

EVALUATION OF A BACTERIAL STIMULANT FOR LACTIC STARTER
CULTURES IN COTTAGE CHEESE MANUFACTURE

by

DERALD DEAN VINCENT

B. S., Kansas State University, 1961

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

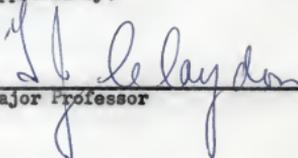
MASTER OF SCIENCE

Department of Dairy Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

Approved by:


Major Professor

LD
2668
TH
1963
V76
C.2

11

TABLE OF CONTENTS

Document

INTRODUCTION.....	1
LITERATURE REVIEW.....	2
Some Factors Affecting Activity of Lactic Starter Cultures.....	3
Cottage Cheese Manufacturing Methods.....	4
Factors Affecting Cottage Cheese Curd Characteristics.....	5
Use of Reconstituted Nonfat Milk Solids.....	11
Recent Approaches to Cottage Cheese Manufacture.....	13
EXPERIMENTAL PROCEDURES.....	14
Bacteriological Methods.....	14
Preparation of Pseudomonas Proteolysate from the Pseudomonas Culture.....	15
Preliminary Testing for Stimulation.....	17
Procedures for Manufacturing Cottage Cheese on a Laboratory Scale.....	17
Procedures for Manufacturing Cottage Cheese on a Pilot Scale....	20
Quality Tests.....	25
RESULTS AND DISCUSSION.....	31
Effect on Lactic Starter Activity of Adding Pseudomonas Proteolysate.....	31
Effect of Heating the Pseudomonas Proteolysate on Its Coagulatory and Stimulatory Activity.....	34
Effect of Pseudomonas Proteolysate on Processing Cottage Cheese on a Laboratory Scale.....	35
Effect of Pseudomonas Proteolysate on Cottage Cheese Manufacture on a Pilot Scale.....	37
SUMMARY AND CONCLUSIONS.....	54
ACKNOWLEDGMENTS.....	58
LITERATURE CITED.....	59

INTRODUCTION

The large increase in cottage cheese production during the past few years has stimulated an interest in methods of accelerating manufacturing procedures and improving the quality of this product. Also, there has been a search for rennet substitutes which will produce a more standardized, uniform skimmilk coagulation.

A significant decrease in manufacturing time without a decrease in quality would be of economic value. Reduction in overall manufacturing time might be achieved by a shorter ripening time, shorter cooking period, or faster washing and draining operations. Using a fast acid-producing culture, the normal time for making "short set" cottage cheese is about 7 to 8 hr. Numerous variations encountered in making cottage cheese makes it impossible, even for experienced operators, to always follow a consistent pattern of procedures without modifications. The operator must rely on skill and experience to control these variations and achieve the desired results.

During the ripening period of making cottage cheese acid development occurs. Since the ripening period accounts for over 50% of the total manufacturing time, it would be the most logical area in which to achieve a reduction in time. Normal cottage cheese manufacturing is dependent on the acid produced by the lactic starter culture. Frequently, a slow acid-producing lactic culture will disrupt the entire production schedule. Many times this results in a low quality product. It is known that 80 to 90% of the problems in making cottage cheese can be traced to the lactic starter culture.

If slow lactic starter cultures could be stimulated to faster and more

uniform acid production, it would accomplish a reduction in ripening time and greater uniformity in the finished product. Although substances have been tested which will stimulate lactic starter organisms or similar organisms (18, 25, 27, 39, 40), they have not received much application in commercial cottage cheese manufacture. Many of these stimulants produce undesirable reactions in milk. This alone prevents their use. Also, the lactic starter organisms vary in their response to such materials.

Previous work by Koburger and Claydon (27) showed that acid production by starter organisms could be significantly stimulated by a filtrate obtained from a proteolyzed milk culture of Pseudomonas fluorescens. This material was found to contain coagulative and possibly stimulatory enzyme(s), as well as peptides and smaller protein fractions. At 70 F and 14 to 16 hr of incubation, acid production was increased 36% by addition of 1% stimulatory filtrate.

The work reported in this manuscript was carried out to evaluate the practical aspects of the bacterial stimulant from Ps. fluorescens in cottage cheese manufacture. Rate of acid production, ripening, cooking and draining time, and effect on curd properties and flavor were the main areas of investigation. Some of the possibilities and limitations of the stimulant as a milk coagulator for cottage cheese were also studied.

LITERATURE REVIEW

In milk, the rate of acid production by lactic starter cultures may be influenced by a variety of factors. Among these are heat treatments applied to the milk, the milk composition, particularly with respect to protein fractions, and the addition of substances that stimulate or inhibit

the culture organisms. Keogh (24) observed variations in acid production among different starters from day to day. The cultures he tested also varied in their sensitivity to stimulatory and inhibitory factors in the milk.

Some Factors Affecting Activity of Lactic Starter Cultures

Much of the information relating to heat treatment, milk composition, and addition of stimulatory or inhibitory substances which influence the activity of the lactic starter cultures, has been reviewed by Koburger (26).

Anderson et al. (3) found that the ability of whole milk from healthy cows to promote growth of lactic streptococci depended on its content of peptide fractions. However, they noted that high protein milk did not necessarily promote better growth than low protein milk. In most cases, different cultures of lactic streptococci showed differences in response to the peptides present.

Garvie and Mabbitt (18) concluded that milk contains the necessary substances for rapid acid production, but these substances are not always available for immediate use. This applies mainly to the protein. It has been shown by several workers (18, 39, 41) that useable, degraded proteins, mainly peptides and smaller fractions, would stimulate acid production of lactic streptococci when added to milk.

Koburger and Claydon (27) used a stimulatory preparation from a proteolysed milk culture of Ps. fluorescens to stimulate acid production by lactic starter cultures. In 14 hr incubation at 70 F an unfractionated Seitz filtrate, an acetone-ethanol precipitate, and an amino acid fraction containing 17 amino acids, showed average increases in titratable acidity over the control of 36, 28, and 13%, respectively. The amino acids and

unknown material were responsible for the stimulation. A loss in stimulation from autoclaving the precipitate suggested the presence of an enzyme or some other heat-labile material.

Speck and Williamson (42) concluded that stimulatory peptides added to the milk eliminated the necessity for the organisms to hydrolyze the milk protein before initial growth could occur. Slow acid-producing cultures, which possess less ability to hydrolyze nitrogen compounds, showed more stimulation than fast acid-producing cultures. Garvie and Mabbitt (18) also observed more stimulation in slow cultures than fast cultures from addition of peptides, but noted that peptones produced no effect with either type culture. Stimulation may be effective only in the initial stage of acid development. Anderson and Elliker (2) found no differences in titratable acidity between stimulated fast cultures and stimulated slow cultures in 24 to 36 hr incubation periods, using 1 drop of lactic starter in 5 ml of milk and incubating at 70 F.

Cottage Cheese Manufacturing Methods

Cottage cheese is made from fresh skimmilk, fortified skimmilk, wholly reconstituted skimmilk, or concentrated skimmilk. The curd may be small or large in the dry form or creamed form. Although the main steps and procedures are well standardized (20, 46), almost every manufacturer and research worker adopts modifications in making cottage cheese and handling the problems involved (9, 19, 34, 35, 37, 38). No one specific method can be used invariably due to differences in milk and other manufacturing conditions.

Factors Affecting Cottage Cheese Curd Characteristics

Physical characteristics of cottage cheese curd are of vital importance in consumer acceptance. Curd may vary from soft and mushy to hard and rubbery. Acceptable curd must be tender, yet sufficiently firm to withstand handling and packaging operations. It also must contain the optimum amount of moisture. Curd characteristics also govern the yield and losses sustained during manufacturing. Accordingly, the many factors that influence the nature of the curd have received considerable attention by manufacturers and research workers.

Pasteurization. Skimmilk for cottage cheese making is subjected to pasteurization to kill the majority of the microorganisms and prolong the keeping quality. Pasteurization of the skimmilk will influence the amount of calcium chloride and rennet that must be added to the milk to obtain proper curd firmness. Several workers (13, 45) have found that low-temperature, long-time pasteurization usually gives the most desirable results, but high-temperature, short-time pasteurization can be used successfully.

Calcium Chloride. Calcium chloride is sometimes added to skimmilk to assist in firm setting of the milk and firming of the curd during cooking. If the milk has a low calcium level, the addition of 0.02% calcium chloride, which is the maximum level allowed, is definitely recommended. Dill and Roberts (13) observed that more calcium chloride was required to reach maximum curd tension when the heat treatment on the skimmilk was increased. They concluded that this was due to partial precipitation of the calcium chloride salts during the heat treatment. They also found that further decreases in curd firmness may be caused by (a) pancreatic enzymes, (b) addition of sodium salts, (c) homogenization, and (d) dilution of milk

with water.

Emmons et al. (17) found that 0.02% calcium chloride was not beneficial in cottage cheese making when using "high heat" powder, "low heat" powder, or excessively heated skim milk.

Coagulating Agents. Coagulating agents are used in cottage cheese making to produce a firmer curd. This cuts down on curd losses, allows more vigorous handling of the curd, and gives a firmer texture to the finished product. The most commonly used coagulant for cottage cheese making is rennet. However, other coagulating preparations are frequently used. Hales (20) recommends the use of commercial coagulators with, or in place of, rennet for a more standardized and uniform coagulation from vat to vat. These coagulators vary in composition but usually contain pepsin, rennin, or both, plus calcium salts.

Koburger and Claydon (27) found that the *Pseudomonas proteolytica* obtained from a *Ps. fluorescens* milk culture would coagulate milk. This was attributed to a proteolytic enzyme. If this proteolytic enzyme was used in cottage cheese making, it would be expected to increase the rate of coagulation and possibly modify curd characteristics.

Almost any proteolytic enzyme will coagulate milk, but most of these if applied to cottage cheese making would produce some undesirable characteristic(s) in the curd. Many of these enzymes produce a bitter or unclean flavor. Some are so highly proteolytic that their use would cause a loss in yield and a deleterious effect on the curd firmness. Most proteolytic enzymes have an optimum pH and temperature for maximum reaction. Bahadur and Kumari (7, 8) obtained several fractions from alcohol precipitation and acetone precipitation of commercial papain. All fractions had milk clotting

properties, but each fraction had an optimum temperature and a definite reaction time. In most cases, a bitter flavor was produced when these fractions were added to milk.

Starter. Due to variations in milk composition, Horrall et al. (23) used nonfat milk solids (NMS) to carry lactic starter cultures. Even then they found that it was necessary to check each batch of powder for support of starter activity. Using 2 to 10% starter, Price (34) found that 90 F was the optimum temperature for acid production.

In the "short-set" method of making cottage cheese, 5% starter is the normal amount used. Several investigators (20, 46) have found that using over 5% starter markedly reduces the strength of the curd and wastes starter. Other workers (22, 35) have observed that the rate of acid production, the proteolytic activity, and the particular strain of the starter may affect the curd firmness.

Stage of Cutting the Curd. After the milk has coagulated it is cut into cubes to permit expulsion of whey and contraction of the curd. The stage at which the curd is cut influences its subsequent characteristics. One, two, or a combination of three methods is usually used to determine the proper time for cutting the curd. Hales (20) lists these three methods as pH of the whey, titratable acidity of the whey, and the acid coagulation test (A-C Test). To perform the acid coagulation test, a small amount of the cheese milk, with all additives present, is placed in a container and held in the vat milk. When whey appears from breaks in the curd in the container, the vat milk is ready to be cut. Hales recommends this test to adjust for varying solids content in the milk. Price et al. (35) also recommend the acid-coagulation test for best results. Reid and Maughan (37)

in their work, used the curd splitting technique to determine the proper cutting point. The criteria used will vary considerably among research workers and manufacturers.

When titratable acidity of the whey is used as a measure of cutting time, Price et al. (35) recommend that the whey be drawn from 3 to 6 inches below the surface of the curd and centrifuged before titrating. VanSlyke and Price (46) found that surface whey had a lower titratable acidity than whey separated from centrifuged curd. Among various workers (35, 43, 46), each recommend a different titratable acidity for the proper cutting point. This will depend on the solids content of the milk, the size of the curd being made, and possibly other factors. For 11% solids, the proper whey acidity for cutting will range from 0.48 to 0.56%. The proper pH for cutting is about 4.7.

Price et al. (35) observed that cutting time affects the yield, composition, uniformity of the curd particles, the per cent solids recovered, and the quality of the cottage cheese.

Cooking. The purpose of cooking is to expel the whey from the curd, and to control the starter organisms and any spoilage organisms which might be present. Angevine (4) and Collins (10) recommend cooking temperatures of 120 F and 130 F for 15 to 20 min at pH 4.6 for controlling spoilage and starter organisms.

Hales (20) describes four methods which can be used for cooking the curd. The most common method is raising the temperature of the whey and curd by a heated water jacket. Cooking is stopped when the curd reaches a meaty, tender consistency and all of the interior whey is expelled. If the curd is too soft after draining, VanSlyke and Price (46) recommend

allowing the curd to remain on the warm surface of the vat until it becomes sufficiently firm.

Lundstedt (30) describes an apparatus used to determine the proper firmness for cooked curd. This apparatus measures the curd firmness on a scale graduated in 1/4 inch divisions and numbered from 0 to 26. Each division is converted to deg. on the calibrated stem. Each curd characteristic such as rubbery, fragile, etc., is assigned a value. Emmons and Price (14) also describe an apparatus which can be used for the same purpose, but primarily was designed for testing finished curd.

Effect of Washing and Draining the Curd. The purpose of washing is to sweeten, shrink, firm and cool the curd. The temperature and number of wash waters recommended varies among workers (5, 20, 46). If three wash waters are used, the temperature of the first, second, and third water is usually about 80, 65, and 40 F, respectively.

Various investigators (5, 45, 46) also recommend different degrees of chlorination and acidification of the wash water. Generally, the wash water is acidified to pH 4.8 to 5.0 and chlorinated to contain 5 ppm available chlorine. This prevents contamination of the cheese with poor bacterial quality water and prevents an increase in curd pH. After washing, the curd is allowed to drain. VanSlyke and Price (46) recommend at least 1 hr for draining.

The firmness of the finished curd affects the palatability of the cottage cheese. As mentioned above, Emmons and Price (14) have devised a modification of the Cherry-Burrell curd tension meter which they used to determine the toughness or firmness of the drained curd. The values obtained from this tester are correlated with the curd characteristics.

Creaming the Curd. Creaming the curd produces a more tender, palatable product, and provides the $\frac{1}{4}$ % legal fat content required in creamed cottage cheese. Angevine (5) recommends that the cream dressing be fortified with NMS, pasteurized at 165 F for 30 min, with salt included, then homogenized at 1500 to 2000 lb pressure. VanSlyke and Price (46) found that the curd should be in contact with the dressing for at least 16 hr to obtain maximum absorption.

Emmons and Price (15) found that cream retention by the curd was due to (a) absorption of serum from the dressing, and (b) physical clinging of the cream to the outside of the curd. In general, any factor which decreases the curd firmness will increase the amount of cream retained. These investigators measured cream retention by placing creamed curd in a screened-bottom funnel, then weighing the free cream dressing drained from the curd to determine the amount of cream dressing retained.

Yield. Curd characteristics throughout the manufacturing procedure directly affect the yield. Hales (20) found that the yield of cottage cheese varies with the casein content of the skimmilk, the moisture content, and the solids lost in the whey. Cordes (11) listed three ways to state yield. These are: (a) lb of curd per 100 lb of skimmilk, (b) lb of curd per lb of solids in the skimmilk, and (c) lb of curd per lb of casein. A desirable yield for 9% solids milk is about $\frac{1}{4}$ to 16 lb of curd per 100 lb of skimmilk.

Storage and Keeping Quality. The curd properties and characteristics will be greatly altered if the curd is not properly stored. Storage at low temperature will help prevent spoilage by microorganisms and extend the shelf-life. Several investigators (4, 45, 46) found that curd stored at 30 to 40 F had a keeping quality of 7 to $\frac{1}{4}$ days.

Use of Reconstituted Nonfat Milk Solids

Use of Skimmilk Fortified with Extra NMS. The use of NMS for fortifying skimmilk has greatly increased during the past few years. The cottage cheese industry has found that extra solids in the skimmilk will give a better yield and a more desirable curd. Cordes (11) observed that in fortifying, 11% solids is maximum for good curd characteristics and a profitable return from extra overhead. Such practice necessitates modification in procedures.

The proper pH and titratable acidity for cutting will vary with the per cent solids in the skimmilk. It was recommended to Wilkowske (48) through personal communication that the final reading for proper cutting acidity be increased by 0.05% for each per cent solids over 9%. The American Dry Milk Institute (1) recommends developing 0.34 to 0.36% acidity above the initial titratable acidity of the milk and cutting at this point. Other investigators (43, 47) have recommended different titratable acidities for cutting curd of different solids content. Reid and Maughan (37) were fairly successful in using 20% solids and cutting at whey acidities ranging from 0.60 to 0.90%. However, the "curd split" criteria was used to determine the proper cutting point.

Use of NMS Entirely. Whitaker (47) and Remaley (38) point out that reconstituted skimmilk shows less day to day variation than fresh skimmilk. Since NMS are readily available, produce less variation, and give higher yields, they are often reconstituted and used for cottage cheese making.

Before NMS are used, tests should be made to determine if they possess desirable properties for cottage cheese manufacture. The Harland-Ashworth test for whey protein nitrogen (33, 44, 47) plus rennet-hydrochloric acid curd tension tests are used for this purpose. These tests are used to

separate "low heat" powder from "high heat" powder, the latter being undesirable for cottage cheese making. Several investigators (38, 47) have used whey protein nitrogen values ranging from 6.5 to 7.0 mg to indicate "low heat" powder. However, some workers (33, 36, 44) have concluded that the whey protein nitrogen and supplementary tests do not predict the curd-making quality of the powder.

The amount of rennet required to set reconstituted skimmilk may be different from that used in setting fresh skimmilk. Several workers (28, 38, 43) used 1 ml of rennet per 1000 lb of skimmilk. Stone et al. (44) used 2.5 ml of rennet for the same amount of skimmilk. In early work, Reid and Maughan (37) used 1.2 ml of rennet per 100 lb for 20% solids reconstituted skimmilk.

Observations by Emmons et al. (16) showed that the amount of whey expelled by the curd made from reconstituted skimmilk decreased with decrease in pH. Randolph and Kristoffersen (36) found that cutting the curd at lower than normal whey acidity resulted in a shorter cooking time. Kosikowski (28) noted that surface water is held more tenaciously by curd made from wholly reconstituted skimmilk than curd made from fresh skimmilk, resulting many times in a pasty curd.

The yield is usually different when using wholly reconstituted NMS. Stiles (43) considered 17 to 18 lb of curd per 100 lb of skimmilk as a good yield when using 11% solids skimmilk. However, Tuckey (45) considers 23 lb of cottage cheese for the same amount of 11% solids skimmilk as a good yield.

Recent Approaches to Cottage Cheese Manufacture

Although the general method of cottage cheese making has changed little in many years, there has been more recent interest in new approaches to lactic starter stimulation and milk coagulators.

Speck and Ledford (40) successfully utilized pancreas extract for stimulation of lactic starter organisms in cottage cheese manufacture. At 90 F, using 5% lactic starter and 0.06% added pancreas extract, the time saved in ripening, cooking, and consequently overall was 17.7, 9.1, and 16.6%, respectively. The time saved depended on the amount of extract added. A level of 0.06% extract produced the best overall results. The curd had to be cut at lower than normal acidity to prevent a tough curd when cooked. With high concentrations of pancreas extract, the curd was sufficiently firm at the start of cooking; consequently, the cooking period necessary was too short to properly kill the microorganisms present. The flavor and texture of the experimental cheese was comparable to that of the control cheese, according to the authors.

Hammond and Deane (21) made cottage cheese curd by using an acidogenic agent (an isomer of lactic acid lactide or D-glucone-delta-lactone) to lower the pH of the skimmilk to the desired point. No starter was used, but rennet and calcium chloride were added as desired. Deane and Hammond (12) have also made cottage cheese by using D-glucone-delta-lactone and meso-lactide. These compounds hydrolyze to produce a pH of 4.6 when added to warm skimmilk. With addition of rennet, coagulation was faster, so the curd was cut earlier with acid being developed during cooking. The report indicated that the curd had a bland flavor and a good appearance.

McNurlin and Ernstrom (31) reported suitable formation of curd from

adding concentrated lactic or hydrochloric acid to 40 F skim milk, and then warming by electric heating to 70 to 80 F without agitation. For improved body, 1 ml of rennet per 1000 lb of skim milk was added. Tests showed that the curd firmness increased with decrease in pH and with increased setting temperature and time.

EXPERIMENTAL PROCEDURES

Since other work (26) demonstrated that preparations from milk cultures of Ps. fluorescens at a 1% level would stimulate lactic starter organisms at 70 F, experimental procedures were primarily limited to evaluating this material in cottage cheese making. Test tube stimulation tests using 5% lactic starter at 90 F were carried out on a limited scale. To control the coagulation properties of the pseudomonas proteolysate, preparatory procedures involved certain heat treatments in an attempt to standardize the coagulative enzyme(s).

Bacteriological Methods

Media. Reconstituted skim milk was used as the substrate for the Ps. fluorescens milk cultures; for carrying the lactic starter cultures; and also for test tube stimulation tests. Each batch of NMS was pretested for inhibitory substances by the penicillin disc method (6). The method described by Kuramoto et al. (29) was used to determine the whey protein nitrogen of each batch of NMS. The NMS were reconstituted to 9% solids with distilled water and dispensed into one-l Erlenmeyer flasks in 250 ml quantities for the pseudomonas milk cultures, and into six-oz screw-cap bottles in 100 ml quantities for the lactic starter cultures. These preparations were autoclaved

at 15 lb pressure for 16 min.

Lactic Starter Cultures. The six cultures used in the experiment were dual-purpose commercial cultures. They were transferred twice weekly in reconstituted skim milk and incubated at 72 F for 16 hr, then held in the refrigerator. Unless it was desired to test stimulation on old lactic starter cultures, a fresh transfer of starter was made the night before an experiment and incubated at 72 F for 16 hr.

Preparation of Pseudomonas Milk Cultures. The stock cultures of Ps. fluorescens were carried in litmus milk and transferred weekly at 78 F, then held in the refrigerator. For production of the proteolysate, 250 ml of sterile reconstituted skim milk, prepared as previously described, was inoculated with the stock culture and incubated for 10 to 12 days at 78 F. Each day the contents of the flask were thoroughly agitated. At the end of the incubation period, the milk appeared to be almost completely proteolyzed.

Preparation of Pseudomonas Proteolysate from the Pseudomonas Culture

Koberger (26) showed that fractionation of the pseudomonas proteolysate resulted in a loss of active components in the supernatant. Seitz filtration of the proteolyzed milk eliminated this problem and gave a sterile product, but it was too slow and laborious to yield large quantities needed for cottage cheese making. For these reasons, and since a sterile product was necessary, the following alcohol treatment was devised. This method allowed the proteolysate to be prepared in relatively large quantities.

Alcohol Treatment. In early trials, a 25% alcohol-acetone mixture,

containing 18% ethanol and 7% acetone, was used to fractionate and sterilize the proteolysate. It was found that the acetone caused the proteolysate to produce an unclean, bitter flavor when added to skimmilk. However, using ethanol alone eliminated this problem. At the 25% level, the ethanol sterilized the culture material and caused little precipitation or other loss in stimulatory activity.

After incubation, the pseudomonas milk culture was mixed with 25% ethanol then centrifuged at 600 G for 25 min. The supernatant was decanted from the small amount of precipitate into a sterile one-l flask and placed in a 120 F water bath under aspirator vacuum to remove most of the alcohol. A small amount of sterile, commercial anti-foaming agent was added to prevent excess foaming. After most of the alcohol had been removed, as indicated by odor, the remaining material was transferred aseptically to sterile bottles and refrigerated at 32 F.

To test the alcohol treated proteolysate for sterility and proteolytic activity, a small amount was placed on sterile litmus milk agar, then incubated at 78 F and observed for 2 days.

Freeze Drying. Lypholizing the alcohol-treated proteolysate and storing it in moisture free containers reduced the problems of growth from any bacterial contaminant. It also provided a concentrated preparation. However, the lypholized material produced an objectional cheesy flavor when added to milk and hence, was unsuitable for use in making cottage cheese. Also, the lypholizing procedure required a considerable amount of time.

Preliminary Testing for Stimulation

To determine the activity of each stimulatory preparation, test tube titration techniques similar to those described by Koburger (26) were used. Reconstituted skim milk of 9% solids was dispensed in 100 ml quantities into screw-cap bottles. After adjusting to 90 F, each lot was inoculated with 5% starter and 1% of the proteolysate being tested unless otherwise indicated. Control lots contained only the starter. After mixing the prepared samples, 9 ml quantities from each sample were dispensed into six screw-cap tubes, providing duplicate samples for three incubation periods. The tubes were placed in a 90 F water bath. At 3, 4, and sometimes 5 hr intervals, titratable acidities were measured by direct titration in the tubes with 0.1 N sodium hydroxide and phenolphthalein indicator. The coagulation time was recorded when the milk reached a soft gel consistency.

Procedures for Manufacturing Cottage Cheese on a Laboratory Scale

In order to obtain preliminary information on the effect of the proteolysate on cottage cheese, trials were made on a miniature scale in the laboratory. Plate I shows the equipment used in making these small lots of cottage cheese. The equipment consisted of three small stainless steel vats, each with a capacity of approximately 2 gal. The vats were placed in a thermally controlled water bath.

High-temperature, short-time pasteurized skim milk from the University creamery, freshly reconstituted skim milk, or a mixture of the two was used in all trials. Two l of milk were added to each vat and prewarmed to 90 F. After prewarming, the desired amount of starter, rennet, and proteolysate was

PLATE I



added to the proper vat and thoroughly mixed. Since rennet was required in very small amounts, it was diluted in water to obtain the desired concentration. Each vat contained an aluminum cup filled with milk from the vat to be used for subsequent testing of curd tension.

The desired cutting point was determined by pH, whey acidity, curd tension, or a combination of these criteria. The curd was cut by using a modified curd knife made of a 7 inch by 7 1/2 inch steel frame with fine wire divisions at 1/4 inch intervals.

Cooking the curd was accomplished by raising the temperature of the external water bath. If the curd in different vats was ready for cooking at different times, the vats were removed and heated in separate water baths. The temperature of the whey was raised slowly to 110 F. It was then rapidly increased to 120 F and maintained at this temperature until the curd developed a springy consistency and was free of whey pockets.

For washing the curd, water was added directly to the vats. In most cases, three wash waters at 85, 65, and 40 F, respectively, were used. After the curd was washed and cooled, it was placed in a large strainer to drain. The finished curd was placed in jars and held at 40 F. In these trials, general observations were made on cutting conditions, cooking efficiency, body and texture, and flavor of the finished curd.

Procedures for Manufacturing Cottage Cheese on a Pilot Scale

Following laboratory scale preparations of cottage cheese, production was carried out on a pilot scale that closely resembled commercial conditions. Plate II shows the equipment used in making cottage cheese on the pilot scale. The four stainless steel vats each had approximately a 16 gal capacity

PLATE II



and were equipped with separately regulated steam and water supplies for heating and cooling.

Fresh, grade A skimmilk from the University Creamery was fortified to 11% solids with "low heat" NMS. It was pasteurized at 165 F for 15 sec, then cooled to 40 F. This milk was placed in carefully sanitized milk cans and held overnight at 40 F. When wholly reconstituted skimmilk was used, 5 gal of skimmilk was prepared by thoroughly mixing 4.7 lb of "low heat" NMS with 38.3 lb of 90 F tap water. In each trial two such lots were prepared, one for the control vat and one for the experimental vat. Since the NMS and the water used for reconstituting had very low bacterial counts, and minimum heat treatments were desired, the reconstituted skimmilk was not pasteurized.

Before rennet or starter was added the milk was adjusted to 90 F. This temperature was maintained until the curd was ready to be cut.

Adding Starter and Rennet. Except where otherwise noted, 5% fresh lactic starter was added to the milk, then mixed thoroughly. One of six lactic starters (A, B, C, D, E, or F) was used in each trial. The rennet solution was freshly prepared for each trial. Since only small amounts were needed for the control lot, the rennet was diluted 1:99 with water. The amount of rennet added ranged from 5.0 to 6.5 ml from this dilution for the 5 gal of skimmilk.

Adding Pseudomonas Proteolysate and Hydrochloric Acid. The proteolysate was added at the rate of 1% of the volume of skimmilk to each experimental vat immediately after adding the starter. One series of trials involved the addition of hydrochloric acid to the experimental vats. Enough 5% hydrochloric acid solution was added to the cold (50 F) skimmilk to lower the pH

to 5.8 to 5.9 before the starter and proteolysate were added. To prevent precipitation of some of the milk constituents, constant stirring was necessary while adding the hydrochloric acid solution. In all trials, the vats were covered to help maintain the milk at a constant temperature during the ripening period.

Cutting the Curd. The desired cutting point was based on pH, whey acidity, curd break, and curd tension, either singly or in combination. The criteria used depended mainly on the coagulatory activity of the proteolytic enzyme(s) in the proteolysate. Curd break and whey acidity were often used either separately or together. The pH of the curd was determined on a Beckman glass electrode pH meter, and the curd tension was measured on a Cherry-Burrell curd tension meter and recorded in grams. The whey acidity was determined by drawing 9 ml of clear whey from a whey pocket, then titrating with 0.1 N sodium hydroxide and phenolphthalein indicator. Curd break was judged by splitting the curd over a thermometer.

At the desired cutting point, the curd was cut with 3/8 inch curd knives. If the necessary whey acidity had not been reached at this point, more acidity was allowed to develop before cooking was started. When the curd was cut at low acidities, frequent stirring was necessary to prevent matting.

Cooking the Curd. The same procedure was used as in cooking the curd in laboratory trials. However, in some trials the experimental curd was sufficiently firm at the start of cooking; therefore, the temperature was raised quickly to 120 F and held for 25 min to inhibit further growth of the lactic starter organisms.

When necessitated by curd conditions, certain modifications in cooking were used. In rare cases, it was necessary to drain the whey from the curd

and allow the curd to lie on the warm surface of the vat to expel the excess whey. The proper firmness of the curd was determined by dropping the curd on a hard surface to check for shattering, by placing a small amount of curd in cold water for 5 min then checking for a springy consistency, and by breaking open the curd to check for absence of whey pockets.

Washing and Draining. When cooking was finished, the whey was drained from the vat and 3 inch of 80 F water was added and the curd was gently stirred. The procedure for washing the curd as described by Hales (20) was used with the exception that the third wash water was chlorinated with five ppm chlorine. After washing, the curd was piled and allowed to drain.

Storage and Preparing Samples. After draining, the curd was stored in stainless steel pails overnight at 40 F. Samples were then prepared for testing cream retention, flavor and texture, keeping quality of creamed and uncreamed curd, and finished curd firmness.

Creaming the Curd. A cream dressing of 14% fat was prepared with added salt to give a 1.5% salt concentration to the curd. This cream dressing was added to the weighed samples to give the mixture a 4% fat content. The samples were held at 40 F and frequently inverted to assure contact of the cream dressing with the curd.

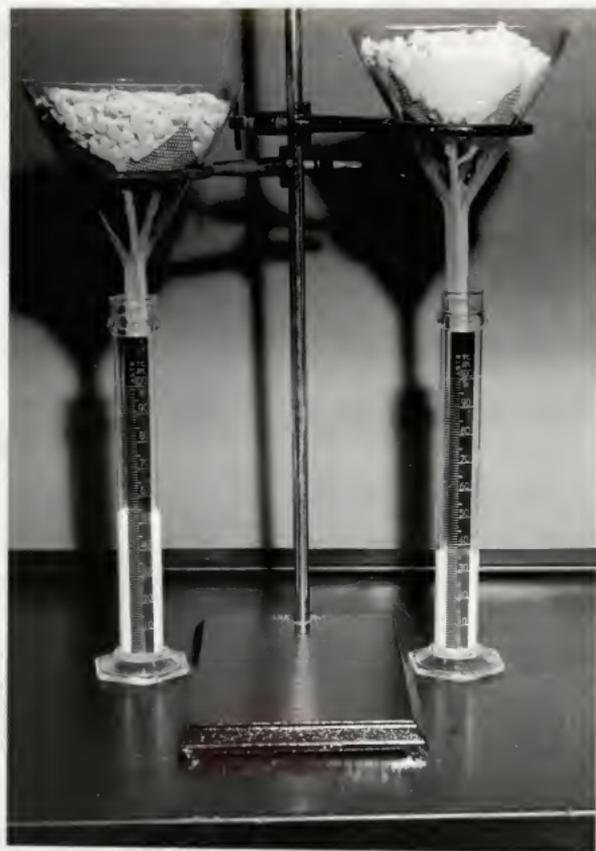
Quality Tests

Measuring Cream Retention. The cream retention of the finished curd was measured by using a slight modification of the procedure described by Emmons and Price (15). Plate III shows the equipment used. A 340 g sample, which had been mixed with 140 ml of cream dressing and held for 20 hr at 40 F, was placed in a funnel with an inner screen support for the curd and inserted

EXPLANATION OF PLATE III

Apparatus used in measuring cream retention
of the finished curd.

PLATE III



into a graduated cylinder. After 30 min at room temperature, the ml of cream dressing drained from the samples were subtracted from the initial amount of cream dressing added. The difference indicated the ml of cream dressing retained by the curd.

Measuring Finished Curd Firmness. Plate IV shows the apparatus used in testing finished curd firmness. This is a modification of the method used by Emmons and Price (14). The container consisted of a stoppered $4 \frac{3}{4}$ inch long by $1 \frac{1}{2}$ inch diameter sanitary pipe, with four 3 inch vertical slots opposite each other in the upper portion of the pipe. A 75 g sample of curd was placed in the unstoppered end of the container with frequent packing with a 380 g tamping weight. By placing the filled container on a dietetic scale and forcing a taut wire, held by two strips of steel, slowly and steadily through the curd, the amount of resistance offered by the curd could be read in g on the scale. The reading was used as the measure of curd firmness. By slightly repacking the curd, a second reading was made by passing the wire through the opposite two slots.

Moisture. Moisture content was determined in a Brabender Moisture Tester using a 10 g mascerated sample. The samples were held in the machine for 1 hr before readings were taken.

Flavor and Texture. Flavor and texture preferences were obtained from a panel of six members. A sample of the creamed control and creamed experimental cheese from each trial was judged by each member approximately 24 hr after creaming. In the system of judging, the panel member indicated a preference for A, or B, or whether he considered there to be no difference.

The results from six trials in each series were pooled, and where a preference was indicated, the control and experimental judgements were treated

EXPLANATION OF PLATE IV

Apparatus used in measuring finished curd firmness, showing the cutting wire, the curd container, and the dietetic scale.

PLATE IV



with a chi-square test using a 5% alpha level with 1 degree of freedom (D/F).

Keeping Quality. Creamed and uncreamed samples were held at 40 F. Each sample was tested organoleptically every other day until objectional defects occurred. The time required for such change to develop was recorded.

RESULTS AND DISCUSSION

Data were obtained on effects of the pseudomonas proteolysate on lactic starter stimulation; on manufacturing procedures for cottage cheese; and on curd characteristics of the finished product.

Effect on Lactic Starter Activity of Adding Pseudomonas Proteolysate

At 1% concentration, the proteolysate gave varying amounts of stimulation in acid production using 5% lactic starter at 90 F. As shown in Table 1, the largest amount of stimulation was generally at 4 hr incubation; however, several proteolysates produced the most stimulation at 5 hr. The amount of stimulation varied considerably among the various proteolysates. The increase in titratable acidity over the controls ranged from 0 to 15.6%, 8.2 to 31.1%, and 1.4 to 25.0% at 3, 4, and 5 hr, respectively. The average increase in acidity at 3, 4, and 5 hr was 9.1, 18.3, and 12.1%, respectively. It was observed that the proteolysate which gave most stimulation also caused most rapid coagulation.

Even though the proteolysates varied in their stimulation activity, the variation was not as great as that of the coagulatory activity. The time for various proteolysates to coagulate skim milk containing 5% lactic starter, ranged from 1 hr:35 min to 4 hr:30 min, with an average of 2 hr:40 min

Table 1. Effect of various pseudomonas proteolysates on the activity of lactic starters at 90 F.

Starter culture	Age of starter culture ^b	Pseudomonas proteolysate	Titratable acidity ^a at		
			3 hr	4 hr	5 hr
A	2 day	Cont. ^c	0.32	0.45	0.63
		K	0.37	0.59	0.71
B	1 day	Cont.	0.28	0.38	0.52
		U	0.28	0.45	0.62
C	Fresh	Cont.	0.35	0.52	0.69
		A	0.38	0.59	0.70
F	Fresh	Cont.	0.32	0.45	0.63
		D	0.37	0.59	0.71
F	Fresh	Cont.	0.33	0.49	0.63
		Q	0.37	0.53	0.65
F	4 day	Cont.	0.27	0.32	0.47
		P	0.28	0.36	0.52
F	5 day	Cont.	0.24	0.30	0.36
		X	0.27	0.34	0.43
		U	0.26	0.33	0.43
		W	0.27	0.33	0.45

^aAverage of duplicate samples.

^bPeriod of refrigerated storage following incubation.

^cControl - no proteolysate.

comparing 15 different proteolysate preparations (data not shown). Results suggested that the use of the proteolysate would accelerate acid production and coagulation in cottage cheese making.

The results from using different quantities of proteolysates with different quantities of lactic starter are recorded in Table 2. Again the amount of stimulation obtained varied with the different proteolysates. Stimulation also varied with the age of lactic starters used. More stimulation usually resulted from using old or slow starters. In all but one trial, the 4% lactic starter plus 1% proteolysate produced acidities

Table 2. Effect of different levels of pseudomonas proteolysate and lactic starter inoculum on acid development at 90 F.

Trial	Starter culture	Age of starter culture	Pseudomonas proteolysate		5% lactic starter		4% lactic starter + 1% Ps. prot. a		3% lactic starter + 1% Ps. prot. a		3% lactic starter + 2% Ps. prot. a	
			4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr	4 hr : 5 hr
1	B	1 day	Q	0.45	0.57	0.48	0.63	0.45	0.63	0.49	0.66	0.66
2	B	1 day	U	0.43	0.61	0.38	0.55	0.32	0.47	0.36	0.54	0.54
3	B	2 day	P	0.39	0.44	0.44	0.60	--	--	--	--	--
4	B	3 day	O & L	0.32	0.40	0.32	0.47	--	--	--	--	--
5	B	4 day	O & L	0.39	0.49	0.44	0.62	0.42	0.60	0.41	0.61	0.61
6	C	Fresh	O & L	0.61	0.69	0.60	0.70	0.56	0.69	0.57	0.70	0.70
7	F	Fresh	P	0.46	0.63	0.49	0.64	0.43	0.61	0.48	0.66	0.66
8	F	2 day	O & L	0.43	0.52	0.41	0.54	--	--	--	--	--
Avg.				0.44	0.55	0.44	0.59	0.44	0.60	0.46	0.63	0.63

^aPseudomonas proteolysate.

^bAverage of duplicate samples.

^cPeriod of refrigerated storage following incubation.

equal to or slightly greater than the control containing 5% starter alone. Acidities equal to those of the control were also produced when using 3% lactic starter plus 1% and 2% proteolysate (Table 2).

Since a smaller amount of starter plus the proteolysate produced as much acid as 5% starter alone, a stimulation effect was indicated even though the acidities obtained were about equal. Hence, the use of the proteolysate might permit the use of less starter in cottage cheese making.

Effect of Heating the Pseudomonas Proteolysate on Its Coagulatory and Stimulatory Activity

As shown in Table 3, heating the proteolysate had a slight inhibitory effect on its stimulatory property and also decreased its coagulatory activity. The decrease depended on the degree of heat treatment.

Since there was very little stimulation of acid production noted before heating with the particular proteolysate and starter used, there was only a slight decrease possible between heat treatments of 160 and 200 F for 5 to 30 min, but the coagulation time increased 3 hr;20 min. Similar observations were made by Koburger (26) who suggested that a heat-labile factor was involved. It was found that autoclaving the proteolysate completely destroyed its coagulatory activity. Since coagulation rate is important in cottage cheese making, heat treatment of the proteolysate offered a means of partially standardizing this property.

Table 3. Effect of heating the pseudomonas proteolysate on its coagulatory activity and stimulation of lactic starters at 90 F.

Heat treatment		Titratable acidity ^a at		Coagulation
Temp (F)	Time (min)	3 hr	4 hr	time ^b
(Cont.)	No heat	0.33	0.49	4:30
(Exp.)	No heat	0.37	0.53	2:00
160	5	0.38	0.51	4:40
	10	0.38	0.52	4:40
	20	0.38	0.54	4:40
	30	0.36	0.52	4:45
170	5	0.34	0.50	4:45
	10	0.34	0.50	4:45
	20	0.35	0.52	4:45
	30	0.30	0.50	4:45
180	5	0.34	0.50	4:45
	10	0.34	0.50	4:45
	20	0.34	0.50	4:45
	30	0.34	0.50	4:45
200	5	0.34	0.48	4:50
	10	0.33	0.48	5:00
	20	0.33	0.48	5:00
	30	0.34	0.48	5:20

^aAverage of duplicate samples.

^bTime recorded in hr:min.

Effect of Pseudomonas Proteolysate on Processing Cottage Cheese on a Laboratory Scale

Some of the main processing characteristics of cottage cheese made on a miniture scale with pseudomonas proteolysate are presented in Table 4.

Comparing six control and eleven experimental lots, the average values show that the experimental curd was cut at a slightly higher curd tension and 0.3 point higher pH value than the control curd. It was necessary to cut the experimental curd at the higher pH because of the fast coagulation

Table 4. Effect of pseudomonas proteolysate on processing of cottage cheese on a laboratory scale.^{a, b}

Processing characteristics	Cont.		Exp.	
	Range	Avg.	Range	Avg.
Curd tension at cutting (g)	55-100	75	50-125	90
pH at cutting	4.7-5.1	4.8	4.9-5.2	5.1
Whey acidity at cutting	0.35-0.45	0.40	0.24-0.50	0.38
Whey acidity at cooking	0.44-0.48	0.46	0.45-0.50	0.46
Time required for cooking	1:00-3:00 ^c	1:40	0:50-2:00	1:25
Overall time	7:00-8:45	7:45	5:40-7:40	6:45
Time saved				1:00

^aAverage of six trials including 6 controls and 11 experimental lots.

^bAll but 1 trial included 2 experimental vats.

^cTime recorded in hr:min.

of the skimmilk by the proteolysate. At the recommended cutting pH, the curd would have been too firm for satisfactory cutting. Even though the whey acidity of the experimental curd averaged 0.02% below the controls at cutting, it increased to the same acidity as the control before cooking was started. This prevented a rubbery experimental curd. Since the cheese vats were very small, it was difficult to cut the curd into uniform cubes. With a clean cut, the experimental curd was easier to cook than the control; however, when the curd was broken at cutting both curds were hard to cook. With the curd being shattered in the laboratory vats, only a small amount of time was saved in cooking the experimental curd (Table 4). However, an average of 1 hr was saved in the overall processing time. The time saved

did not include draining time. Due to the small laboratory vats many difficulties were involved, especially in cutting and cooking, so other characteristics of the curd were not measured.

**Effect of Pseudomonas Proteolysate on Cottage
Cheese Manufacture on a Pilot Scale**

In further evaluating the pseudomonas proteolysate in cottage cheese manufacture, a total of 24 trials were made on a pilot scale. They were divided into four series of six trials each, with each trial containing a control and experimental lot. The list below indicates the principal processes involved in the four series.

- Series I. Fresh skimmilk and 5% lactic starter.
- Series II. Reconstituted skimmilk and 5% lactic starter.
- Series III. Fresh skimmilk with 5 and 4% lactic starter for the control and experimental cheese, respectively.
- Series IV. Fresh skimmilk, 5% lactic starter, and added hydrochloric acid.

Series I

Processing Characteristics. Data on principal processing characteristics are presented in Table 5.

As was the case in the previous laboratory trials, it was found that the proteolysate caused early coagulation of the skimmilk before the desired cutting acidity was reached. Hence, in order to avoid too firm a curd and prevent shattering on cutting, it was necessary to cut the curd at pH of 5.1 instead of the normal pH of 4.7. The average curd tension of the controls at cutting was 90. This was less than the 105 value for the experimental curd. Since different proteolysate preparations were used, the curd tension

Table 5. Processing data of cottage cheese made from fresh skim milk and *Pseudomonas proteolytica*.^{a,b} (Series I)

Processing characteristics	Cont.		Exp.	
	Range	Avg.	Range	Avg.
Time required for setting	3:00-4:35 ^c	3:51	2:50-4:00	3:36
Time required until cutting	3:55-5:30	4:51	3:45-4:00	4:14
Curd tension of curd at cutting (g)	70-150	90	60-130	105
pH of curd at cutting	4.8-5.1	4.9	4.9-5.4	5.1
Whey acidity at cutting	0.35-0.52	0.46	0.32-0.49	0.39
Whey acidity at cooking	0.47-0.52	0.49	0.48-0.51	0.49
Time required for cooking	0:35-2:40	1:40	0:35-2:30	1:00
Time required for draining	0:25-1:05	0:45	0:20-0:55	0:40
Overall time	6:00-8:50	7:50	5:50-8:35	6:40
Time saved				1:10

^aTwo different lactic starters were used in this series.

^bResults of six trials.

^cTime recorded in hr:min.

at cutting varied considerably.

The average whey acidity of the experimental curd at cutting was 0.39% compared with 0.46% for the controls. It was necessary to hold the experimental curd in the whey for further acid development. At the start of cooking, both experimental and control curd had the same whey acidity of 0.49%. Nevertheless, the experimental curd expelled whey faster during cooking and became tough if heating was prolonged. Generally, the experimental curd had become sufficiently firm by the time the proper whey acidity was reached; consequently, the experimental curd required a shorter cooking time than the

control (1 hr versus 1 hr:40 min). This time reduction accounted for the major portion (57%) of the time saved by the experimental procedure. Due to its firmer texture, the experimental curd drained slightly faster. The average overall time saved during the manufacturing process was 1 hr:10 min or a 14.9% reduction.

Curd Characteristics. Table 6 shows that the average curd firmness value (267) for the dry experimental curd was definitely higher than that for the controls (202). Creaming reduced firmness and lessened the difference between the experimental and control curd, even though the control curd absorbed an average of 77 ml of cream dressing compared to 68 ml absorbed by the experimental. However, even after creaming, the experimental curd remained firmer than the controls with an average value of 147 compared to 105. These values compared to an average curd firmness of 108 for various creamed, commercial cottage cheese samples.

Table 6. Curd characteristics of cottage cheese made from fresh skim milk and pseudomonas proteolytate. (Series I)

Trial	Moisture		Yield		Curd firmness (dry)		Curd firmness (creamed)		Cream retention	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
	%		%		g		g		ml	
1	79.6	75.0	20.0	16.0	170	330	120	160	85	68
2	79.6	76.0	20.0	16.0	200	280	130	180	25	20
3	79.7	81.0	17.4	14.0	160	170	60	70	107	87
4	78.6	79.0	19.0	14.3	150	220	60	130	97	66
5	79.8	77.0	20.0	16.4	180	220	100	160	77	77
6	78.5	77.5	14.3	13.0	350	380	160	180	70	90
Avg.	79.3	77.6	18.4	14.9	202	267	105	147	77	68

The lower average moisture content of 77.6% for the experimental curd, compared to 79.3% for the controls, could account for only a small portion of the lower yield of the experimental curd. The average yields of 18.4% and 14.9% for the controls and experimental curd, respectively, show a somewhat lower yield for the experimental curd. Part of the lower yield could be attributed to the loss from shattered curd caused by the high curd tension at cutting, as shown in Table 5.

Organoleptic Quality. Table 7 shows the preference of the taste panel for flavor and texture of the finished curd.

Table 7. Organoleptic quality of cottage cheese made from fresh skim milk and pseudomonas proteolytate. (Series I)

Trial	Panel preference						Keeping quality			
	Flavor			Texture			Creamed		Uncreamed	
	Cont.	Exp.	No. diff.	Cont.	Exp.	No. diff.	Cont.	Exp.	Cont.	Exp.
No. of judgements							Time in days			
1	1	4	1	2	3	1	16	13	20	14
2	5	1	0	5	1	0	19	19	12	15
3	1	0	5	2	2	2	16 ^b	13 ^b	13 ^b	9 ^b
4	0	2	4	4	1	1	6 ^b	13 ^b	11 ^b	6 ^b
5	1	4	1	3	2	1	15	14	14	18
6	0	0	6	0	0	6	23	23	14	12
Avg.	1.3	1.8 ^a	2.8	2.7 ^a	1.5	1.8	15.8	15.8	14.0	12.3

^aChi-square nonsignificant at 5% level with 1 D/F.

^bStorage cooler not operating properly.

The average value of 2.8 judgements in the no-difference column for flavor as compared to 1.3 and 1.8 in the control and experimental columns, respectively, shows that the panel members generally had no flavor preference for one curd over the other. However, the 2.7 average value in the control column under texture indicates a preference for the control, when compared with the value of 1.5 in the experimental column and 1.8 in the no-difference column. None of the preferences were significant at the 5% level using a chi-square test with 1 D/F. Since the chi-square test does not account for interaction and the judgements in the no-difference column, it may not be a reliable index of significance in this case.

At refrigerator temperature of about 45 F, the average keeping quality of 14 days for the uncreamed controls was only slightly higher than the 12.3 days for the experimental curd. For the creamed curd, the control and experimental lots both had an average keeping quality of 15.8 days. The relatively long keeping quality for both types of curd in several trials, might be explained by the conditions of handling and testing the curd which limited most possibilities for contamination. The longer keeping period of the creamed curd may have resulted from a masking of off-flavors by the cream dressing; consequently, prolonging detection of unfavorable quality.

Series II

Processing Characteristics. This series was designed to evaluate the proteolysate as a means of preventing a soft, pasty curd commonly associated with cottage cheese made from reconstituted skim milk. The reconstituted skim milk was used to counteract the strong coagulatory activity of the proteolysate. Since curd made from reconstituted skim milk is often hard

to cook-out, it was felt that the syneresis effect of the proteolysate would be beneficial during the cooking period.

Even though reconstituted skim milk was used, the experimental curd had to be cut in 3 hr:16 min as compared to an average of 4 hr:5 min for the controls, to prevent tough curd formation (Table 8). Nevertheless, the

Table 8. Processing data of cottage cheese made from reconstituted skim-milk and pseudomonas proteolysate.^{a,b} (Series II)

Processing characteristics	Cont.		Exp.	
	Range	Avg.	Range	Avg.
Time required for setting	2:55-4:00 ^c	3:18	1:45-3:10	2:26
Time required until cutting	3:35-5:05	4:05	2:25-3:50	3:16
Curd tension of curd at cutting (g)	80-110	88	90-110	100
pH of curd at cutting	4.8-5.2	5.0	4.9-5.6	5.2
Whey acidity at cutting	0.36-0.50	0.46	0.25-0.54	0.37
Whey acidity at cooking	0.49-0.52	0.51	0.48-0.55	0.51
Time required for cooking	1:00-2:40 ^c	1:49	0:25-3:20	1:14
Time required for draining	0:20-0:45	0:35	0:15-1:15	0:33
Overall time	6:35-8:15	7:28	5:15-8:40	6:31
Time saved				0:57

^aThree different lactic starters were used in this series.

^bResults of six trials.

^cTime recorded in hr:min.

strong coagulatory activity of the proteolysate produced an average curd tension value of 100 for the experimental curd as compared to 88 for the controls at time of cutting. Even at this point, the cutting pH was higher

than normal (5.2). As was the case in Series I, the experimental curd was cut at a higher pH and lower whey acidity than the controls, but acid developed in the experimental curd to equal that of the controls before cooking was started.

By using the proteolysate, 35 min was saved in cooking the experimental curd, which accounted for 61% of the overall time saved. However, the time required for draining was almost the same for both curds. The overall time saved by using the proteolysate was 57 min or a 12.7% reduction in processing the experimental curd.

Curd Characteristics. There was wide variation in firmness of uncreamed curd in different trials, with the experimental curd averaging considerably firmer than the controls. Because of a high average cream retention by both control and experimental curd, 89 and 99 ml, respectively, the curd firmness of the dry curd was reduced over 50% after creaming (Table 9). The difference in curd firmness of 55 g between the dry curds was reduced to 28 g after creaming.

Since curd from reconstituted skim milk has a high water holding capacity, the controls averaged only 0.3% below the legal moisture maximum of 80%, but the experimental curd averaged lower with 75.8% moisture (Table 9). This difference in moisture could be a contributing factor to the 20.2% average yield from the controls as compared to 15.7% from the experimental curd.

Organoleptic Quality. Even though the curd firmness was only slightly higher for the experimental curd than for the control curd after creaming (Table 9), the taste panel showed a significant preference for the texture of the control curd when a preference was indicated. The preference for

Table 9. Curd characteristics of cottage cheese made from reconstituted skim milk and pseudomonas proteolysate. (Series II)

Trial	Moisture		Yield		Curd firmness (dry)		Curd firmness (creamed)		Cream retention	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
	%		%		g		g		ml	
1	81.0	82.2	20.0	17.0	175	145	90	50	87	105
2	81.0	71.4	20.5	11.4	210	300	110	200	107	105
3	79.8	76.2	20.0	15.0	180	330	70	150	82	72
4	78.8	77.8	22.0	20.0	140	160	80	70	70	90
5	78.0	68.4	18.3	12.1	220	320	60	140	100	104
6	79.4	79.0	20.6	18.8	130	130	80	50	90	117
Avg.	79.7	75.8	20.2	15.7	176	231	82	110	89	99

the flavor of the control curd was also statistically significant. It was felt (since the flavor of the curds was much alike) that the preference for the control flavor resulted more from differences in texture than from differences in taste.

With the average keeping quality at refrigeration temperature being almost the same for both uncreamed curds (Table 10), there was no logical explanation for the longer average keeping period of 22.7 days for the creamed controls as compared to 17.8 days for the creamed experimental curd.

Table 10. Organoleptic quality of cottage cheese made from reconstituted skim milk and pseudomonas proteolysate. (Series II)

Trial	Panel preference						Keeping quality			
	Flavor		Texture		No.		Creamed	Uncreamed		
	Cont.	Exp.	diff.	Cont.	Exp.	diff.	Cont.	Exp.	Cont.	Exp.
No. of judgements							Time in days			
1	2	2	2	6	0	0	26	22	21	21
2	6	0	0	6	0	0	14	20	20	14
3	5	1	0	3	3	0	19	11	11	10
4	1	3	2	0	1	5	23	17	17	24
5	5	1	0	6	0	0	25	11	20	22
6	2	1	3	5	1	0	29	26	26	25
Avg.	3.5 ^a	1.3	1.2	4.3 ^a	.83	.83	22.7	17.8	19.2	19.3

^aChi-square significant at 5% level with 1 D/F.

Series III

Processing Characteristics. This series was conducted to evaluate the use of 4% lactic starter plus 1% proteolysate, as compared to 5% lactic starter alone in making cottage cheese.

Because of slower acid production from using 4% lactic starter in making the experimental curd, the average time required until cutting was longer than in the previous two series and only 19 min less than the control in this series (Table 11). The average curd tension at cutting was quite low for both curds, and the reading for the experimental curd was only 16 g higher than for the controls (75 g versus 91 g). The low curd tension of both curds resulted in smooth, uniform cutting with very little shattered curd. As observed in Series I and II, the experimental curd was cut at a

Table 11. Processing data of cottage cheese made from fresh skim milk, 4% lactic starter, and pseudomonas proteolysate.^{a,b} (Series III)

Processing characteristics	Cont. ^c		Exp.	
	Range	Avg.	Range	Avg.
Time required for setting	3:05-4:50 ^d	3:48	2:30-4:05	3:18
Time required until cutting	3:50-6:00	4:36	3:30-5:10	4:17
Curd tension of curd at cutting (g)	70-90	75	60-120	91
pH of curd at cutting	4.8-5.0	4.9	4.8-5.4	5.1
Whey acidity at cutting	0.45-0.55	0.50	0.34-0.48	0.41
Whey acidity at cooking	0.49-0.55	0.53	0.49-0.54	0.52
Time required for cooking	2:00-3:00	2:25	0:30-2:55	1:05
Time required for draining	0:40-2:10	1:23	0:20-1:30	0:53
Overall time	7:50-10:35	8:56	6:10-9:55	7:20
Time saved				1:36

^aFour different lactic starters were used in this series.

^bResults of six trials.

^c5% lactic starter.

^dTime recorded in hr:min.

higher pH and lower whey acidity than the control, but acid developed in the experimental curd to equal that of the control before cooking was started.

As shown in Table 11, 1 hr:20 min was saved in cooking, and 30 min was saved in draining the experimental curd. This time reduction in cooking and draining accounted for 83%, and 31%, respectively, of the 1 hr:36 min saved in the overall processing time of the experimental curd. Since 83% and 31% accounts for more than 100% of the overall time saved, it appears that time was lost early in processing the experimental curd. Using only 4%

lactic starter in this series, it is quite possible that the time loss occurred during the ripening period. The average overall processing time of the experimental curd exceeded that of the previous two series by 40 min or more. This was undoubtedly caused by the lower amount of starter used and consequently the longer ripening period.

Curd Characteristics. In this series, the average curd firmness of the control curd (123 g) and the experimental curd (160 g) was lower than it was in Series I or II. The low curd firmness plus the high average cream retention values of 64 and 90 for the control and experimental curd, respectively, contributed to a very low curd firmness in the creamed curd (Table 12). However, creaming the curd only slightly reduced the difference in curd firmness noted between the dry curds.

Table 12. Curd characteristics of cottage cheese made from fresh skim milk, 4% lactic starter, and pseudomonas proteolysate. (Series III)

Trial	Moisture		Yield		Curd firmness (dry)		Curd firmness (creamd)		Cream retention	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
	%		%		g		ml		g	
1	80.2	78.0	21.4	16.3	80	180	62	75	40	130
2	73.6	73.4	17.8	17.8	130	160	103	103	65	80
3	66.2	67.2	19.0	20.0	100	110	109	112	50	50
4	76.4	73.0	17.9	15.5	170	200	117	97	80	110
5	75.4	74.4	20.9	17.4	120	160	115	111	60	90
6	76.4	75.4	22.9	20.9	140	150	101	106	90	80
Avg.	74.7	73.6	20.0	18.0	123	160	101	101	64	90

As Table 12 shows, the average moisture content of the controls and experimental curd was almost the same. Despite the moisture content being considerably lower than the 80% maximum allowed, the yield was good for the control (20.0%) and the experimental curd (18.0%). The adverse effect on yield by the relatively low moisture content may have been counteracted by the low loss of curd at cutting.

Organoleptic Quality. The data in Table 13 show that there was no significant preference for either the control or experimental curd in flavor or texture. The more nearly equally divided preference for both curds, than in prior series, was most likely due to lower curd firmness of the experimental curd, which would also influence the preference for its flavor.

Table 13. Organoleptic quality of cottage cheese made from fresh skim milk, 4% lactic starter, and pseudomonas proteolysate. (Series III)

Trial	Panel preference						Keeping quality			
	Flavor			Texture			Creamed		Uncreamed	
	Cont.	Exp.	diff.	Cont.	Exp.	diff.	Cont.	Exp.	Cont.	Exp.
1	2	3	1	2	3	1	16	16	13	13
2	4	1	1	1	4	1	17	18	23	16
3	0	4	2	0	1	5	13	20	18	19
4	3	3	0	5	1	0	15	14	22	18
5	4	2	0	3	3	0	13	12	23	21
6	1	2	3	1	2	3	11	13	26	15
Avg.	2.3	2.5 ^a	1.2	2.0	2.3 ^a	1.4	14.2	15.5	20.8	17.0

^a Chi-square nonsignificant at 5% level and 1 D/F.

At 45 F there was very little difference in keeping quality between the control and experimental lots for the creamed curd. However, the control had an average shelf-life of 3.8 days longer than the experimental when the curd was dry. Both lots of curd from this series had exceptionally good keeping quality.

Series IV

Processing Characteristics. In initial tests, it was found that the addition of hydrochloric acid solution to the skim milk tended to produce a softer curd at the coagulation point. Also, the acid had a slight tendency to flocculate some of the skim milk constituents, giving a softer curd. This additional effect promised to be beneficial since the curd in the earlier series was firmer than desired.

As the data in Table 14 show, the experimental curd still had to be cut at a slightly higher pH and lower whey acidity than the controls to prevent tough curd formation. At the start of cooking, the average whey acidity of the experimental curd (0.49%) was slightly lower than the controls (0.52%). After the initial increase in acid from adding the hydrochloric acid solution, the development of acid by the lactic starter in the experimental curd appeared to be slower than that in the controls, particularly during the first 3 hr. After cutting however, the acid development in the experimental curd was rapid.

The overall time saved by the experimental procedure was 1 hr:43 min. Part of this saving (29%) was contributed by the slightly shorter time needed for cooking the curd. Since no time was saved in draining the experimental curd, the 21.8% reduction in overall processing time resulted mainly

Table 14. Processing data of cottage cheese made from fresh skim milk, pseudomonas proteolysate, and added hydrochloric acid.^{a, b}
(Series IV)

Processing characteristics	Cont.		Exp.	
	Range	Avg.	Range	Avg.
Time required for setting	3:45-4:10 ^c	3:58	1:50-2:30	2:11
Time required until cutting	4:45-5:45	5:05	3:25-4:50	3:47
Curd tension of curd at cutting (g)	70-115	88	60-150	130
pH of curd at cutting	4.8-4.9	4.85	5.0-5.0	5.0
Whey acidity at cutting	0.47-0.55	0.52	0.44-0.48	0.46
Whey acidity at cooking	0.49-0.55	0.52	0.47-0.50	0.49
Time required for cooking	1:20-2:00	1:41	0:45-1:35	1:11
Time required for draining	0:20-1:00	0:32	0:20-0:45	0:31
Overall time	7:20-8:30	7:52	5:15-7:50	6:09
Time saved				1:43

^aFour different lactic starters were used in this series.

^bResults of six trials.

^cTime recorded in hr:min.

from a shorter ripening period. The 21.8% reduction was the largest obtained of all four series.

Curd Characteristics. The curd firmness of the dry controls and experimental curd varied considerably, but the experimental curd averaged only slightly higher than the controls (199 g versus 192 g). As shown in Table 15, both curds retained a large portion of the cream dressing. Perhaps the 20% greater cream retention by the experimental curd than by the control curd resulted from some effect of the hydrochloric acid on its cream

Table 15. Curd characteristic of cottage cheese made from fresh skim milk, pseudomonas proteolytate, and added hydrochloric acid. (Series IV)

Trial	Moisture		Yield		Curd firmness (dry)		Curd firmness (creamed)		Cream retention	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
	%		%		g		ml		g	
1	71.0	73.0	17.2	18.4	200	180	120	65	117	129
2	76.2	75.8	16.3	15.2	200	190	130	140	103	107
3	74.0	73.2	18.0	15.5	220	230	120	130	100	107
4	71.0	69.0	20.0	16.5	120	135	90	85	102	117
5	77.6	75.0	19.7	16.0	160	210	90	110	90	105
6	79.0	69.0	20.4	11.6	250	250	75	180	67	130
Avg.	74.8	72.5	18.6	15.5	192	199	104	118	97	116

retention properties.

The curd firmness of both control and experimental curd was greatly reduced by the large amount of cream dressing retained. An average curd firmness of 104 g for the controls as compared to 118 g for the experimental, gave both curds a moderately tender texture. The lower yield by the experimental curd may have resulted partially from the lower moisture content (72.5% as compared to 74.8% for the controls), but it was more likely due to a loss of shattered curd at cutting.

Organoleptic Quality. An average of 3.2 judgements were made in favor of the control flavor as compared to 1 judgement for the experimental flavor (Table 16). Statistically this difference was significant. The higher preference for the control texture was also significant; however, the almost equal number of judgements in the no-difference column indicates only a

apparent.

Each series of cottage cheese showed different characteristics. However, some similar conditions and problems were encountered in each series. The coagulatory activity of the proteolysate was hard to regulate. Each lot of proteolysate varied in activity and in most cases had to be pre-tested before use. The strong coagulatory activity of the proteolysate frequently produced a very firm experimental curd at the time of cutting. This, in addition to the difficulty of cutting curd in small vats resulted in shattering, and subsequently a lower yield for the experimental curd. In many cases before the proper cooking temperature and time could be reached, the experimental curd was too firm. This was due either to slow acid production, which did not coincide with the rate of coagulation, or to too rapid coagulation by the proteolysate.

From the results, it is evident that a definitely shorter time was required for processing the experimental cheese compared to the control procedure. Even though the control cheese was generally preferable from the stand point of flavor and texture, in some trials the experimental curd was preferred, and in many cases no preference was indicated. It appeared that a relationship existed between curd firmness and flavor preference for the creamed curd. Hence, a less firm experimental curd might considerably increase the preference for its flavor. The proteolysate produced no off-flavors and had no adverse effect on the keeping quality of the experimental curd. Also, the experimental curd was usually easier to handle during manufacture.

From the results obtained, it appears that the proteolysate also has possibilities as a skimmilk coagulant for cottage cheese manufacture, but

further work is needed to provide a concentrated form of uniform activity.

SUMMARY AND CONCLUSIONS

Experiments were conducted to evaluate a bacterial stimulant from proteolyzed milk cultures of *Ps. fluorescens* in cottage cheese manufacture. The proteolyzed milk cultures were sterilized by adding 25% by volume of ethanol, centrifuged, then exposed to vacuum treatment at 120 F to remove most of the alcohol. The treated proteolysate was stored at 32 F until used.

In testing the effect of the proteolysate on lactic starter activity in milk, it was used at the rate of 1% in test tube stimulation techniques. At 90 F using 5% inoculum, the proteolysate produced varying degrees of stimulation of acid production. Generally, the most stimulation occurred at 1/2 hr and with slow lactic starters. Under all conditions coagulation was accelerated.

Heat treatment, which was used in an attempt to standardize the proteolysate for cottage cheese making, had a definite effect on its coagulatory properties but only slightly affected its stimulatory activity. The results indicated that a heat-labile enzyme(s) was the coagulatory agent but was not a principal factor in stimulating acid production by lactic starters.

In making cottage cheese on a laboratory scale with the proteolysate, the experimental curd had to be cut at a higher pH than the controls to prevent tough curd formation. However, acid development in the experimental curd equalled that of the controls before cooking was started. Processing time was 1 hr less for the experimental curd than for the controls.

Making experimental cottage cheese on a pilot scale from fresh skim milk, by the short set method, using 5% lactic starter and 1% pseudomonas proteolysate,

resulted in a 14.9% reduction in processing time over the control procedure. The shorter cooking time of the experimental curd accounted for 57% of the overall savings in time. Using a chi-square test at the 5% level with 1 D/F, no significant preference was indicated by the taste panel for flavor or texture of either the control or experimental cottage cheese.

When experimental cottage cheese was made from reconstituted skim milk, 5% lactic starter, and 1% pseudomonas proteolysate, a 12.7% reduction was obtained in the overall processing time. The greatest part of this saving (61%) was contributed by the shorter cooking period. In this series, the preference for the flavor and texture of the control cheese was significant at the 5% level.

In Series III, fresh skim milk, 1% pseudomonas proteolysate, and 4% lactic starter were used in the experimental cottage cheese. Acid production was slightly slower in the experimental lots during the ripening period than in the controls, which contained 5% lactic starter and no proteolysate. However, a 17.9% reduction in manufacturing time was obtained from the overall experimental procedure. As noticed in the first two series, a shorter cooking period for the experimental curd was responsible for a large portion of the time saved (83% in this series). There was no significant preference for either the control or experimental curd in flavor and texture.

The largest saving in overall time was obtained when fresh skim milk, 1% pseudomonas proteolysate, 5% lactic starter, and added hydrochloric acid solution were used in making the experimental cheese. The added acid helped form a softer curd which was desired and gave an initial decrease in pH. The decrease in pH shortened the ripening period and consequently accounted for most of the 21.8% reduction in processing time. The time saved in cooking

the experimental curd in this series was considerably less than in Series I, II, and III. A significant preference at the 5% level was shown by the taste panel for the flavor and texture of the control curd. However, a large number of judgements indicating no difference in preference suggests that the preference for the control was slight. A relationship between flavor preference and curd firmness suggested that the preference for the control flavor was due more to a lower curd firmness, rather than a better flavor than the experimental curd.

In general, the experimental curd in all four series had to be cut at a lower whey acidity than the controls to prevent tough curd formation. However, the whey acidities were approximately the same before cooking was started. Except in Series IV, the main portion of the overall time saved was in the shorter cooking and draining periods. Overall, the curd firmness of the dry curd was greatest for the experimental curd, but the difference was lessened by creaming. In Series II, III, and IV, the cream retention was higher for the firmer experimental curd and almost the same in Series I as the control curd.

In each series, the use of the proteolysate resulted in a considerable reduction in time in processing the experimental curd. No off-flavors were produced nor was the normal keeping quality affected. Even though the control curd was often favored from the stand point of flavor and texture, in many cases no preference was indicated. Furthermore, the experimental curd was often easier to handle during manufacture. Stimulation of acid production by the proteolysate in its present form is questionable, when using 5% lactic starter at 90 F. However, it was observed in some trials, especially with added hydrochloric acid, that acid production after cutting the experimental

curd was faster than any time during the control procedure. Also, the proteolysate was quite effective for slow starters.

If the proteolysate can be improved from the stand points of concentration and uniform activity, it is believed that processing conditions can be adjusted to produce an acceptable cottage cheese with a marked saving in processing time. It also appears that the proteolysate has possibilities as a skimmilk coagulant.

ACKNOWLEDGMENTS

The author wishes to express his most sincere appreciation to Dr. T. J. Claydon, Department of Dairy Science, for his helpful suggestions, advice, and encouragement during the course of his study, and for the assistance and guidance in preparing this manuscript.

Thanks is given to Mrs. Richard Chansy for her help in running routine experimental tests.

Acknowledgment is also made to those persons who so faithfully served as members of the taste panel and to those persons in the dairy plant who so willingly helped provide material needed in the research project.

LITERATURE CITED

- (1) American Dry Milk Institute.
Nonfat dry milk solids in dairy manufacturing. Bull. 650. 1951.
- (2) Anderson, A. W., and Elliker, P. R.
The nutritional requirements of lactic streptococci isolated from starter cultures. II. A stimulatory factor required for rapid growth of some strains in reconstituted nonfat milk solids. J. Dairy Sci., 36:608-614. 1953.
- (3) Anderson, A. W., Parker, R. B., and Elliker, P. R.
The nutritional requirements of lactic streptococci isolated from starter cultures. III. Variations in the growth-promoting properties of fresh whole milks. J. Dairy Sci., 38:1083-1089.
- (4) Angevine, N. C.
Keeping quality of cottage cheese. J. Dairy Sci., 42:2015-2021. 1959.
- (5) Angevine, N. C.
Present day problems in the manufacture of cottage cheese. The Milk Dealer, 46(4):37. 1957.
- (6) Arret, B., and Kirshbaum, A.
A rapid disc assay method for detecting penicillin in milk. J. Milk and Food Technol., 22(11):329-331. 1959.
- (7) Bahadur, K., and Kumari, I.
Fractionation of commercial papain by acetone and investigations of the proteolytic and milk clotting properties of the different fractions. Enzymologia, 21(2):114-123. 1959.
- (8) Bahadur, K., and Kumari, I.
Fractionation of commercial papain by ethyl alcohol and investigation of the proteolytic and milk clotting properties of different enzymic fractions. Enzymologia, 21(3):145-155. 1959.
- (9) Bergford, H.
Manufacturing cottage cheese. The Milk Dealer, 49(7):28. 1960.
- (10) Collins, E. B.
Resistance of certain bacteria to cottage cheese cooking procedures. J. Dairy Sci., 44:1989-1997. 1961.
- (11) Cordes, W. A.
Factors affecting the yield of cottage cheese. J. Dairy Sci., 42:2012-2015. 1959.

- (12) Deans, D. D., and Hammond, E. G.
Coagulation of milk for cheese making by ester hydrolysis. *J. Dairy Sci.*, 43:1421-1430. 1960.
- (13) Dill, C. W., and Roberts, W. M.
Relationships of heat treatment, solids-not-fat, and calcium chloride to the curd tension of skim milk. *J. Dairy Sci.*, 42:1792-1800. 1959.
- (14) Emmons, D. B., and Price, W. V.
A curd firmness test for cottage cheese. *J. Dairy Sci.*, 42:553-557. 1959.
- (15) Emmons, D. B., and Price, W. V.
Observations on creaming of cottage cheese. *J. Dairy Sci.*, 43:931-945. 1960.
- (16) Emmons, D. B., Price, W. V., and Swanson, A. M.
Tests to measure syneresis and firmness of cottage cheese coagulum, and their application in the curd-making process. *J. Dairy Sci.*, 42:866-869. 1959.
- (17) Emmons, D. B., Swanson, A. M., and Price, W. V.
Effects of skim milk heat treatments on cottage cheese manufacture. *J. Dairy Sci.*, 42:1020-1032. 1959.
- (18) Garvie, E. I., and Mabbitt, L. A.
Acid production in milk by lactic cultures-The effect of peptone and other stimulatory substances. *J. Dairy Research*, 23:305-315. 1956.
- (19) Hales, M. W.
Cottage cheese from reconstituted nonfat dry milk solids. *Butter, Cheese and Milk Prod. J.*, 43(6):22. 1952.
- (20) Hales, M. W.
Sweet curd cottage cheese. 3rd ed. Chr. Hansen's Laboratory, Milwaukee 14, Wisconsin.
- (21) Hammond, E. G., and Deane, D. D.
Cheesemaking process. U. S. Pat. 2,982,654. 1961. (*Dairy Sci. Abstr.*, 24:41). 1962.
- (22) Heinemann, B.
Influence of cottage cheese starter organisms on strength of curd at time of cutting. *J. Dairy Sci.*, 40:437-438. 1957.
- (23) Horrall, B. E., Elliker, P. R., and Kensler, G.
The use of reconstituted non-fat-dry milk solids for propagating mother and batch starter cultures. *J. Dairy Sci.*, 32:250-257. 1950.

- (24) Keogh, B. P.
Variations in lactic acid production in milk. *The Australian J. Dairy Technol.* 13(3):132-138. 1958.
- (25) Kizer, D. E., Hankin, L., Speck, M. L., and Aurand, L. W.
Stimulation of lactic acid bacteria by substances concomitant to certain proteolytic enzymes. *J. Dairy Sci.*, 38:303-310. 1955.
- (26) Koburger, J. A.
Identification of substances in milk cultures of *Pseudomonas fluorescens* which stimulate lactic starter cultures. Unpublished M. S. Thesis, Kansas State University, Manhattan, Kansas. 1960.
- (27) Koburger, J. A., and Claydon, T. J.
Identification of substances in milk cultures of *Pseudomonas fluorescens* which stimulate lactic starter cultures. *J. Dairy Sci.*, 44:1811-1818. 1961.
- (28) Kosikowski, F. V.
Cottage cheese from low-heat, "low-temp" and instant spray-dried milks. *Milchwissenschaft*, 13(8):351-356. 1958.
- (29) Kuramoto, S., Jenness, R., Coulter, S. T., and Choi, R. P.
Standardization of the Harland-Ashworth Test for whey protein nitrogen. *J. Dairy Sci.*, 42:28-39. 1959.
- (30) Lundstedt, E.
New curd meter takes guesswork out of cheese making. *Food Eng.*, 27(11):97. 1955.
- (31) McMurlin, T. F., and Ernstrom, C. A.
Formation of curd by direct addition of acid to skimmilk. (Abstract) *J. Dairy Sci.*, 45:647. 1962.
- (32) Moir, G. M.
The effect of heat upon the rennin coagulation. I. *J. Dairy Research*, 2:68-76. 1930.
- (33) Morris, H. A., Coulter, S. T., Combs, W. B., and Heinzl, L. R.
Estimation of the serum protein content as a method for evaluating nonfat dry milk solids for use in cottage cheese manufacture. (Abstract) *J. Dairy Sci.*, 34:487. 1951.
- (34) Price, W. V.
Shortening the method of making cottage cheese. *Proc. 25th Ann. Conv., Intern. Assoc. Milk Dealers, Detroit, Mich.*, p. 35-45. 1932.
- (35) Price, W. V., Swanson, A. M., and Emmons, D. B.
Recent developments in cottage cheese manufacturing procedures. *J. Dairy Sci.*, 42:2005-2009. 1959.

- (36) Randolph, H. E., and Kristoffersen, T.
Characteristics of commercial nonfat dry milk for cottage cheese.
J. Dairy Sci., 44:833-844. 1961.
- (37) Reid, W. H. E., and Laughan, M. O.
Manufacture of cottage cheese from nonfat dry milk solids. Mo.
Agr. Expt. Sta., Bull. 505. 1947.
- (38) Remaley, R. J.
Using nonfat dry milk for cottage cheese. The Milk Dealer, 47(5):64.
1958.
- (39) Sandine, W. E., Speck, M. L., and Aurand, L. W.
Identification of constituent amino acids in a peptide stimulatory
for lactic acid bacteria. J. Dairy Sci., 39:1532-1542. 1956.
- (40) Speck, M. L., and Ledford, R. A.
Acceleration of cottage cheese manufacture by use of starter culture
stimulants. The Milk Dealer, 48(8):53. 1959.
- (41) Speck, M. L., McAnelly, J. K., and Wilbur, Jeanne D.
Variability in response of lactic streptococci to stimulants in
extract of pancreas, liver, and yeast. J. Dairy Sci., 41:502-509.
1958.
- (42) Speck, M. L., and Williamson, W. T.
The influence of stimulatory peptides on proteolysis of milk by
lactic streptococci. (Abstract) J. Dairy Sci., 41:705-706. 1958.
- (43) Stiles, J. C.
Use of nonfat dry milk solids in the making of cottage cheese and
cultured buttermilk. The Milk Dealer, 39(8):132. 1950.
- (44) Stone, W. K., Barge, P. M., and Graf, G. C.
Determination of curd making quality of nonfat dry milk. J. Dairy
Sci., 43:48-54. 1960.
- (45) Tuckey, S. L.
Problems in cottage cheese production for 1959. J. Dairy Sci.,
42:1246-1251. 1959.
- (46) VanSlyke, L. L., and Price, W. V.
Cheese. p. 382. Orange Judd Co., New York, N. Y. 1952.
- (47) Whitaker, R.
The selection and use of nonfat dry milk solids in the manufacture
of cottage cheese. J. Dairy Sci., 39:231-234. 1956.
- (48) Wilkowske, H. H.
Relationship between titratable acidity and pH during lactic acid
fermentation in reconstituted nonfat milk. J. Dairy Sci., 37:22-30.
1954.

EVALUATION OF A BACTERIAL STIMULANT FOR LACTIC STARTER
CULTURES IN COTTAGE CHEESE MANUFACTURE

by

DERALD DEAN VINCENT

B. S., Kansas State University, 1961

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

A bacterial stimulant (proteolysate) obtained from milk cultures of Pseudomonas fluorescens was evaluated in cottage cheese manufacture. The objectives were to determine if an acceptable product could be made, and to test the proteolysate as a skim milk coagulant and for stimulation of lactic starter acid production.

Previous work has shown that various materials, particularly proteins or protein fractions, stimulate acid production by lactic starter organisms. However, the commercial application of these materials in cottage cheese manufacture has been limited. In addition to the problem of response variation by the lactic organisms, is that of altered curd characteristics and the consequent need for modifying manufacturing procedures.

To obtain the proteolysate for use in cottage cheese manufacture, re-constituted skim milk was inoculated with the Ps. fluorescens culture then incubated 10 to 12 days at 78 F. The proteolysed skim milk was then treated with 25% by volume of ethanol, centrifuged, and subjected to vacuum treatment at 120 F to remove the ethanol. It was stored at 32 F.

Preliminary testing of the proteolysate was followed by pilot scale cottage cheese manufacture. Four different series with six control and six experimental trials each were evaluated. Data were recorded on the processing characteristics, yield, moisture content, curd firmness, cream retention, keeping quality, and panel preference for the flavor and texture of both curds.

Preliminary tests showed that the stimulatory and coagulatory effects of the proteolysate were variable and often unpredictable. In pilot scale cottage cheese manufacture, 1% added proteolysate was used in all four series. It was found that this necessitated cutting the experimental curd at a higher

pH and lower whey acidity than normal. When the experimental cheese was made from fresh skim milk and 5% lactic starter (Series I), a 14.9% reduction in processing time was obtained. The taste panel indicated no significant preference for the flavor or texture of either curd. In Series II where the experimental curd was made from reconstituted skim milk and 5% lactic starter, the reduction in processing time was 12.7%. Statistically a significant preference was shown for the flavor and texture of the control curd. When using fresh skim milk and 4% lactic starter for making the experimental cheese (Series III), the overall processing time was reduced 17.9%. As found in Series I, no significant preference was shown for the flavor or texture of either curd. The largest savings obtained in overall processing time was 21.8% in Series IV, where fresh skim milk, 5% lactic starter, and added hydrochloric acid were used in making the experimental cheese. The taste panel indicated a significant preference for the control flavor and texture. However, as with the other three series, a large proportion of judgements indicated no preference which suggests only a slight difference between the two curds.

In general, the experimental curd was easier to handle during manufacture. It was firmer than the control curd but retained more cream dressing. The keeping quality was about the same as that of the control curd. If the proteolysate can be concentrated and standardized to give uniform activity, it is believed that manufacturing procedures can be modified to produce an acceptable product with a considerable saving in processing time.