.37/1000 kg) cheese milk (Appendix C Table 16). Increased costs of yeast extract are outweighed by the value of cottage cheese yield increases. It is imperative that individual strains and blends be evaluated for potential yield increase prior to use in commercial cottage cheese manufacture.

CONCLUSIONS

This study has shown that Prt- variants of selected strains of S. cremoris can provide increased cottage cheese yields. Increases are determined by a comparison of yields of cottage cheese manufactured with Prt- and Prt+ starter cultures. Direct acidification procedures provide maximum cottage cheese yields, but Prt- cultures can reduce differences between culture and direct acidification manufacturing methods.

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APPENDIXES

Appendix A. Activity Test Data

TABLE 2. Inoculum level, incubation temperature and incubation time influence on culture activity as measured by pH, for UL21A Prt- in 10% RNDM[1]

Inoculum Level	Incubation Temp(°C)	Incubation Time (h) 0	2	4	5	6
2%	32	6.51	6.15	5.74	5.68	5.62
	35	6.51	6.06	5.78	5.71	5.60
	38	6.51	6.36	5.89	5.73	5.62
	41	6.51	6.25	6.17	6.16	6.09
4%	32	6.51	5.98	5.66	5.62	5.57
	35	6.51	5.98	5.66	5.56	5.46
	38	6.51	6.12	5.76	5.59	5.51
	41	6.51	6.21	6.08	5.94	5.95
6%	32	6.58	5.91	5.55	5.48	5.42
	35	6.58	5.84	5.54	5.45	5.37
	38	6.58	6.08	5.73	5.53	5.41
	41	6.58	6.17	5.94	5.85	5.75

[1]Reconstituted Nonfat Dry Milk

TABLE 3. Inoculum level, incubation temperature and incubation time influence on culture activity as measured by pH, for UC73 Prt- in 10% RNDM[1]

Inoculum Level	Incubation Temp(°C)	Incubation Time (h) 0	2	4	5	6
2%	32	6.36	6.02	5.67	5.52	5.44
	35	6.36	5.88	5.57	5.45	5.28
	38	6.36	5.92	5.73	5.70	5.58
	41	6.36	5.96	5.73	5.71	5.53
4%	32	6.33	5.81	5.38	5.30	5.26
	35	6.33	5.68	5.36	5.20	5.02
	38	6.33	5.76	5.54	5.43	5.35
	41	6.33	6.72	6.48	5.44	5.38
6%	32	6.33	5.71	5.27	5.08	5.00
	35	6.33	5.52	5.23	5.10	4.95
	38	6.33	5.65	5.40	5.26	5.22
	41	6.33	5.61	5.43	5.33	5.26

[1]Reconstituted Nonfat Dry Milk

TABLE 4. Inoculum level, incubation temperature and incubation time influence on culture activity as measured by pH, for UC310 Prt- in 10% RNDM[1]

Inoculum Level	Incubation Temp(°C)	Incubation Time (h) 0	2	4	5	6
2%	32	6.37	5.99	5.65	5.51	5.40
	35	6.37	5.94	5.61	5.45	5.40
	38	6.32	5.87	5.68	5.53	5.41
	41	6.32	6.05	5.88	5.81	5.74
4%	32	6.36	5.80	5.45	5.32	5.23
	35	6.36	5.74	5.41	5.30	5.23
	38	6.31	5.84	5.50	5.34	5.25
	41	6.31	5.93	5.74	5.59	5.57
6%	32	6.34	5.68	5.33	5.23	5.15
	35	6.34	5.68	5.33	5.15	5.07
	38	6.29	5.63	5.39	5.18	5.11
	41	6.29	5.87	5.60	5.52	5.35

[1]Reconstituted Nonfat Dry Milk

TABLE 5. Inoculum level, incubation temperature and incubation time influence on culture activity as measured by pH, for UC73 Prt- in 10% RNDM[1]

Inoculum Level	Incubation Temp(°C)	Incubation Time (h) 0	2	4	5	6
6%	35	6.37	5.49	5.11	5.04	4.98
	38	6.37	5.61	5.25	5.25	5.12
8%	35	6.24	5.31	5.07	4.98	4.94
	38	6.24	5.48	5.23	5.09	4.98
10%	35	6.19	5.28	5.03	4.96	4.91
	38	6.19	5.42	5.13	4.94	5.00

[1]Reconstituted Nonfat Dry Milk

TABLE 6. Inoculum level, incubation temperature and incubation time influence on culture activity as measured by pH, for UC310 Prt- in 10% RNDM[1]

Inoculum Level	Incubation Temp(°C)	Incubation Time (h) 0	2	4	5	6
6%	35	6.26	5.61	5.23	5.21	5.07
	38	6.26	5.66	5.28	5.17	5.09
8%	35	6.21	5.52	5.07	5.02	5.01
	38	6.21	5.54	5.16	5.08	5.00
10%	35	6.17	5.46	5.09	5.03	4.98
	38	6.17	5.53	5.23	5.10	5.01

[1]Reconstituted Nonfat Dry Milk

TABLE 7. Inoculum level, incubation temperature and incubation time influence on culture activity as measured by pH, for UC310 Prt- in 10% RNDM[1] pre-acidified with Phosphoric acid

Inoculum Level	Incubation Temp(°C)	Incubation Time (h) 0	2	4	5	6
2%	35	5.25	5.07	4.99	4.91	4.85
4%	35	5.28	5.06	4.94	4.89	4.85
6%	35	5.28	5.04	4.91	4.87	4.83
8%	35	5.30	5.02	4.91	4.86	4.83
10%	35	5.32	5.00	4.91	4.87	4.84

[1]Reconstituted Nonfat Dry Milk

TABLE 8. Inoculum level, incubation temperature and incubation time influence on culture activity as measured by pH, for UC310 Prt- in 10% RNDM[1] enriched with .4% AYE-Light[2] and .1% casein hydrolysate, and pre-acidified with Phosphoric acid

Inoculum Level	Incubation Temp(°C)	Incubation Time (h) 0	2	4.5h	5h
2%	35	5.20	5.00	4.60	4.49
4%	35	5.22	4.95	4.57	4.50
6%	35	5.23	4.92	4.61	4.49
8%	35	5.25	4.88	4.61	4.53
10%	35	5.26	4.87	4.66	4.60

[1]Reconstituted Nonfat Dry Milk
[2]AYE-Light Yeast Extract

TABLE 9. Inoculum level, organic stimulant level and incubation time influence on culture activity as measured by pH, for UC310 Prt- in enriched 10% RNDM[1] incubated at 35°C

Inoculum Level	Milk Stimulant	Incubation Time (h) 0	2	4	5	6
2%	.1% CH[2] & .4% AYE[3]	6.52	5.48	----	4.70	----
	.1% CH	6.70	5.99	5.71	5.10	----
	.1% AYE	6.64	5.65	5.28	4.90	4.76
	.2% AYE	6.58	5.49	5.05	4.63	4.51
	.4% AYE	6.52	5.69	5.02	4.66	----
4%	.1% CH & .4% AYE	6.51	5.44	----	4.66	----
	.1% CH	6.70	5.62	5.19	4.87	----
	.1% AYE	6.63	5.43	5.01	4.78	4.68
	.2% AYE	6.58	5.22	4.80	4.61	4.51
	.4% AYE	6.57	5.33	4.77	4.60	----
6%	.1% CH & .4% AYE	6.50	5.31	----	4.64	----
	.1% CH	6.69	5.50	4.98	4.80	----
	.1% AYE	6.63	5.30	4.93	4.75	4.65
	.2% AYE	6.57	5.11	4.77	4.63	4.53
	.4% AYE	6.50	5.17	4.76	4.61	----

[1]Reconstituted Nonfat Dry Milk
[2]Casein Hydrolysate
[3]AYE-Light Yeast Extract

TABLE 10. Inoculum level, mineral stimulant level and incubation time influence on culture activity as measured by pH, for UC310 Prt- in enriched 10% RNDM[1] incubated at 35°C

Inoculum Level	Milk Stimulant	Incubation Time (h) 0	2	4	5h
2%	.1% Mg[2] & .1% K[3]	6.66	6.28	6.00	5.87
	.1% Mg, .1% K & .01% Zn[4]	6.24	5.89	5.58	5.50
4%	.1% Mg & .1% K	6.66	6.11	5.73	5.71
	.1% Mg, .1% K & .01% Zn	6.26	5.72	5.41	5.18
6%	.1% Mg & .1% K	6.66	6.02	5.60	5.40
	.1% Mg, .1% K & .01% Zn	6.28	5.68	5.31	5.15

[1]Reconstituted Nonfat Dry Milk
[2]Magnesium chloride
[3]Potassium chloride
[4]Zinc chloride

Appendix B. Analysis of Variance Data

TABLE 11. Analysis of variance for Total Nitrogen (calculated as protein) content of cottage cheese whey manufactured with Prt+ and Prt- cultures.

Source	df	Mean Squares	F Stat.	Expected Mean Square
Run	2	.00008623	.715	See
Strain	1	.00344835	1267.776***	Table 15
Variant	1	.04253637	1249.967***	
Strain*Variant	1	.00069650	8.640*	
Run*Strain	2	.00000272	.226	
Run*Variant	2	.00003403	.282	
Run*Strain*Variant	2	.00008061	.669	
Pooled Error	24	.00012057		
Pooled Sample Err.	36	.00005118		
Total	71			

*P < .1
**P < .01
***P < .001

TABLE 12. Analysis of variance for Non-Protein Nitrogen (calculated as protein) content of cottage cheese whey manufactured with Prt+ and Prt- cultures.

Source	df	Mean Squares	F Stat.	Expected Mean Square
Run	2	.00006557	.905	See
Strain	1	.00006841	1.522	Table 15
Variant	1	.11776064	2072.521***	
Strain*Variant	1	.00034212	7.151	
Run*Strain	2	.00004495	.621	
Run*Variant	2	.00005682	.784	
Run*Strain*Variant	2	.00004784	.660	
Pooled Error	24	.00007244		
Pooled Sample Err.	36	.00008830		
Total	71			

*P < .1
**P < .01
***P < .001

TABLE 13. Analysis of variance for Soluble Nitrogen (calculated as protein) content of cottage cheese whey manufactured with Prt+ and Prt- cultures.

Source	df	Mean Squares	F Stat.	Expected Mean Square
Run	2	.00005556	.226	See
Strain	1	.00448812	497.574**	Table 15
Variant	1	.01874687	330.167**	
Strain*Variant	1	.00006233	10.935*	
Run*Strain	2	.00000902	.037	
Run*Variant	2	.00005678	.231	
Run*Strain*Variant	2	.00000570	.023	
Pooled Error	24	.00024627		
Pooled Sample	36	.00011554		
Total	71	.00047354		

*P < .1
**P < .01
***P < .001

TABLE 14. Analysis of variance for Casein Nitrogen (calculated as protein) content of cottage cheese whey manufactured with Prt+ and Prt- cultures.

Source	df	Mean Squares	F Stat.	Expected Mean Square
Run	2	.00008627	.715	See
Strain	1	.00344823	126.680**	Table 15
Variant	1	.04253715	1249.256***	
Strain*Variant	1	.00069658	8.644*	
Run*Strain	2	.00002722	.226	
Run*Variant	2	.00003405	.282	
Run*Strain*Variant	2	.00008059	.668	
Pooled Error	24	.00012058		
Pooled Sample Err.	36	.00005113		
Total	71			

*P < .1
**P < .01
***P < .001

TABLE 15. Expected mean square equations for analysis of variance on cottage cheese whey analysis.

Source	Expected Mean Square
Run	$\sigma^2_D + d\sigma^2_E + svnd\sigma^2_R$
Strain	$\sigma^2_D + d\sigma^2_E + vnd\sigma^2_{RS} + rvndK^2_S$
Variant	$\sigma^2_D + d\sigma^2_E + snd\sigma^2_{RV} + rsndK^2_V$
Strain*Variant	$\sigma^2_D + d\sigma^2_E + nd\sigma^2_{RSV} + rndK^2_{SV}$
Run*Strain	$\sigma^2_D + d\sigma^2_E + vnd\sigma^2_{RS}$
Run*Variant	$\sigma^2_D + d\sigma^2_E + snd\sigma^2_{RV}$
Run*Strain*Variant	$\sigma^2_D + d\sigma^2_E + nd\sigma^2_{RSV}$
Pooled Error	$\sigma^2_D + d\sigma^2_E$
Pooled Sample Err.	σ^2_D

Appendix C. Economic Estimates

TABLE 16. Econonmic estimations of increased profitability of cottage cheese manufacture using Prt- variants of two strains of Streptococcus cremoris.

	UC310		UC320	
	Prt+	Prt-	Prt+	Prt-
Theoretical Yield (kg/100 kg milk)	15.544	15.857	15.498	15.743
Dry Curd (kg/1000 kg milk)	155.44	158.57	154.98	157.43
Finished Product (dry curd/.60)	259.07	264.28	258.30	262.38
Retail Sales Value[1]	$117.75	$120.12	$117.40	$119.25
Retail Price Difference	$2.37		$1.85	
Added Yeast Extract (%)	.2	.1	.2	.1
Added Yeast Extract (kg/1000 kg milk)	2	1	2	1
Yeast Extract Cost ($1.00/kg)	$2.00	$1.00	$2.00	$1.00
Extra Profit of Prt- Cottage Cheese/1000 kg milk (retail price diff. - yeast ext. cost)	$.37	$1.37	-$.15	$.85

[1] $.4545/kg cottage cheese ($1.00/lb)

Appendix D. Protein Analysis of Cottage Cheese Milk and Whey from Laboratory-scale Cheese Vats

TABLE 17. Fractional protein analysis of cottage cheese milk and whey obtained from trial manufacturing runs using direct acidification (D.A.), UC310 proteinase positive cultures (Prt+) or UC310 proteinase negative cultures (Prt-) for acid production in laboratory-scale vats using 6 kg of cheese milk (Tad Butterfield, unpublished data)

Method of Acidification	Protein Type	Reps	Mean	LSD	Standard Deviation	C.V. (%)
Prt+	CP[1]	5	2.802	A[4]	.104	3.7
	SP[2]	5	.772	A	.044	5.7
	NPN[3]	5	.250	A	.015	6.0
Prt-	CP	5	2.835	A	.077	2.7
	SP	5	.738	A	.072	9.8
	NPN	5	.239	A	.028	11.7
D.A.	CP	5	2.872	A	.097	3.4
	SP	5	.701	A	.021	3.0
	NPN	5	.139	B	.012	8.6

[1]Casein protein nitrogen expressed as % protein
[2]Soluble protein nitrogen expressed as % protein
[3]Nonprotein nitrogen expressed as % protein
[4]Protein types with same letter in LSD column are not significantly different

TABLE 18. Fractional protein analysis of cottage cheese milk and whey obtained from simulated manufacturing runs using UC310 proteinase positive cultures (Prt+) or UC310 proteinase negative cultures (Prt-) for acid production in 100 ml of cheese milk

Method of Acidification	Protein Type	Reps	Mean	LSD	Standard Deviation	C.V. (%)
Prt+	CP[1]	9	2.648	A[4]	.008	0.3
	SP[2]	9	.483	A	.014	2.9
	NPN[3]	9	.466	A	.013	2.8
Prt-	CP	9	2.703	B	.012	0.4
	SP	9	.514	B	.015	2.9
	NPN	9	.381	B	.007	1.8

[1]Casein protein nitrogen expressed as % protein
[2]Soluble protein nitrogen expressed as % protein
[3]Nonprotein nitrogen expressed as % protein
[4]Protein types with same letter in LSD column are not significantly different