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Seasonal Variation in the Ability of Milk and Whey to Support Lactic Culture Growth

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SEASONAL VARIATION IN THE ABILITY OF MILK AND WHEY TO SUPPORT LACTIC CULTURE GROWTH

by

Rick Cameron Norton

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This thesis is dedicated to my loving and encouraging parents. And finally to my wife, Esther, for giving of her love, time, patience, and diversions to sustain me in this assignment, I extend my love and gratitude.

Rick C. Norton

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ABSTRACT

Seasonal Variation in the Ability of Milk and Whey
to Support Lactic Culture Growth

by

Rick Cameron Norton, Master of Science

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Milk samples from two cheese plants with overlapping milk supplies were collected monthly for one year in an attempt to measure seasonal variation in the ability of milk and whey to support lactic culture growth. Treatments to control and minimize variability of milk or whey were evaluated to optimize stability in starter culture performance.

Raw milk samples were tested for somatic cell counts, activity tests (modified Horrall-Elliker), acid degree values, and total plate counts. Activity (modified Horrall-Elliker) and inhibitory tests were also performed on pasteurized, pasteurized-vacuumized and high heat milk treatments.

Rennet whey (heated and unheated) was collected from raw and pasteurized-vacuumized milk and tested for lactic culture performance by monitoring growth under pH control for 16 h and measuring milli-equivalents of neutralizer (NaOH) added.

Lactic culture performance and stability in raw milk was poor in all seasons.

Culture performance in high heat milk was poor, but demonstrated good repeatability.

Pasteurized milk supported good lactic culture performance and stability.

Pasteurized-vacuumized milk provided excellent lactic culture performance and stability throughout the year.

Culture performance during December through March demonstrated the greatest variation. The cultures performed more uniformly during April through August. September was a transition month. Cultures demonstrated uniformity and optimum culture activity during October and November.

Whey substrates without heat sterilization demonstrated similar results to their milk counterparts. Heat treated whey samples showed seasonal variation, but was less than the non-heat treated whey.

(86 pages)

INTRODUCTION

The consumption of cheese and related fermented products (i.e., buttermilk, cottage cheese, and yogurt) has had a marked increase in recent years (2). Manufacture of these products has experienced remarkable changes and advancements, but milk remains as the major constituent.

Milk, being high in moisture, nearly neutral in pH, and rich in microbial foods, is very susceptible to contamination by undesirable microorganisms. Control of microflora in milk has been improved by the excellent refrigeration methods used by the dairy industry.

Variation of milk composition (i.e., compositional variation due to breed of the cow, period of lactation, feed changes, temperature changes in the environment, age of the cow, and changes of composition during milking and quarters of the udder) is evident according to the season of the year. Even with great efforts to standardize the medium, inoculation, and incubation conditions associated with the production of bulk cultures, variations exist in the ability of lactic organisms to develop in milk for cheese making. Thus, inocula volumes vary seasonally.

Recognition of milk's high susceptibility to contamination and variation due to seasonal conditions, therefore, affecting its ability to support lactic culture growth, is essential. The ability to standardize and anticipate culture performance in milk will provide economic advantages to the fermented milk products manufacturer.

The purpose of this work was to measure seasonal variation of lactic culture performance in milk and whey substrates for one year. Factors for reducing variation were also examined.

REVIEW OF LITERATURE

Effect of Seasonal Variation of Milk on Lactic Culture Performance

Solids

Researchers worldwide have examined protein, non-protein nitrogen, total solids, butterfat, and butterfat composition to evaluate variation in milk due to season (3, 5, 17, 25, 38, 52, 53, 64, 66).

Overman (53) provided a representative view of the above researchers' work illustrating solids variability in milk. His work incorporated milk from six breeds of cows: Ayshire, Jersey, Guernsey, Brown Swiss, Holstein, and Guernsey-Holstein cross. Because the number of samples was large and from individual cows of different breeds during all stages of lactation, the data represents seasonal fluctuations. Lactose is the least variable of all components on a percentage basis. Due to the osmotic pressure relationship between milk and blood, lactose increases as other soluble solids decrease, and vice versa. Osmolarity, which can effect lactic bacteria, is controlled in milk mainly by lactose concentration (34). Lactose is the least variable milk component (53). Thus, osmotic pressure variations are minimal. Fat, protein, and ash decrease most in milk from late May to early August. Although this decrease is often attributed to a change

in feed or temperature (5, 45, 46), Johnson et al (38) has shown that cows fed the same ration month after month and housed under slight temperature variation still produce milk exhibiting seasonal variations in total solids.

The effect of compositional variations in milk on lactic bacteria performance is not well defined. Lindquist (44) reported no correlation between seasonal variations in protein composition or free amino acids in cheese milk and the occasional slow growth of starters.

Rancidity

Hydrolytic rancidity. Hydrolytic rancidity is caused by cleavage of free fatty acids from the glycerol moiety of milk fat under the catalytic influence of lipase (35) enzymes. These enzymes are normally present in milk, and vary with season (68). Tarassuk and Frankel (68) described at least two lipase enzymes in bovine milk. The "membrane lipase" is irreversibly absorbed on the fat globule as milk is cooled. It is abundant and active in milk from cows late in lactation. "Plasma lipase" is associated with the caseinate system and can be activated by agitation or homogenization to produce lipolysis. Milk from cows late in lactation and on dry feed contains more "membrane lipase" and is subject to spontaneous lipolysis (68). Not all investigators agree that lipolytic activity increases toward the end of lactation (28, 59).

Chen and Bates (12), Hileman and Courtney (29), and Jensen (35) support Tarassuk and Frankel (68) in attributing seasonal variation of lipase to feed. Feed experiments and practical observations have demonstrated that green pasture decreases and dry feed increases the incidence of rancidity.

The inhibitory action of rancid milk on the growth of Streptococcus lactis has been confirmed by Tarassuk and Smith (69). Costilow and Speck (15) reported that the inhibitory effect of rancid milk on streptococci is due to several specific fatty acids. Caprylic, capric, and lauric acids inhibited growth of S. lactis. Degree of inhibition increased as the concentration of acid increased. Oleic, butyric, linoleic, linolenic, arachidic, palmitic, and .05% or less caproic and stearic acids did not have an effect on S. lactis.

Anders and Jago (1), elucidated in 1971 the detrimental effect of fatty acids on streptococci. They showed that oleic acid is harmful to lactic streptococci whereas Costilow and Speck (15) had previously indicated that oleic acid was not harmful. Treatment of cells with oleic acid appears to alter the permeability of the cell membrane so that the cell can no longer regulate the intracellular pH independently from the external pH. Oleic acid also appears specifically to inhibit an enzyme involved in the formation of acetate. The uptake of oxygen and the formation of acetate were inhibited by oleic acid. In the absence of oleic acid, acetate and carbon dioxide were the only products detected.

Anders and Jago (1) concluded that accumulation of fatty acids in milk and milk products could inhibit lactic streptococci growth.

Branen et al (9) also related antimicrobial properties of lipids to the bacterial cell membrane. The fatty acids appear to form a monolayer around cell walls which blocked the transport of nutrients into the cell. Gram-negative bacteria are less susceptible than gram-positive bacteria to the action of lipids because the gram-negative bacteria can metabolize lipids more efficiently. The lipopolysaccaride layer which typically surrounds the cell wall of gram-negatives can screen out fatty acids. The fatty acids are thus prevented from accumulating.

Oxidative rancidity. Oxidative rancidity, the lipid deterioration of milk fats resulting in saturated and unsaturated aldehydes which impart off-flavors to milk, appears to increase at low temperatures (7, 10, 18). With the advent of stainless steel equipment, oxidative deterioration in fluid milk as a result of copper contamination has decreased significantly, but spontaneous oxidation still exists. Bruhn and Franke (10) showed that 38% of samples collected from a Los Angeles milkshed were susceptible to spontaneous oxidation.

The catalytic effect of natural light on promoting off-flavors in fluid milk has been recognized for years (18). Efforts to inhibit or retard off-flavors resulting from sunlight exposure led to the introduction of light impermeable doorstep coolers and bottles.

Data on the effect of oxidative rancidity in milk on lactic streptococci performance is not conclusive, nor has seasonal variability been established. It is not likely that milk with "sunlight flavor" would be used in bulk culture production. Kulshrestha and Marth (42) indicated that certain aldehydes, ketones, and fatty acids are inhibitory to S. lactis. Correlation of these products with oxidative rancidity products was not determined.

Vitamins and Minerals

Fluctuation of vitamins and minerals in milk exists, but appears to be minimal. A nationwide survey carried out by USDA indicated that summer milk contained 1.6 times as much vitamin A as winter milk. Vitamin B_{12}, biotin, pantothenic acid, thiamin, and vitamin C are fairly constant throughout the year. Riboflavin, niacin, vitamin B_6, and vitamin E are highest in spring and/or summer milk (27).

Relatively large proportions of natural elements exist in milk (27). They include potassium, calcium, sodium, magnesium, phosphorus, chlorine, and sulfur. Mineral variation in milk due to season has not been established.

Nutritional requirements for lactic bacteria in relation to vitamins and minerals is not well documented. Kozak and Dobrzanski (41) describe nutritional requirements of lactic bacteria but for the enhancement of nisin production. They describe the need for B vitamins,

mineral salts, glucose or lactose, and certain amino acids. Postulation that lactic bacteria may perform better in summer milk due to a higher concentration of certain needed B vitamins (41) has not been confirmed and other factors could also affect the increased lactic bacteria performance.

Microflora

Bacteriological quality of milk, particularly when stored for long periods (13), is more important than generally accepted. Seasonal variation of milk microflora is well documented (21, 72). Depletion of low concentrations of free amino acids normally found in raw milk by large numbers of non-starter bacteria might also restrict the initial stages of starter culture growth (33).

Antagonistic Effects Among Lactic Bacteria

Researchers have noted culture inhibition due to antimicrobial substances produced by one strain that adversely affected associate strains (6, 8, 14, 30, 32, 39, 40, 49, 50, 56, 61, 62, 63, 75, 76).

Rogers (61) in 1928 was one of the first to show that lactic streptococci may inhibit the growth of another lactic organism. Beneficial effects from consuming certain cultured dairy products and their inhibition of intestinal flora have been discussed from the early 1900's until today (49, 50, 62).

Hoyle and Nichols (32) in the late 40's warned cheese manufacturers to make appropriate selections of cultures for cheese starters by excluding inhibitory strains among lactic bacteria. Baribo and Foster (6) extended this work and indicated that the inhibitory substance produced by some lactic streptococci was heat stable at pasteurizing temperatures. Hirsch (30) in 1951 named the inhibitory substance nisin and described its production by a Streptococcus lactis. Later that year, he co-authored an article describing the antibiotic lactobacillin produced by lactobacilli (76). Other investigators concentrated on antibiotic characteristics of other lactic cultures (39, 40, 75, 76).

It was not until the 1970's that the isolation, purification, and properties of these antimicrobial substances produced by lactic bacteria were determined (8, 56, 63).

Pretesting of milk quality and its compatability to the starter culture is essential (54). Pearce et al (55) reported that raw milk contained wild lactic streptococci when incubated at above 13C due to a refrigeration breakdown. The nisin-producing streptococci were able to multiply to such an extent that effecting a 100-fold dilution still left a nisin concentration that was inhibitory to the cultures used in cheese making and caused culture failure in the cheese vats.

Support of Lactic Culture Growth in Milk

Naturally occurring compounds in milk may influence the ability of milk to support starter growth. Czulak and Meanwell (16) recognized the depression of certain single strain starter cultures in H.T.S.T. pasteurized milk during October through April, but the effect was removed by momentary boiling. The addition of a small percentage of H.T.S.T. pasteurized milk to autoclaved milk reduced the activity of these cultures. Czulak postulated the existence of a growth stimulating factor in milk throughout the year. During the winter when cattle are off grass and green fodder rich in this stimulatory factor, the concentration of the growth factor was sufficiently low to allow other natural inhibitors in milk to reduce lactic culture activity. No phage analysis was performed in Czulak's studies.

Stadhouders (65) followed up the research of Czulak and Meanwell (16) a decade later. Activity tests were performed on three starters using H.T.S.T. pasteurized milk with and without steaming. Starter activity in the unsteamed milk was slightly different in the winter and summer with highest activity between April and July. The steamed milk showed higher activities in winter than in summer and postulated the presence of thermostable stimulating substances or substances from which thermostable compounds are formed by heating. Stadhouders (65) suggested that peroxidase fluctuation throughout the season might influence peroxidase sensitive bacteria.

Johns (37) reported that 7.3% of samples from herd milk samples collected in the spring showed inhibitory effects against lactic starter organisms, compared with 5.4% of the summer samples. Samples causing inhibition were tested for antibiotics and only 1.4% of the total showed zones of inhibition by the disc assay method. No residual quaternary ammonium compounds were found.

Auclair and Hirsch (4) were among the first to postulate the existence of natural inhibitors referring to them as lactenin I (mainly in colostrum) and lactenin II (mainly in milk). Randolph and Gould (57) tested milk from individual cows and herds and found inhibition of acid production for both single and mixed strain cultures. Pasteurization slightly reduced the inhibitory properties of the milk. Different cultures varied markedly in their susceptibility to the inhibitory properties of milk. Single strain cultures were generally more susceptible than mixed strain cultures. Acid production increased with increasing levels of inoculation, but the inhibitory effects were observable even with 10% inoculum. Use of the various combinations of resistant and susceptible single strain cultures in the inoculum reduced the apparent natural inhibitory properties of milk.

Cerna et al (11) reported that acid development by mixed yogurt cultures of Streptococcus thermophilus and Lactobacillus bulgaricus was inhibited in some raw and pasteurized milks. The inhibitory agent was not found to be an antibiotic, disinfectant, detergent or phage. It

was present in 30-85% of regional milk samples, including aseptically drawn milk, and was prevalent during the spring months.

Miekhejohn (47) reported a periodical seasonal inhibition of yogurt cultures (disappearance of lactobacilli in the second fermentation stage with slowing down or cessation of acid development) in the milk supply. Inhibition of acid development could not be related to antibiotics or to any obvious problem in the factory and occurred over a period of years in widely separated areas of Queensland, Australia. The specific cause was not determined. Some results indicate that relatively high osmotic pressures in products with 25% total solids (including 12% cane sugar) predispose the lactobacilli to inhibition by an inhibitor that becomes apparent during a certain period of the year in heat-concentrated skim milk.

Kulshrestha and Marth (42) reviewed that naturally occurring volatile and non-volatile compounds in milk were inhibitory to starter organisms. Twenty-five milk associated compounds (volatile and non-volatile) including fatty acids, aldehydes, ketones, sulfur compounds, etc., were tested on several bacteria including Streptococcus lactis. Mixtures of all compounds significantly inhibited growth of S. lactis. Distillate obtained from milk at 60, 68.3, or 76.6C had variable effects on growth and activity of S. lactis. When the processing temperature was 68.3C, the distillate inhibited S. lactis in autoclaved milk or in raw milk heated to 68.3C. Kulshrestha and Marth (42) concluded that

volatile and non-volatile compounds, present in fresh milk or generated by heat, can retard growth of different organisms including starter bacteria. They also concluded that volatile compounds may be removed from milk by vacuum treatment and the treated milk would be made more suitable for growth of starter cultures.

Seasonal Variation of Whey Composition

Solids

Glass and Hedricks (24) analyzed sweet and acid type dry whey products for one year to evaluate current standard methods for analysis and to obtain extensive data for product formulations and nutritional labeling.

Jensen and Hansen (36) reported that the protein content of whey differs according to the manufacturing conditions, but that seasonal variations in whey protein correspond to variations in the protein content of milk.

Giroux et al (23) analyzed 153 weekly composite samples of cheddar cheese whey for protein, lactose, ash, and calcium during 1964-1966. Mean values for dried whey in 1964, 65, and 66 were respectively: % protein, 12.12, 12.13, 12.12; % lactose, 74.14, 73.96, and 73.88; % ash, 8.32, 8.37, and 8.25; % calcium, .55, .52, and .66.

The protein content was at its lowest in April and highest from September through January, whereas the lactose content was high in April and at its lowest in September.

Support of Lactic Culture Growth in Whey

Randolph and Gould (57) indicate that culture performance in whey is proportional to the same culture performance in milk from which the whey was collected. They reported that acid production by three lactic cultures was inhibited in both rennet and acid wheys, inhibition ranging from 29% to 40% for acid prepared whey and 38% to 50% for rennet prepared whey. Effects of seasonal trends of inhibition were not studied.

Gillies (22) supported Randolph and Gould (57) indicating that the inhibitory effect of milk is carried over into the whey. Gillies also indicated that sterilization of the whey appeared to eliminate the effects of inhibition.

Vedamutha et al (74) provide the only data to date on the inhibitory compounds in whey but in reference to the propionibacteria. Their isolation procedure of the active components in skim milk whey utilized salt fractionation, Sephadex column separation, and disc-gel electrophoresis. The investigation showed that one of the immune-globulins of milk, pseudoglobulin, was the main inhibitor.

MATERIALS AND METHODS

Cheese Plant Selection

Two cheese plants were selected within close proximity (60 miles) representing similar environmental conditions, breeds of dairy cattle, and feeding techniques. Cheese Plant A used a Manufacturing grade milk in the production of Swiss and Cheddar cheese. Plant B utilized only Grade A milk for fluid milk processing with the excess used for Cheddar cheese production. Samples of milk for cheese production were collected and tested monthly from both plants.

Mastitis Testing

Two methods of mastitis testing were used to determine milk quality throughout the season. The Wisconsin Mastitis Test (WMT) was used as outlined in Standard Methods for the Examination of Dairy Products (73). Materials and apparatus were obtained from Z.D. Roundy, Orem, Utah. As a more rapid test, the New Zealand Rolling Ball Viscometer (procedure, reagents, and equipment were obtained from Automation Engineering, Division of Refrigeration Engineering Co. LTD, Auckland, New Zealand) was also performed. The RBV to date has not been A.O.A.C. approved although it is claimed to have several advantages over the WMT (51). Results were reported as somatic cells per milliliter.

Bacterial Cultures

Streptococcus strains UC77 (Streptococcus cremoris) and UL13 (Streptococcus lactis) were obtained from the Nutrition and Food Sciences Department, Utah State University, Logan, Utah. Original fresh coagulum of stock cultures was propagated at 1% inoculum for 4 h at 30C in 9.45% sterilized reconstituted NDM. Sterile filter paper strips were wetted by capilliary action in the stock culture and placed asceptically into sterilized vials. The vials were allowed to freeze at -20C for 5 h, removed, caps loosened, and placed in a freeze dryer (Unitrap II from Virtis, Gardiner, New York) overnight. Cultures were stored at -40C until used. Lyophilized cultures were removed from -40C storage and transferred twice at 1% inoculum before use.

Reference Activity Test

A modified Horrall-Elliker (31) test was used as a standard reference test. A 0.3 ml inoculum of each UC77 and UL13 culture (3% inoculum) was added to 19.4 ml of sterile reconstituted NDM (9.45% reconstituted NDM for 3.5 h at 30C. An incubation temperature of 30C was selected according to work by Lawrence et al (43) indicating optimum temperature for growth and acid production was between 30 and 33C. Culture activity was measured by both Titratable Acidity (TA) and Potentiometric measurement (pH). Titratable Acidity (measurement of any constituent that will react with and neutralize the

standard alkali) was performed on a Karl Fischer Titration Unit (Multi-Dosimat E415 and Automat 547, Metrohm AG, CH-9100, Herisan, Switzerland) by titrating to 8.80-8.90 pH endpoint. The alkaline titrant (NaOH) was standardized at two-week intervals to determine normality to three decimal places i.e., .054N. The potentiometric measure of hydrogen ion activity (pH) was performed on a Corning model 10 pH meter (Corning Glass Works, Corning, New York). Samples were tested in duplicate, held at 0C for the initial reading, placed in a 30C water bath for 3.5 h, and cooled promptly to 0C for the final activity readings. The above procedure was repeated for all milk treatments (raw, pasteurized, pasteurized-vacuumized, and high heat milks). Results were reported as ΔTA and Δ pH.

Inhibitory Test

A test was devised to examine the inhibition activity of milk against UC77 and UL13. Standard Methods for the Examination of Dairy Products (48) was used as a guide. The selected lactic cultures were unable to grow successfully on Antibiotic Medium No. 1 (48), therefore, M17 medium (70) was substituted. Petri plates (100 x 15 mm) were sectioned into four areas. Six milliliters of sterile M17 agar (cooled to 45C) were mixed with .3 ml of a 1/10 dilution of freshly coagulated lactic culture (UC77 or UL13) and spread evenly over the petri plate bottom. After solidification of the agar, sterile 1/2 inch

filter paper disks were wetted by capilliary action with samples of milk (raw, pasteurized, pasteurized-vacuumized, and high heat) and placed on the agar base. After incubation for 22-26 h, inhibition was evidenced by clear zones surrounding the disks (reported as +). A continuous lawn of growth was reported as -.

Acid Degree Value (ADV)

A test for hydrolytic rancidity (measurement of the amount of base required to titrate 100 g of fat) was used as outlined by Thomas et al (71) only NaOH was used instead of KOH. The ADV was performed for 9 months (Dec-Aug). Results were reported as ADV units.

pH Stat (Whey)

Samples of raw milk whey and pasteurized-vacuumized milk whey were inoculated with .3 ml of UC77 and .3 ml of UL13 lactic cultures into 19.4 ml whey (3% inoculum). Growth was monitored in a pH stat for 16 h using a Sargent-Welch pH recorder with titration and pH stat accessories (Sargent-Welch Scientific Co., 7300 North Linden Avenue, Skokie, Illinois). The system included facilities for agitation of sample, metering of titrant, and continuously recording the pH. A constant 30C temperature was maintained using a Blue M Electric Company constant temperature water bath with a circulating pump (Gorman-Rupp Industries Inc., Bellville, Ohio). The pH was initially

adjusted to 6.4 for all whey samples. When the culture produced sufficient lactic acid to lower the pH to 6.17, .5 N NaOH was automatically added to return the pH to 6.25. The milliequivalents NaOH added over a 16 h period, divided by 16 was reported as average milliequivalents per h (meg/h).

Plate Counts

Plate counts were taken on raw milk samples for a seven-month period (Feb-Aug) using M17 agar (70). Plate counts were also performed on the 16 h pH stat whey samples for seven months (Feb.-Aug.).

Milk and Whey Samples

Raw milk: Raw milk was collected from Plants A and B and stored immediately in ice water for transport to the laboratory. It was maintained at 0-4C until tested. The raw milk had no heat treatment. All milk transfers were made into clean, sterile containers. Tests performed on the samples included seasonal variation in support of specific lactic culture activity, fluctuation of somatic cell count, presence of inhibitory compounds, acid degree value, and bacterial counts.

Pasteurized milk: A portion of the raw milk samples from Plants A and B was heat treated at 62.7C for 30 min (20) and cooled to 0C to determine the effect of pasteurization of milk on seasonal variation of lactic culture activity.

Pasteurized-vacuumized milk: Pasteurized-vacuumized milk was not obtainable from Plant A, therefore, a portion of raw milk was pasteurized at 62.7C for 30 min and vacuum treated under laboratory conditions using a Flash Evaporator (Buchler Instruments, Fort Lee, New Jersey) with 300 ml of milk in the reservoir held at 65C with 16 inches of mercury for one min. This treatment was equivalent to the industrial vacuum treatment of Plant B milk which used 72C and 13 inches mercury for 3-5 sec (Appendix I). This was done to determine the effect of vacuum treatment of pasteurized milk on seasonal variation in lactic culture performance.

High heat milk: A portion of the raw milk from Plants A and B was heat treated to 82C for 3 min to destroy inhibitory compounds naturally present in milk (19).

Nonfat dry milk (NDM): NDM was reconstituted at 9.45% from a single lot of low heat spray processed pasteurized NDM (Western Dairymens Coop. Inc., Richmond, Utah) and sterilized at 15 p.s.i. steam for 15 min. NDM samples were packaged in 30 g sealed containers and stored at -20F for the duration of the project. This served as a reference medium.

Raw whey (no heat): Three hundred milliliter samples of raw milk were treated with rennet (90 ml/1000 lbs. milk), allowed to stand 45 min at 32C and cut using a spatula. Whey was collected following passage through cheese cloth, centrifuged at 3500 rpm for 5 min, and passed through a .45 µm membrane filter (Millex HA) to remove the natural milk flora. Samples of 19.4 ml whey were collected and inoculated with .3 ml UC77 and .3 ml UL13. Lactic culture growth was monitored for 16 h under pH stat conditions. No stimulants were added and a temperature of 30C ± 1 was maintained. This was done to determine the possibility of hold-over inhibitors from milk and solubles in whey.

Raw whey (heat 90C for 45 min): Same as raw whey (no heat) preparation, but heat treated at 90C. for 45 min to determine the possibility of heat labile inhibitors in raw whey.

Pasteurized-vacuumized whey (no heat): Same as raw whey (no heat) preparation, but collected from pasteurized-vacuumized milk. This served to determine the possibility of hold-over inhibitors in the whey after vacuum treatments.

Pasteurized-vacuumized whey (heat 90C for 45 min): Same as raw whey (no heat) preparation but collected from pasteurized-vacuumized milk and heat treated. This was done to determine the possibility of heat labile inhibitors in the whey after vacuum treatment.

A flow diagram of the research design is on Figure 1.

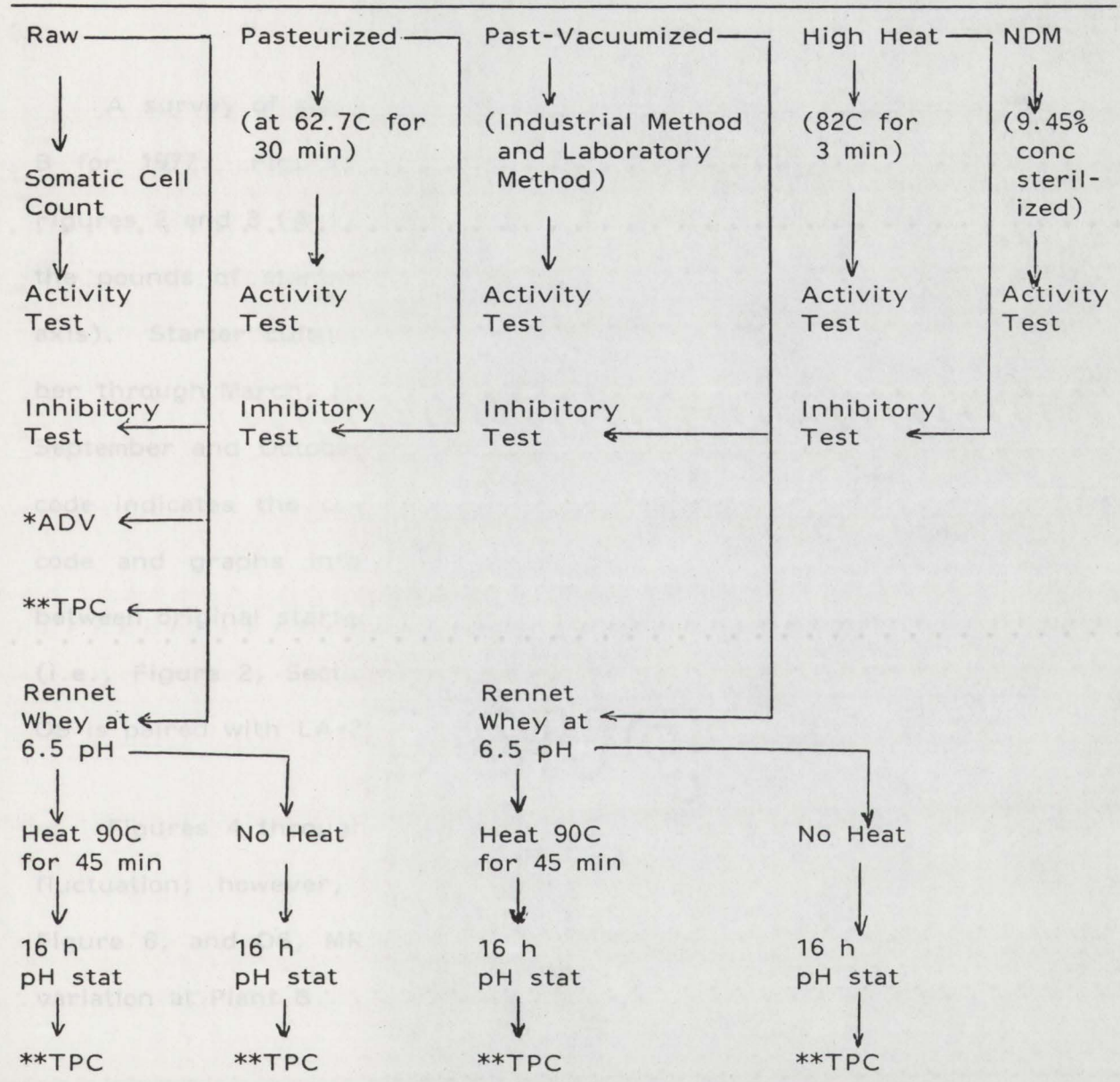

Figure 1. Research design flow diagram

*Acid degree value.
**Total plate count.

RESULTS

Preliminary Industrial Data

A survey of starter usage records was conducted on Plants A and B for 1977. Figures 2 through 8 graphically indicate the results. Figures 2 and 3 (A-1, A-2) of Plant A indicated seasonal fluctuation in the pounds of starter culture (Y axis) used throughout the year (X axis). Starter culture required to set the vats was high from December through March, leveled off from April to August, and decreased in September and October with an increase beginning in November. The code indicates the commercial cultures used. The breakdown of the code and graphs into sections 1, 2, and 3 is used to differentiate between original starter pairs being paired differently later in the year (i.e., Figure 2, Section 1 indicates OS paired with MS, at Section 2, OS is paired with LA-2).

Figures 4 through 7 (B-1, B-2, B-3, B-4) indicate little seasonal fluctuation; however, H-85 of Figure 4, H-72 of Figure 5, H-70 of Figure 6, and OS, MRD, and H-75 of Figure 7 demonstrated seasonal variation at Plant B.

Figure 8 (C) is the accumulation of all figures (Figures 2-8 of Plants A and B) on one graph. Plant A, utilizing Manufacturing grade

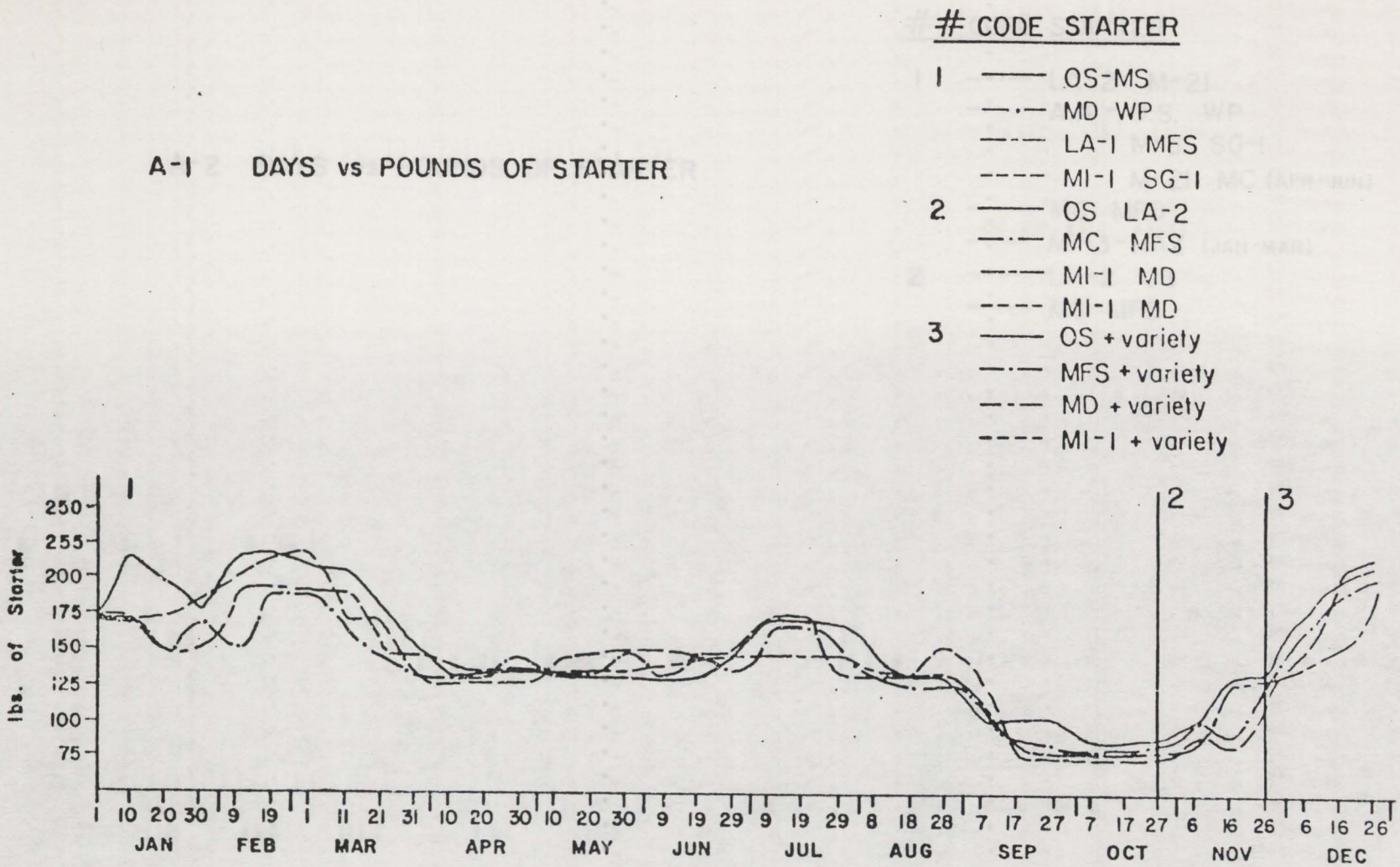

Figure 2. Graphic representation of indicated commercial starter culture inoculum for the year of 1977 at Plant A(1).

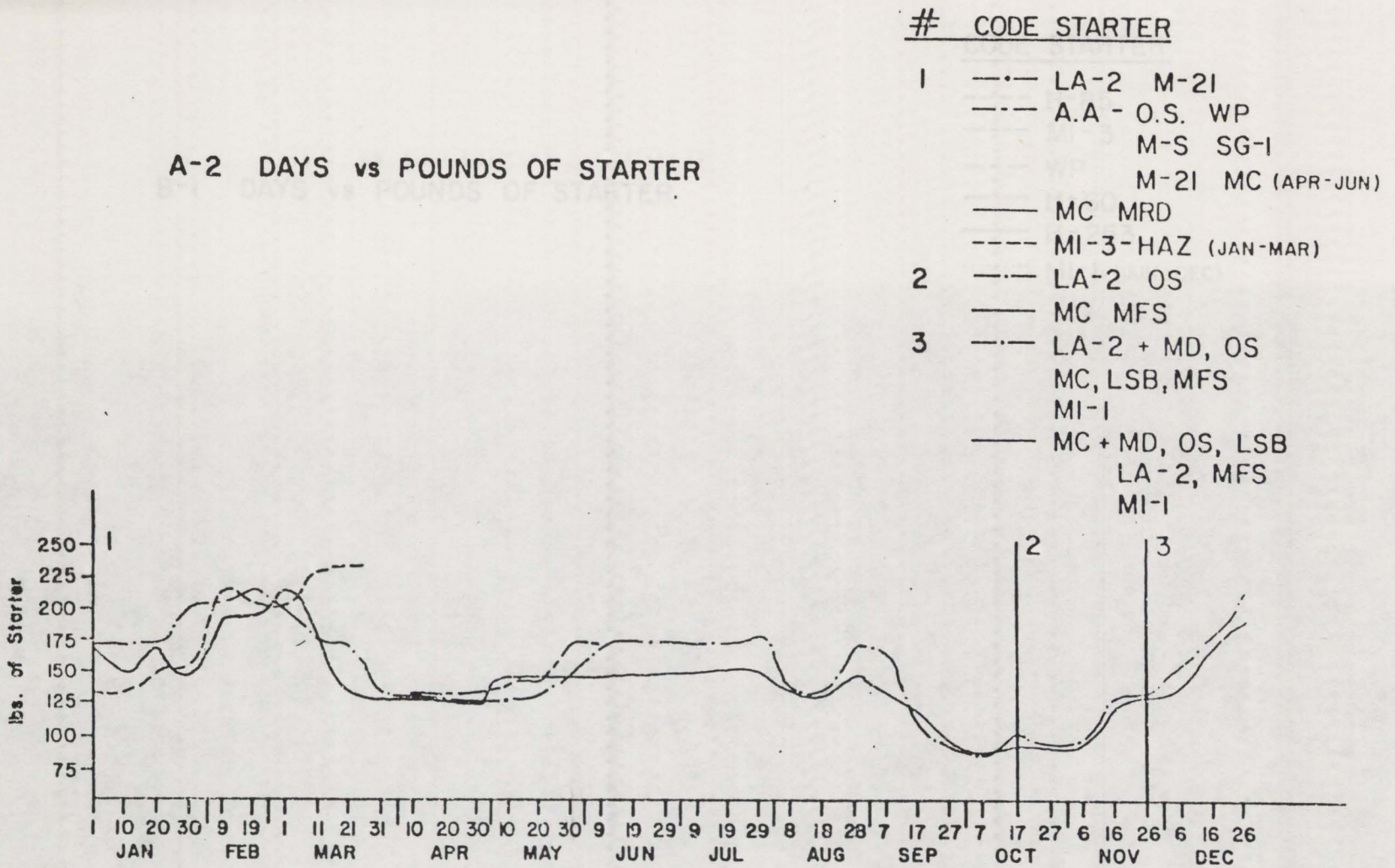

Figure 3. Graphic representation of indicated commercial starter culture inoculum for the year of 1977 at Plant A(2).

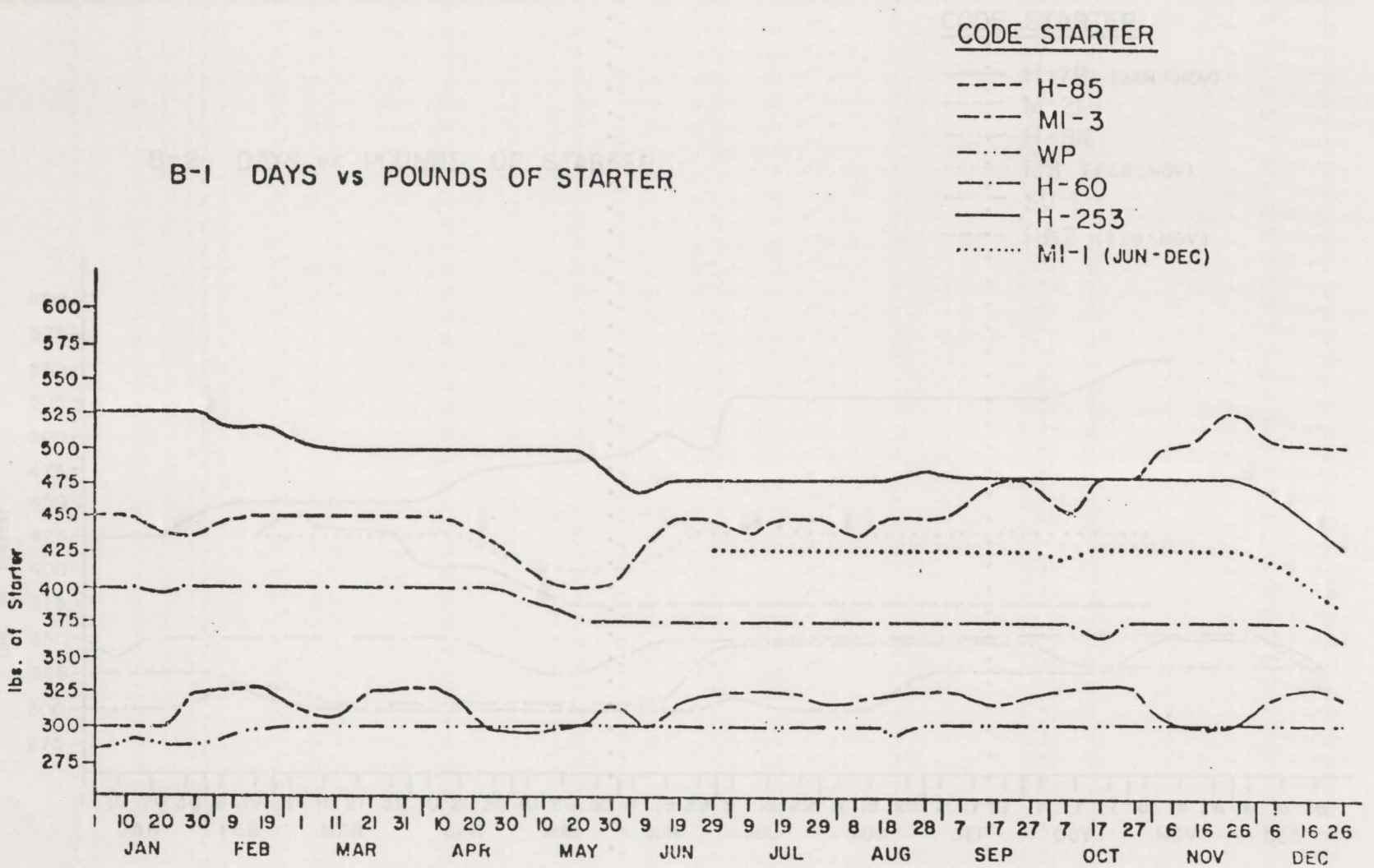

Figure 4. Graphic representation of indicated commercial starter culture inoculum for the year of 1977 at Plant B(1).

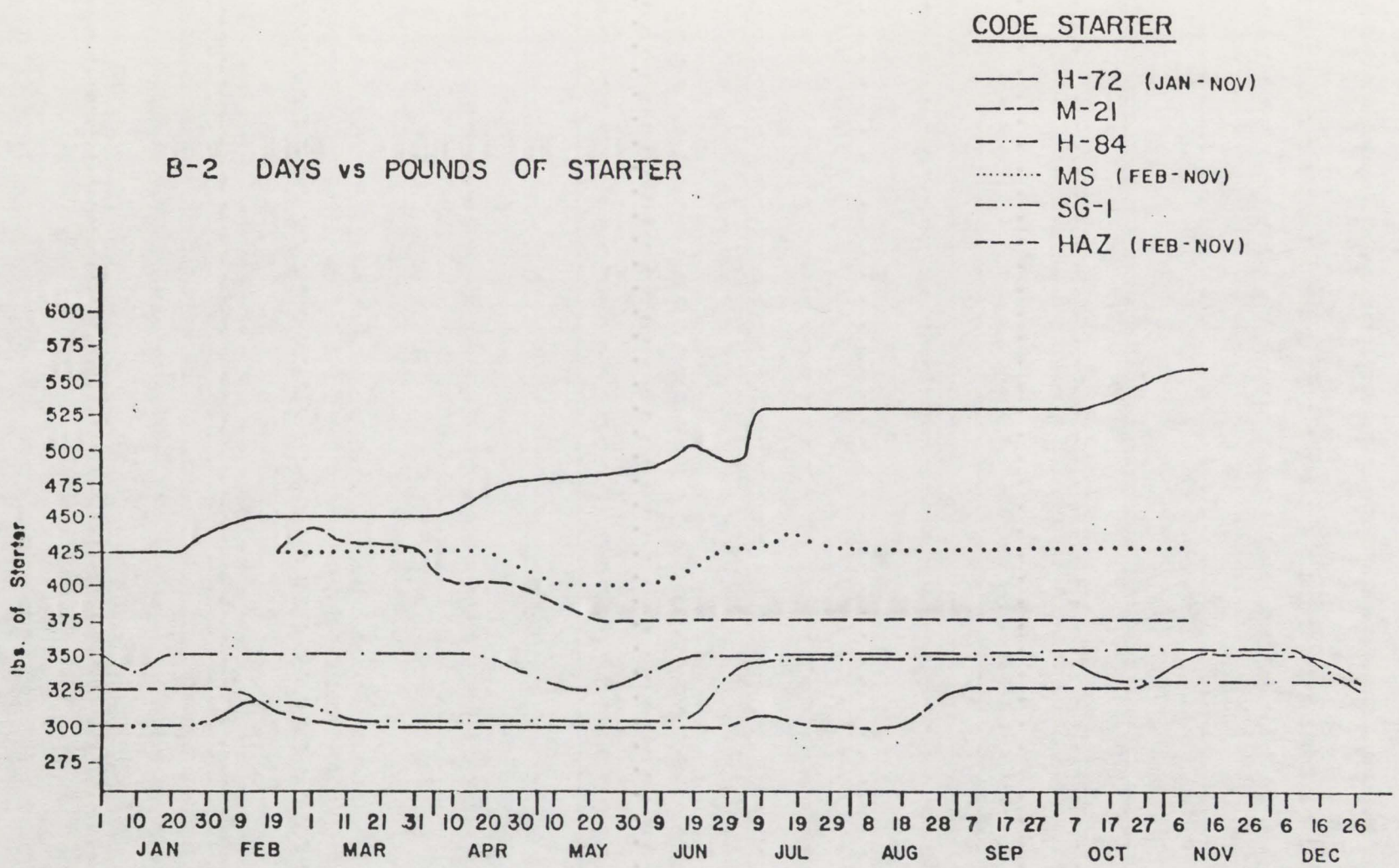

Figure 5. Graphic representation of indicated commercial starter culture inoculum for the year of 1977 at Plant B(2).

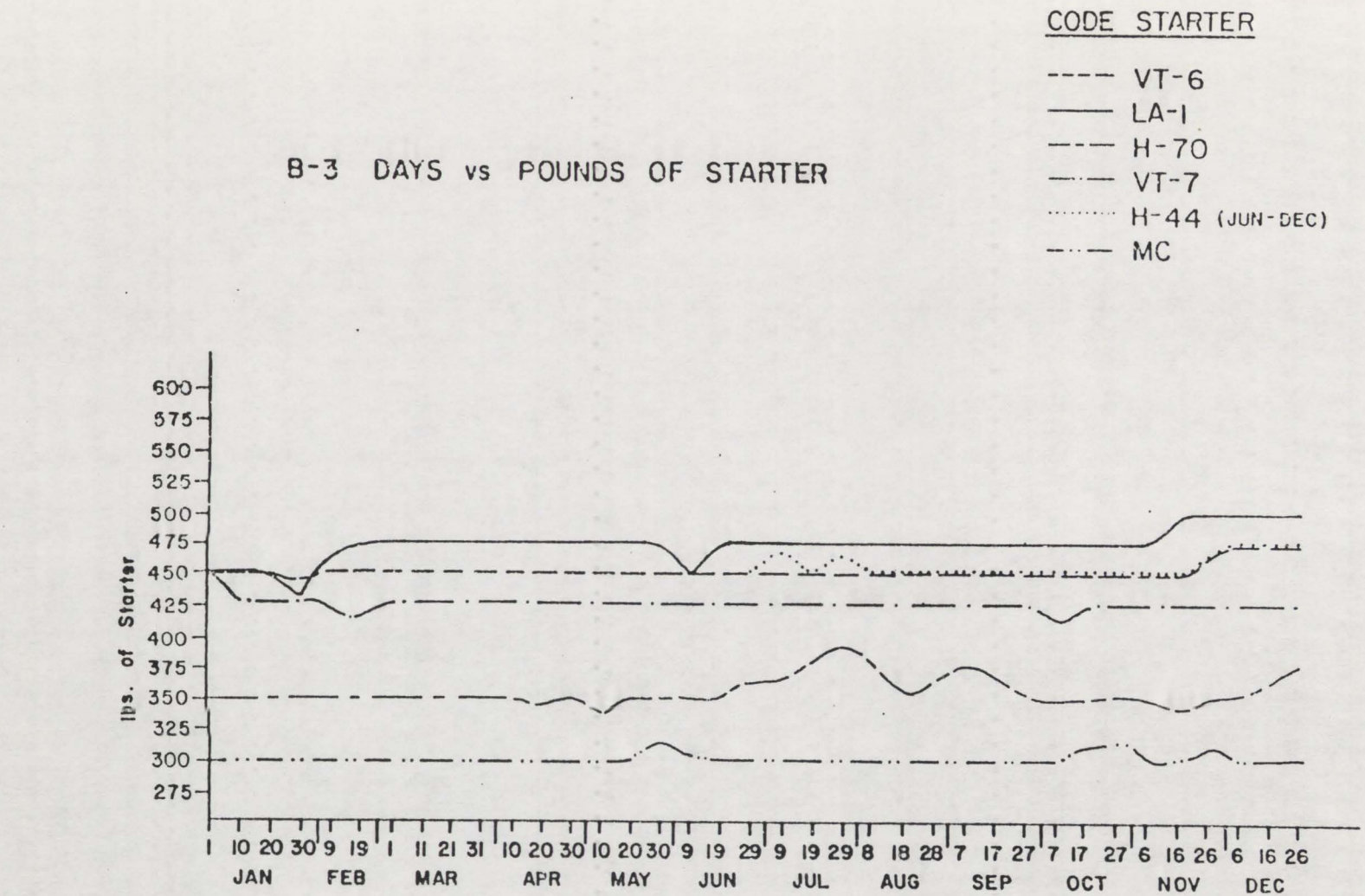

Figure 6. Graphic representation of indicated commercial starter culture inoculum for the year of 1977 at Plant B(3).

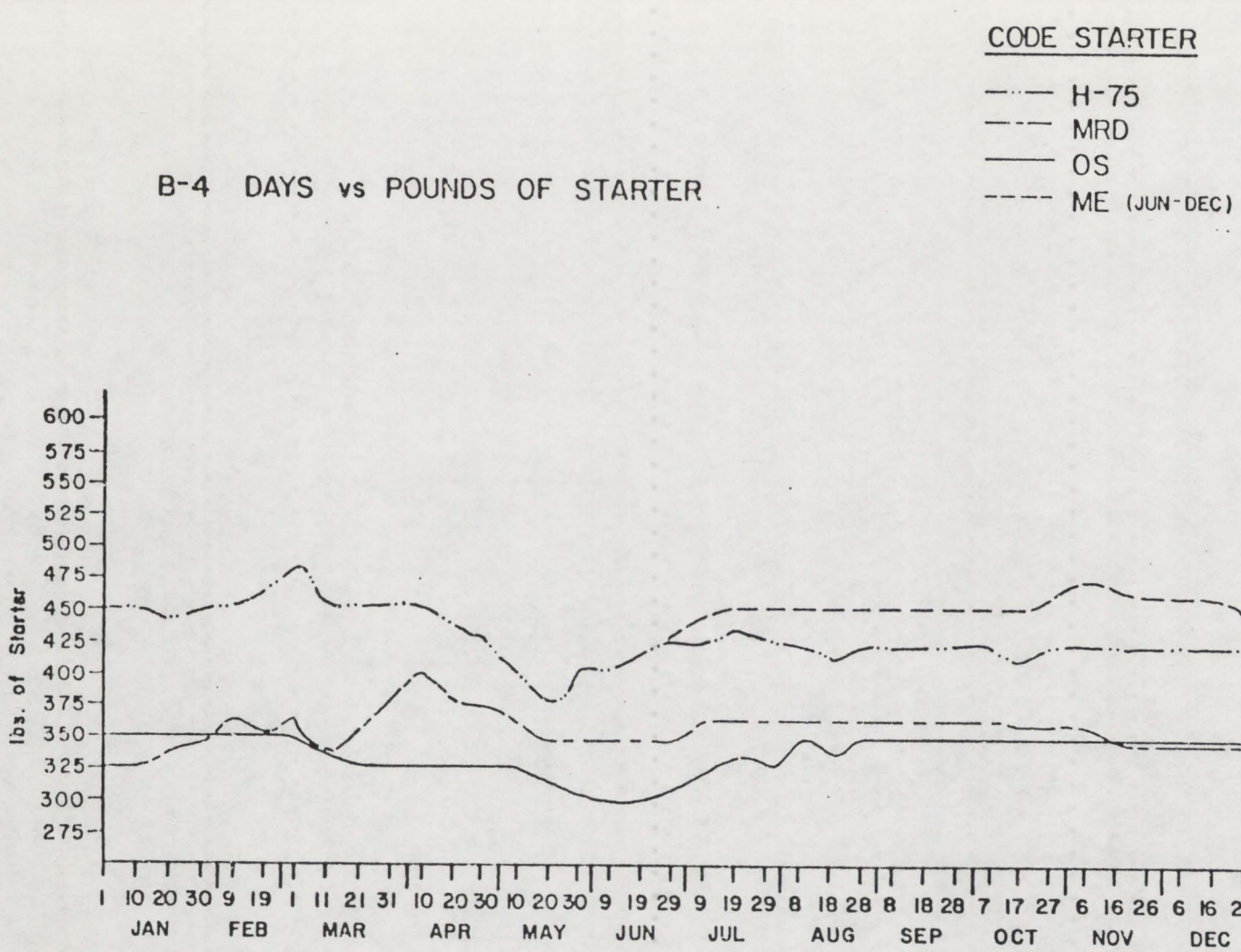

Figure 7. Graphic representation of indicated commercial starter culture inoculum for the year of 1977 at Plant B(4).

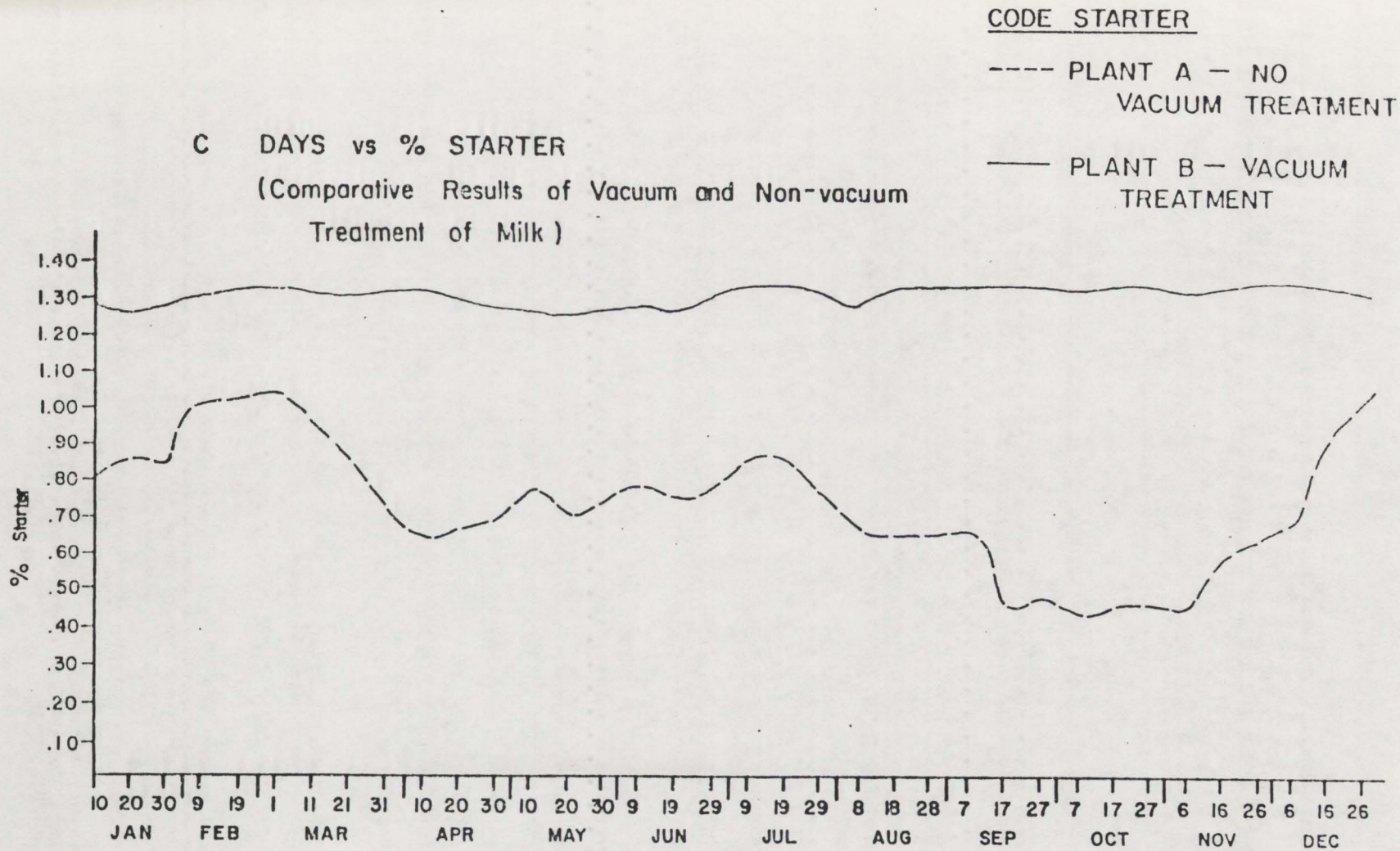

Figure 8. Graphic representation of previous graphs (Figures 2-7) with percent starter culture addition for the year of 1977 at Plants A and B. Comparative results of vacuum and non-vacuum treatment of milk.

milk without vacuum treatment, displayed a higher percent starter requirement from December through March, leveled off from April through August, decreased from September through October and began to increase again in November. Plant B, using Grade A Milk and vacuum treatment demonstrated an extremely uniform starter inoculation throughout the year.

Plant A averaged .85% starter and Plant B averaged 1.3% starter used due to the application of pH controlled propagation of starter cultures at Plant A (58). By using pH control, Plant A was able to obtain higher starter culture numbers and thus less inoculum needed as compared to Plant B using phage inhibitory media without pH control.

Results of preliminary data established the need to test for seasonal variation of milk and its effect on lactic culture performance. From September of 1979 to August of 1980, milk was collected from Plants A and B at monthly intervals and tested.

Milk Analysis

Somatic cell count

Somatic cell counts of milk from Plant A are shown in Table 1. Counts in January and December were lower than in June. The mean

Table 1. Seasonal variation of specified milk parameters for Plant A (Sept. 1979-Aug. 1980)

Month	Somatic Cell Count[1] Somatic Cells/ml WMT	RBV	Acid Degree Value[2] ADV Units	Total Plate Count[3] Raw Milk Colonies/ml
Jan.	400,000	350,000	.65	-
Feb.	300,000	270,000	.65	6.4×10^5
Mar.	300,000	325,000	.60	6.4×10^5
Apr.	300,000	280,000	.60	5.8×10^5
May	300,000	300,000	.65	8.0×10^5
June	200,000	250,000	.60	3.6×10^5
July	300,000	320,000	.65	3.1×10^5
Aug.	300,000	250,000	.60	7.0×10^5
Sept.	275,000	-	-	-
Oct.	325,000	-	-	-
Nov.	300,000	260,000	.65	-
Dec.	300,000	350,000	.65	-
Mean	300,000	295,000	.63	5.8×10^5
Std. Dev.	44,000	39,000	.03	1.8×10^5

[1]Somatic Cell Count was performed in triplicate using the Wisconsin Mastitis Test and the Rolling Ball Viscometer.
[2]Acid Degree Value, as outlined by Thomas et al (71), was performed in duplicate.
[3]Total Plate Count using M-17 (70) with raw milk was performed in duplicate.

for Plant A was 300,000 somatic cells per milliliter. There was excellent uniformity in the somatic cell count in milk from Plant B (Table 2) with one high occurring in August. The mean for Plant B was 108,000 (WMT) or less than 200,000 (RBV) somatic cells per milliliter. Variation of means between the RBV and the WMT of Plant B was due to sensitivity of the RBV (200,000 compared to 100,000).

Acid degree value (ADV)

Data from Table 1 for Plant A showed ADV unit values higher during January, February, May, July, November, and December. The average for Plant A was .63 ADV units. Plant B (Table 2) indicated higher ADV units during February, May, and August. The average was .58 ADV units.

Total plate count - raw milk

Plants A and B (Tables 1 and 2) correlated with respect to high and low plate counts. February, March, April, May, and August showed the higher plate counts. Plant A averaged 5.8×10^5 colonies/ml and Plant B 4.1×10^5 colonies/ml for the seven-month testing period.

Table 2. Seasonal variation of specified milk parameters for Plant B (Sept. 1979-Aug. 1980)

Month	Somatic Cell Count[1] Somatic Cells/ml WMT	RBV	Acid Degree Value[2] ADV Units	Total Plate Count[3] Raw Milk Colonies/ml
Jan.	100,000	< 200,000	.50	-
Feb.	100,000	< 200,000	.65	4.0×10^5
Mar.	100,000	< 200,000	.55	4.7×10^5
Apr.	100,000	< 200,000	.55	6.2×10^4
May	100,000	< 200,000	.65	4.3×10^5
June	100,000	< 200,000	.60	1.2×10^5
July	100,000	< 200,000	.60	2.0×10^5
Aug.	200,000	200,000	.65	6.7×10^5
Sept.	100,000	-	-	-
Oct.	100,000	-	-	-
Nov.	100,000	< 200,000	.60	-
Dec.	100,000	< 200,000	.50	-
Mean	108,000	-*	.58	4.1×10^5
Std. Dev.	29,000	-*	.06	2.0×10^5

[1]Somatic Cell Count was performed in triplicate using the Wisconsin Mastitis Test and the Rolling Ball Viscometer.
[2]Acid Degree Value, as outlined by Thomas et al (71), was performed in duplicate.
[3]Total Plate Count using M-17 (70) with raw milk was performed in duplicate.
*Not Calculable.

Inhibitory test

Tabular data for the Inhibitory Test is not provided. Inhibition was observed only twice. Slight inhibition on the raw milk sample for UC77 was observed in January at Plant B. Inhibition of both UC77 and UL13 was observed for high heat milk (82C for 3 min) during the month of May at Plant A.

Activity test

Review of Figures 9 through 12 illustrates graphically ΔpH and ΔTA of both plants. Interpretation of Figures 9 and 10 (Plant A) indicated the performance of the selected lactic cultures throughout the year. Although Figures 9 and 10 were different measurement parameters of culture performance and cannot be compared directly, one can observe that a high point for any treatment in Figure 9 coincides with a high point for any treatment in Figure 10 as well as low points corresponding between graphs. The ΔTA (Figure 10) measurements appear much more sensitive to change. Interpretation of Figures 11 and 12 (Plant B) was similar to Plant A.

Analysis - months. Statistically there was no difference between milk within plants (Appendix II and III). Least Significant Difference (LSD) analysis of both ΔpH and ΔTA for months can be found in Tables 3 and 4. The means on Tables 3 and 4 were determined by

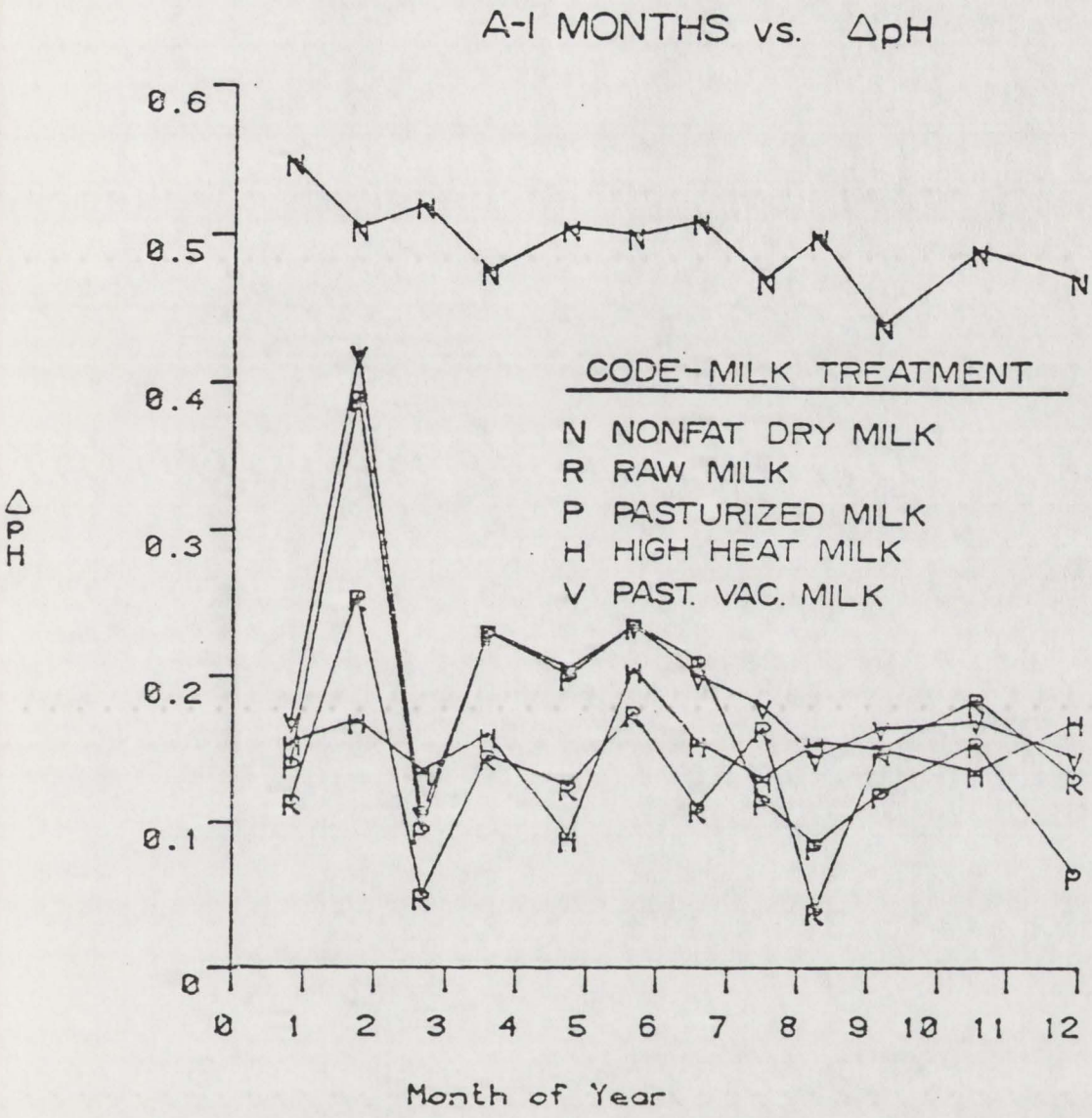

Figure 9. Graphic representation of ΔpH at Plant A for the indicated milk treatments over a year's duration (Sept. 1979-Aug. 1980).

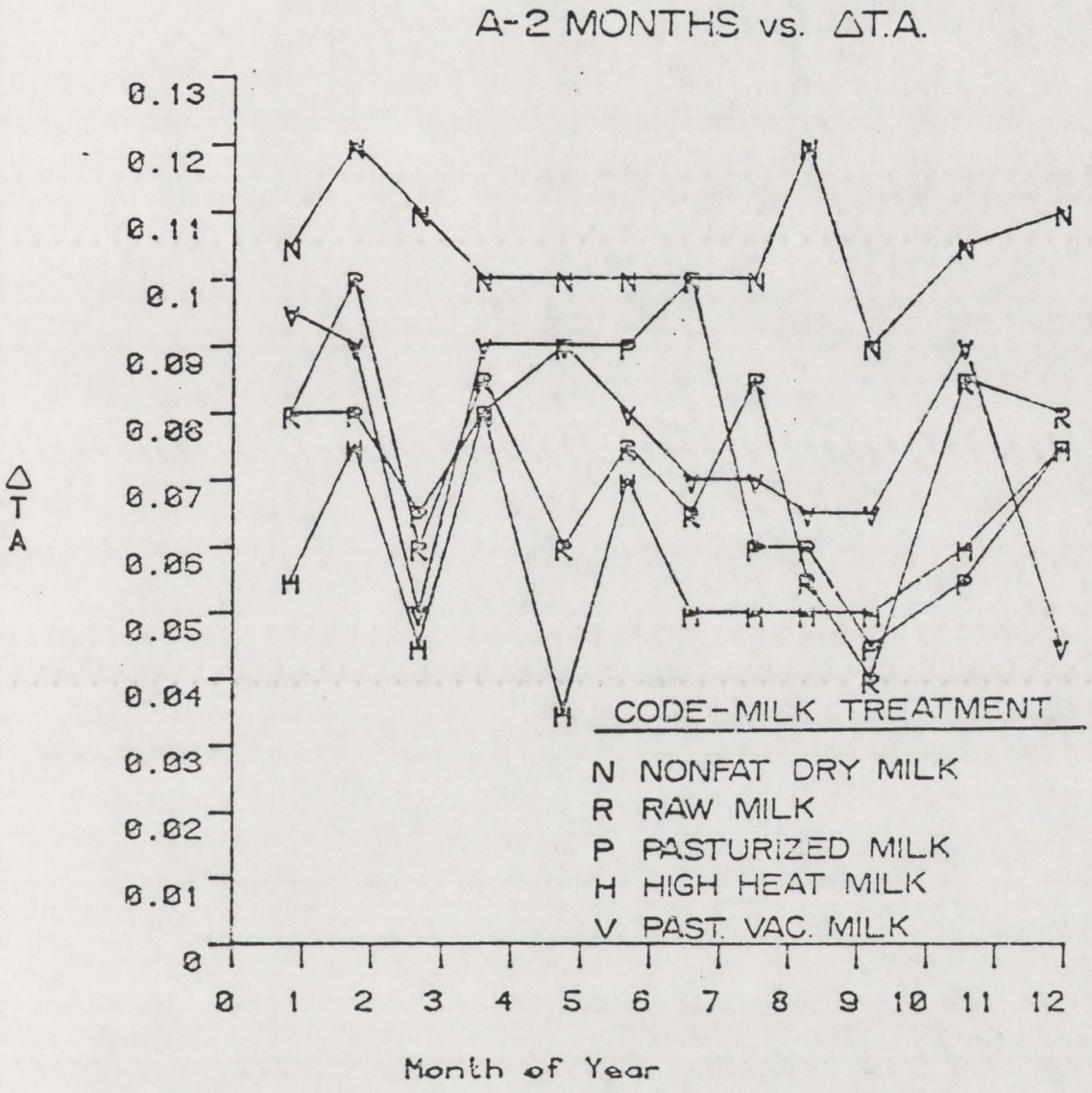

Figure 10. Graphic representation of ΔTA at Plant A for the indicated milk treatments over a year's duration (Sept. 1979-Aug. 1980).

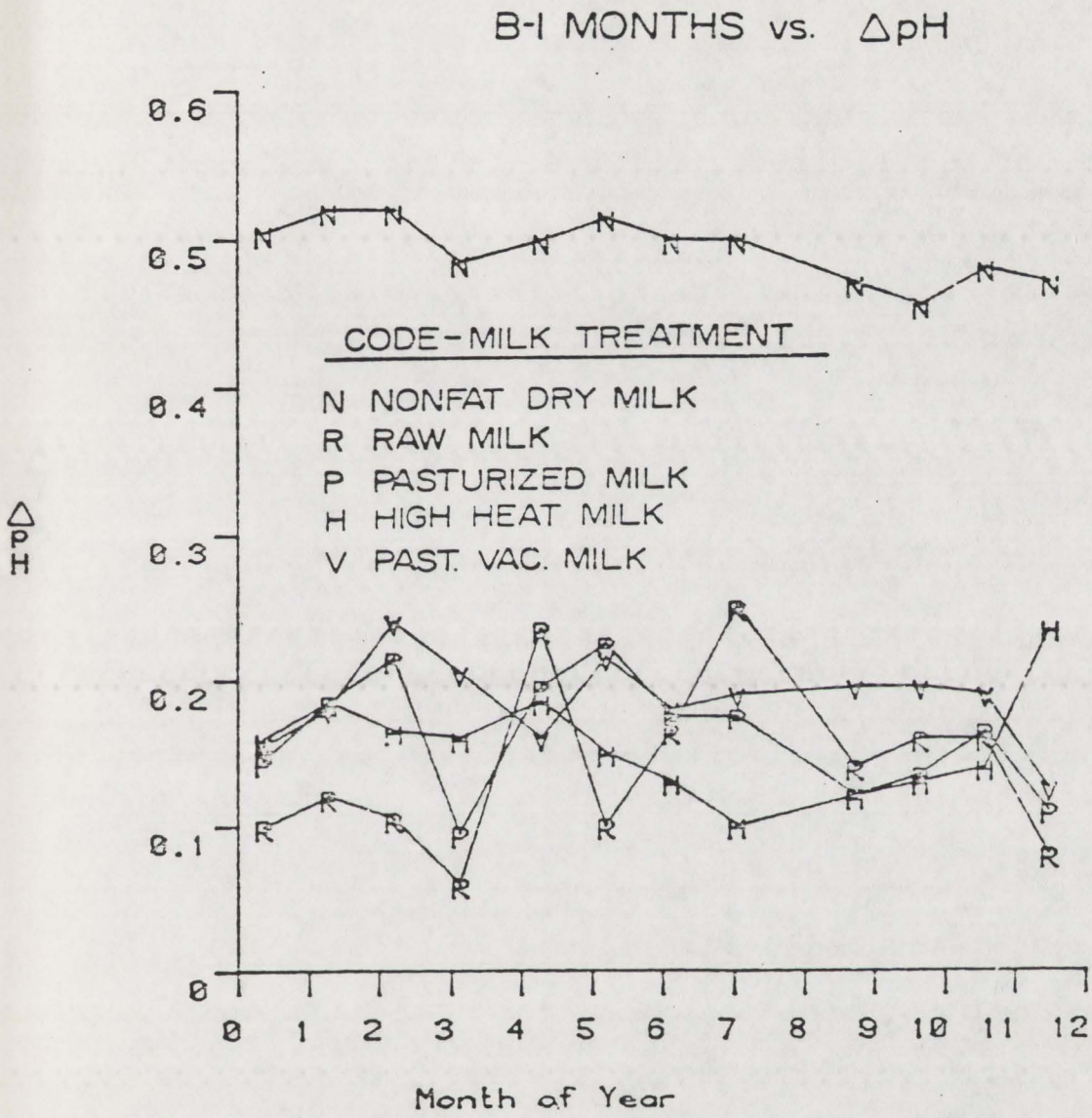

Figure 11. Graphic representation of ΔpH at Plant B for the indicated milk treatments over a year's duration (Sept. 1979-Aug. 1980).

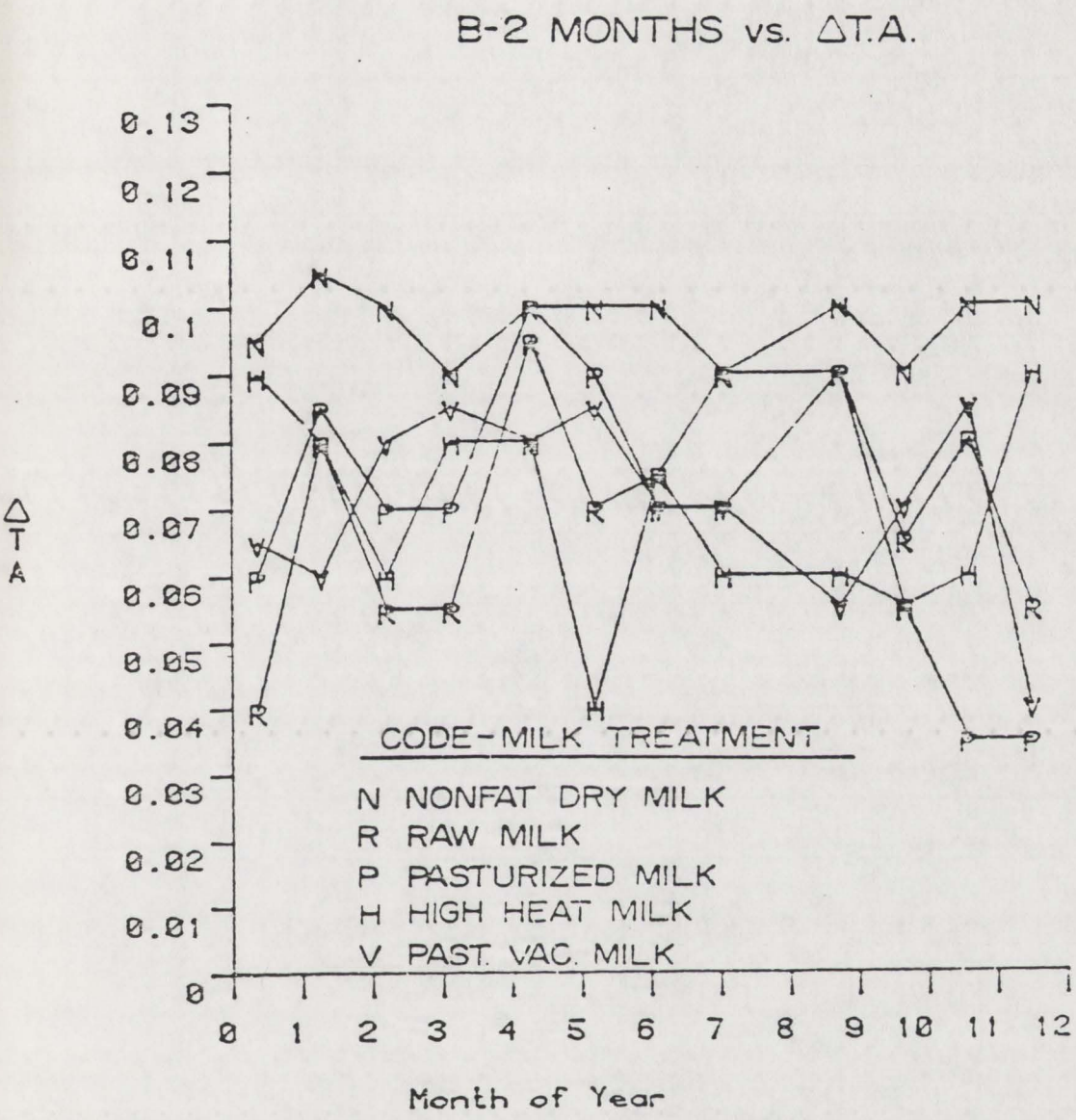

Figure 12. Graphic representation of ΔTA at Plant B for the indicated milk treatments over a year's duration (Sept. 1979-Aug. 1980).

subtracting the milk treatment (raw, pasteurized, pasteurized-vacuumized, and high heat) from the NDM control. Therefore, a low mean on Tables 3 or 4 indicated high culture activity within that month. Results of Tables 3 and 4 indicated high lactic culture performance in February, April, and June. Medium culture performance was observed in November, December, and July. Low culture performance was observed in September, March, and January. October, August, and May showed inverse proportionality between ΔpH and ΔTA. Data of ΔpH values (Table 3) correlated well with Figure 8 for data obtained in 1977 excluding September and February.

Analysis - milk treatments. Data from Tables 5 and 6 illustrates the LSD analysis for milk treatments of both ΔpH and ΔTA. The lower the mean, the higher the culture activity. High heat and raw milk shared the position of the lowest average culture performance. Table 5 representing ΔpH for LSD analysis produced evidence that pasteurized-vacuumized milk has the highest culture performance throughout the year and was statistically different from all other treatments. Pasteurized milk showed no statistical difference from high heat milk. Interpretation of Table 6 indicated no statistical difference between raw, pasteurized, and pasteurized-vacuumized milk although pasteurized-vacuumized milk again demonstrated the highest culture activity.

Table 3. Least significant difference (LSD)* analysis for months - pH measurement (Sept. 1979-Aug. 1980)

Month	Jan.	March	Sept.	July	Dec.	May	Aug.	Nov.	June	Apr.	Oct.	Feb.
Mean	.3825	.3775	.3550	.3381	.3375	.3256	.3231	.3206	.31625	.3150	.2944	.2719

LSD = .0239

*Interpretation of LSD Analysis -
The smaller the numerical mean in this study, indicates better performance of the lactic cultures for that month. Therefore, Feb., Oct., and April indicate good culture performance months while Jan., March, and Sept. are poor culture performance months. A common single line under months indicates no statistical difference between months, therefore, Jan. and March are statistically equivalent months while Jan. and Sept. are statistically different months.

Table 4. Least significant difference (LSD)* analysis for months - TA measurement (Sept. 1979-Aug. 1980)

Month	March	Sept.	Dec.	Jan.	Oct.	Nov.	July	June	Feb.	May	Aug.	Apr.
Mean	.0444	.0437	.0431	.0344	.0331	.0300	.0287	.0250	.0237	.0212	.0212	.0169

LSD = .0025

*Interpretation of LSD Analysis -
The smaller the numerical mean in this study, indicates better performance of the lactic cultures for that month. Therefore, April, Aug., and May indicate good culture performance months while March, Sept., and Dec. are poor culture performance months. A common single line under months indicates no statistical difference between months, therefore, March, Sept., and Dec. are statistically equivalent months while March and Jan. are statistically different months.

Table 5. Least significant difference (LSD)* analysis for milk treatment - pH measurement (Sept. 1979-Aug. 1980)

Milk	Raw	High Heat	Pasteurized	Pasteurized-vacuumized
Mean	.355	.339	.326	.299

LSD = .0163

Table 6. Least significant difference (LSD)* analysis for milk treatment - T.A. measurement (Sept. 1979-Aug. 1980)

Milk	High Heat	Raw	Pasteurized	Pasteurized-vacuumized
Mean	.0371	.0292	.0285	.0271

LSD = .0029

*Interpretation of LSD Analysis -
The smaller the numerical mean in this study, indicates the better treatment. Therefore, pasteurized-vacuumized milk is the best treatment to enhance culture performance for both pH and T.A. measurements (Tables 5 and 6). Raw and high heat milk share the position for the least effective treatment to enhance culture performance for both pH and T.A. measurements (Tables 5 and 6). A common single line under treatments indicates no statistical difference between treatments, therefore, in Table 5, raw and high heat milk treatments are statistically equivalent, while raw and pasteurized milk treatments in Table 5 are statistically different treatments.

Data in Tables 7 and 8 represent means and standard deviations of Δ pH and ΔTA but separated according to Plant A or B. The NDM control was uniform in both ΔpH and ΔTA throughout the year. High heat milk was essentially the same for Δ pH and Δ TA in both plants and also showed uniformity throughout the year although maintaining a low mean average. Raw milk in both plants was the same by observation of ΔpH and ΔTA of Tables 7 and 8 thus supporting the previous statistic that milk within plants was equivalent (Appendices II and III). Variations occurred when examining pasteurized milk and pasteurized-vacuumized milk of both Tables 7 and 8, although in a total overview pasteurized-vacuumized milk showed the best performance of lactic culture activity (a high mean value) and stability (a low standard deviation) throughout the year.

Whey Analysis

Plate count - whey

Data from Tables 9 and 10 represent the fluctuation of the plate count after 16 h under pH control. Raw (heat) and pasteurized-vacuumized (heat) whey showed little variation during the seven-month period. Raw (no heat) and pasteurized-vacuumized (no heat) whey showed variations with high plate counts occurring in May for both Plants A and B. Lower plate counts were observed in March and June for Plant A and March only for Plant B.

Table 7. Mean and standard deviation for Δ pH and Δ TA for Plant A Manufacturing Grade Milk (Sept. 1979-Aug. 1980)

Treatment	Mean and Std. Dev. for Δ pH	Mean and Std. Dev. for Δ TA
NDM	.496 ± .032	.106 ± .009
Raw	.137 ± .058	.072 ± .018
Past	.171 ± .094	.072 ± .015
Past-Vac.	.199 ± .086	.076 ± .018
High Heat	.152 ± .030	.059 ± .015

Table 8. Mean and standard deviation for Δ pH and Δ TA for Plant B Grade A Milk (Sept. 1979-Aug. 1980)

Treatment	Mean and Std. Dev. for Δ pH	Mean and Std. Dev. for Δ TA
NDM	.491 ± .024	.098 ± .005
Raw	.14 ± .058	.071 ± .017
Past	.162 ± .041	.069 ± .021
Past-Vac.	.186 ± .030	.070 ± .014
High Heat	.155 ± .036	.069 ± .015

Table 9. Standard plate count of whey treatments after 16 h pH stat - Plant A (Feb. 1980-Aug. 1980)

Month	Raw (No Heat)	Raw (Heat)	Past.-Vac. (No Heat)	Past.-Vac. (Heat)
Jan.	-	-	-	-
Feb.	3.0×10^8	2.2×10^8	3.0×10^8	2.2×10^8
March	2.3×10^8	2.0×10^8	2.3×10^8	2.0×10^8
April	3.0×10^8	2.2×10^8	3.0×10^8	2.2×10^8
May	4.0×10^8	2.4×10^8	4.3×10^8	2.2×10^8
June	1.8×10^8	1.6×10^8	2.0×10^8	2.0×10^8
July	2.6×10^8	9.5×10^7	2.8×10^8	1.0×10^8
Aug.	3.0×10^8	2.0×10^8	3.4×10^8	2.2×10^8
Sept.	-	-	-	-
Oct.	-	-	-	-
Nov.	-	-	-	-
Dec.	-	-	-	-

Table 10. Standard plate count of whey treatments after 16 h pH stat - Plant B (Feb. 1980-Aug. 1980)

Month	Raw (No Heat)	Raw (Heat)	Past.-Vac. (No Heat)	Past.-Vac. (Heat)
Jan.	-	-	-	-
Feb.	2.4×10^8	2.0×10^8	2.4×10^8	2.0×10^8
March	2.0×10^8	1.8×10^8	2.0×10^8	1.8×10^8
April	2.4×10^8	2.0×10^8	2.0×10^8	2.0×10^8
May	4.0×10^8	2.1×10^8	4.0×10^8	2.1×10^8
June	3.2×10^8	2.0×10^8	3.4×10^8	2.4×10^8
July	2.5×10^8	2.0×10^8	2.4×10^8	2.1×10^8
Aug.	3.3×10^8	2.2×10^8	3.2×10^8	2.1×10^8
Sept.	-	-	-	-
Oct.	-	-	-	-
Nov.	-	-	-	-
Dec.	-	-	-	-

pH stat - whey

Observation of Figures 13 and 14 indicates graphically the seasonal variation of whey in support of lactic culture growth. The Y axis indicates the average milliequivalents of NaOH utilized per h for each of the four treatments collected monthly for a year's duration (X axis). Plants A and B correlate in reference to high and low points on the graphs.

Analysis - months. Statistically there was no difference between plants in the whey analysis (Appendix IV). Observation of Table 11, indicating LSD analysis for months, showed high performance of culture activity during September, October, November, and January. Medium performance occurred in December, August, March, and May. Low performance was observed in June, April, February, and July.

Analysis - whey treatments. Results from Table 12 indicated high performance of lactic cultures in non-heated pasteurized-vacuumized and raw whey with no statistical difference between the treatments. Heating to 90C for 45 min of raw and pasteurized-vacuumized whey decreased the whey's ability to support lactic culture growth evidenced by lower mean values of milliequivalents NaOH added. There was no statistical difference between raw and pasteurized-vacuumized heat treated wheys.

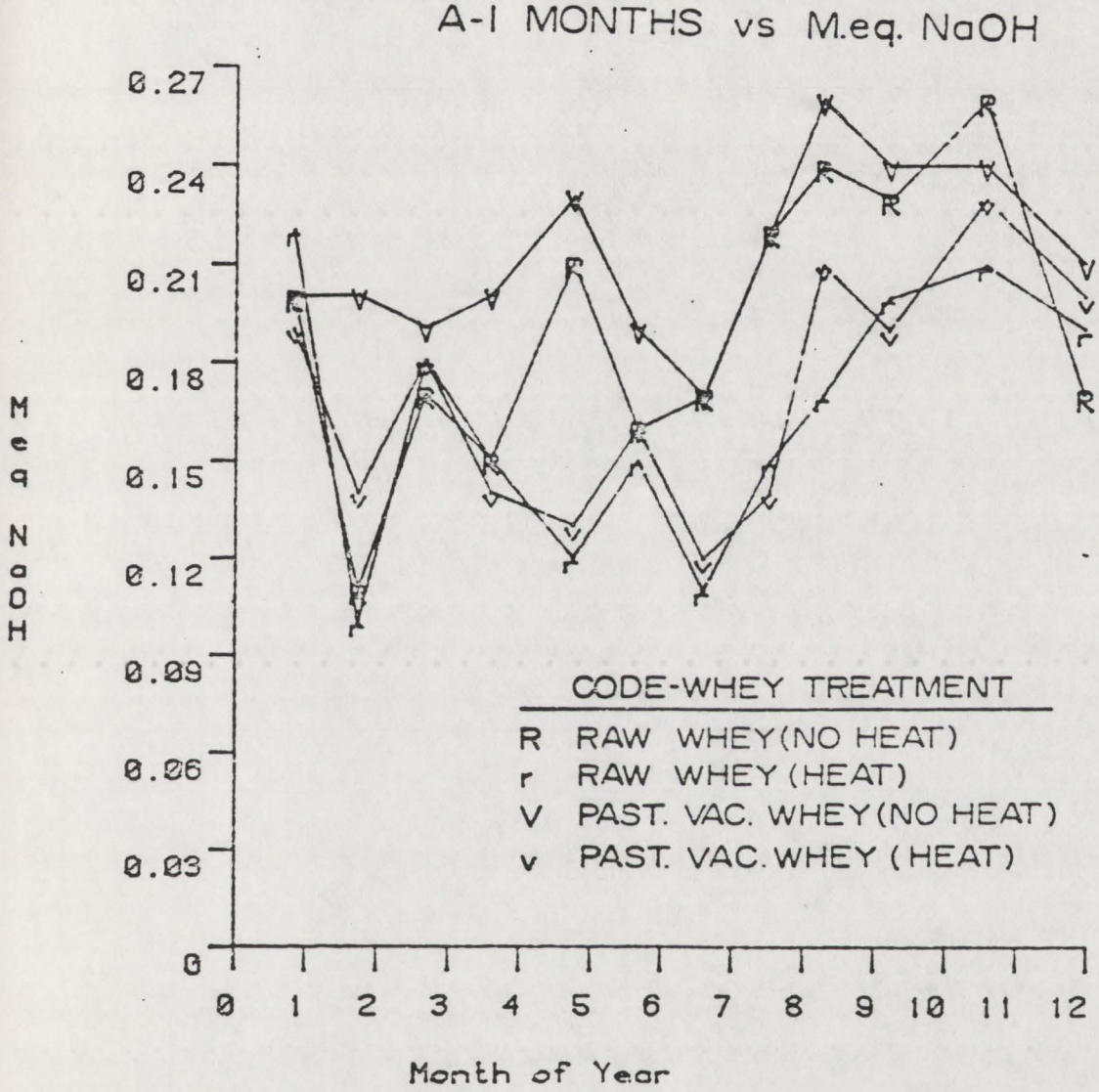

Figure 13. Graphic representation of the average milliequivalents NaOH used under pH stat conditions by the indicated whey treatment for a year's duration at Plant A (Sept. 1979-Aug. 1980).

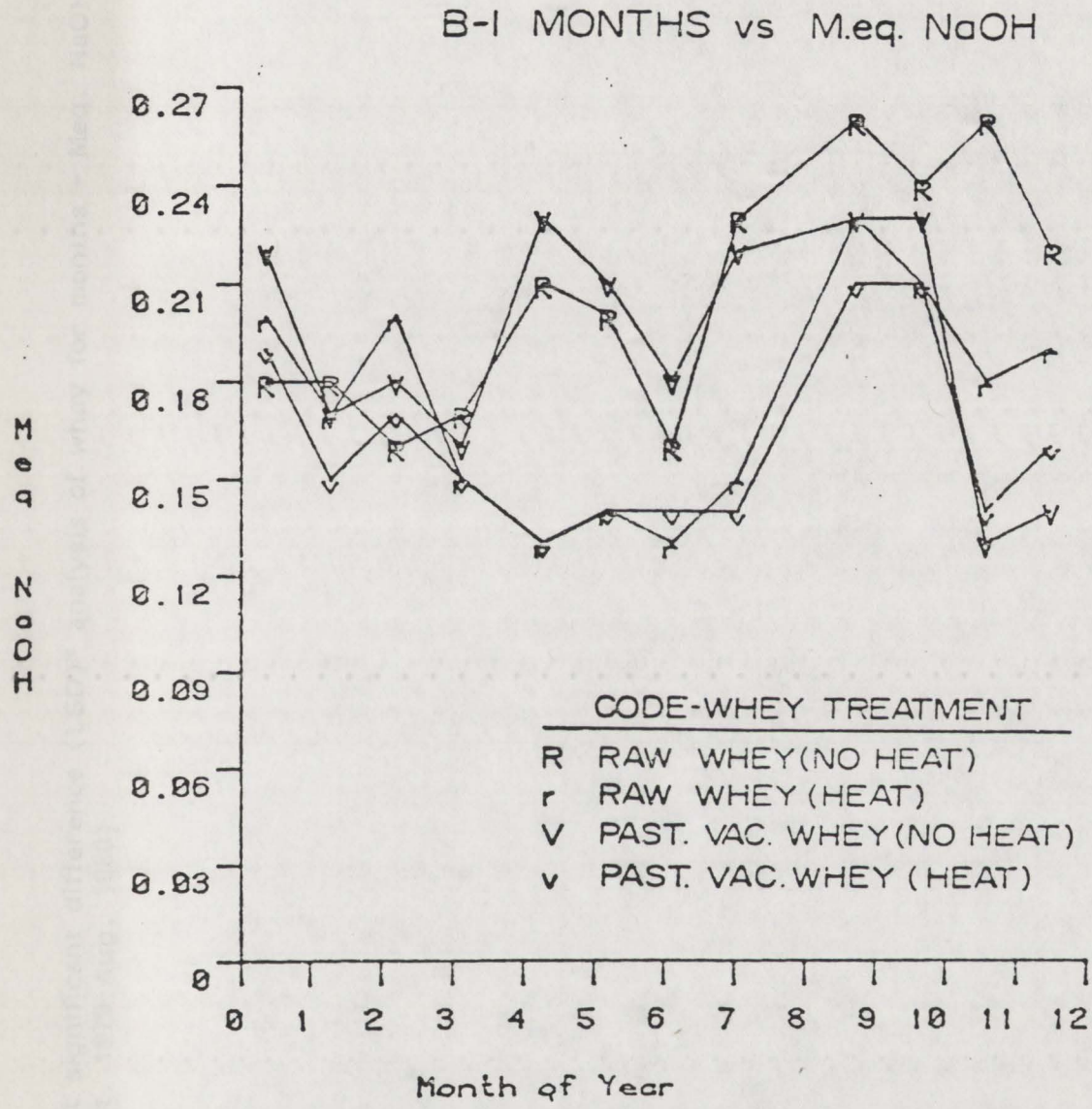

Figure 14. Graphic representation of the average milliequivalents NaOH used under pH stat conditions by the indicated whey treatment for a year's duration at Plant B (Sept. 1979-Aug. 1980).

Table 11. Least significant difference (LSD)* analysis of whey for months - Meq. NaOH (Sept. 1979-Aug. 1980)

Month	Sept.	Oct.	Nov.	Jan.	Dec.	Aug.	March	May	June	April	Feb.	July
Mean	.2262	.2187	.2075	.2000	.1850	.1837	.1787	.1737	.1687	.1587	.1525	.1475

LSD = .01626

*Interpretation of LSD Analysis -
The larger the numerical mean in this study, indicates better performance of the lactic cultures for that month. Therefore, Sept., Oct., and Nov. indicate good culture performance months while April, Feb., and July are poor culture performance months. A common single line under months indicates no statistical difference between months, therefore, Sept. and Oct. are statistically equivalent months while Sept. and Nov. are statistically different months.

Table 12. Least significant difference (LSD)* analysis of whey for treatments - Meq. NaOH (Sept. 1979-Aug. 1980)

Whey	Past.-Vac. (No Heat)	Raw (No Heat)	Raw (Heat)	Past.-Vac. (Heat)
Mean	.2021	.1983	.1679	.1654

LSD = .02769

*Interpretation of LSD Analysis -
The larger the numerical mean indicates the better treatment. Therefore, pasteurized-vacuumized (no heat) whey is the best treatment to enhance culture performance (as measured by Meq. NaOH added). A common single line under treatments indicates no statistical difference between treatments, therefore, pasteurized-vacuumized (no heat) whey is statistically equivalent to raw (no heat) whey while pasteurized-vacuumized (no heat) whey is statistically different from raw (heat) whey treatment.

DISCUSSION

Milk Samples

Raw milk

Raw milk fluctuated in its support of lactic culture growth throughout the year. Of the four treatments studied, raw milk showed the lowest average for total acid production by starter cultures and was the most variable. Somatic cell count on bulk dairy plant samples showed little variation throughout the year. There was a noticeable difference in milk quality between Manufacturing grade milk (Plant A) averaging 300,000 somatic cells per milliliter as compared to Grade A milk (Plant B) averaging slightly over 100,000 somatic cells per milliliter. The Inhibitory Test used to determine the presence of inhibitory compounds was not sensitive enough in comparison to the pH and TA activity tests. Inhibition was observed at points which correlated with the activity test results. Results from the acid degree value (ADV) test do not correlate directly with the activity tests and, therefore, would not indicate culture performance. Manufacturing grade milk (Plant A) showed a higher average ADV unit than Grade A Milk (Plant B) indicating more incidence of hydrolytic rancidity. Plate counts or microflora examination of the raw milk indicated late winter and early spring as high periods of microbial counts but annual data are inconclusive since only seven months of data were obtained.

Performance of lactic cultures in raw milk was equivalent between Plants A and B (Tables 7 and 8).

Pasteurized milk

Pasteurized milk supported lactic culture growth throughout the seasons and was surpassed only by pasteurized-vacuumized milk. A heat treatment of 62.7C for 30 min provided more uniformity and eliminated much of the undesirable microflora. Performance of lactic cultures in pasteurized milk was similar between Plants A and B.

Pasteurized-vacuumized milk

Pasteurized-vacuumized milk best supported lactic culture growth throughout the seasons. It revealed that pasteurization along with a vacuum treatment enhances culture activity. The data supported Kulshrestha and Marth's (42) observation that volatile inhibitory compounds in milk detrimentally affected certain lactic cultures and that they can be removed by vacuum treatment. Both Plants A and B indicated optimum culture activity and seasonal stability using pasteurized-vacuumized treated milk.

High heat milk

High heat milk along with raw milk showed inferior support for lactic culture growth. Of interest is the excellent stability (Tables 7

and 8) of high heat milk. Heat treatment of 82C for 3 min eliminated natural inhibitors in milk (19) but also decreased the performance of lactic cultures (67). A review by Tamine and Deeth (67) indicates that heating of milk can either inhibit or stimulate the activity of lactic starter cultures. The events are (a) stimulation of starter in milk heated between 62C for 30 min and 72C for 40 min; (b) inhibition of starter in milk heated between 72C for 45 min and 82C for 10-120 min, as well as 90C for 1-45 min; (c) stimulation of starter in milk heated to 90C for 60-180 min or autoclaved (120C for 15-30 min); and (d) inhibition of starter in milk autoclaved at 120C for longer than 30 min. The effect of heat treatment of milk on starter behavior, i.e., the apparent stimulation/inhibition/stimulation/inhibition cycle could be duplicated by the addition of denatured serum protein of cysteine hydrochloride. The transition from one phase of the cycle to another in response to different heat treatment exposures occurred as a result of the release of denatured serum protein nitrogen of concentrations of 0.15-0.20 mg/milliliters. When cysteine was added artificially, it augmented the sulfhydryl groups made available by heating. Thus the cysteine became stimulatory in raw and slightly heated milks, but it was inhibitory in highly heated milks.

Nonfat dry milk (NDM)

NDM demonstrated its usefulness as a measurement of lactic culture performance as a controlled medium. Stock cultures performed

evenly throughout the year with minimal variation as observed by graph and statistical analysis.

Overview

Both Manufacturing grade (Plant A) and Grade A (Plant B) milk showed excellent quality parameters with respect to their milk class. Pasteurized-vacuumized milk demonstrated the most stability and highest mean for culture performance. Vacuum treated milk (with pasteurization) did display some variation throughout the year, but that variation was minimal. Cultures would be expected to perform with low activity during January; high activity in February; low activity in March; high activity in April; medium activity in May, June, July, August; low activity in September; medium activity in October and November; and low activity in December.

Whey Samples

Raw and pasteurized-vacuumized whey (no heat)

Whey samples between plants were not statistically different (Appendix IV). Without heat treatment, raw and pasteurized-vacuumized whey were subject to seasonal variation (60), yet total mean performance was better than heat treated wheys. Randolph and Gould (57) and Gillies (22) reported that the inhibitory effect of milk is

carried over into the whey. Results from Plant B utilizing Grade A milk were in agreement. Raw and pasteurized-vacuumized milk coincided in lactic culture performance to raw and pasteurized-vacuumized whey without additional heat treatments. Plant A using Manufacturing grade milk did not correspond during the winter months (January through April) with the unheated raw and pasteurized-vacuumized whey samples of the same months, although the remaining months (May through December) did correlate.

Raw and pasteurized-vacuumized whey (heat)

Heat treatment of raw and pasteurized-vacuumized whey at 90C for 45 min enhanced the respectability of the whey but decreased the total mean performance. Seasonal variation corresponded closely to the non-heat treated wheys but was minimized. The heat treated whey samples showed lower culture performance means than their whey counterparts that were not heat treated. This is explained again by Tamine and Deeth (67). Different heat treatments result in the release of denatured serum protein nitrogen and sulfhydryl groups. These cysteine sulfhydryl groups are inhibitory to culture activity (67). Decreasing the heat treatment from 90C for 45 min to only 62C for 30 min should stimulate culture activity (67) and is recommended in any further study.

Overview

Whey samples coincided with their milk counterpart in respect to seasonal variation of lactic culture performance indicating that the inhibitory effect of milk is carried over into the whey fraction. Of interest is the relationship of Table 11 (LSD analysis of whey for months) to Figure 8. A similar graph could be drawn from the LSD analysis. Inhibitory effects of milk are carried over into the whey (22, 57) and possibly concentrated in the whey. It would be applicable to perform activity tests such as the Horrall-Elliker activity test (31) on rennet whey rather than whole milk to determine the projected culture activity.

No stimulants were added to the whey composites in this research. This was to allow measurement of the inhibitory effects (transferred from milk) which may have been negated if added stimulants were present.

Addition of stimulants to heat treated wheys establish this media as an excellent culture medium and is widely used today (58).

Phage. Direct phage analysis was not performed in this study. Plate counts of lactic colonies were performed after each 16 h pH stat analysis of whey. No significant changes in total numbers were observed even in recognition that if phage did exist, highest numbers would occur in whey. Heap et al (26) in 1978 found few contributions

of phage infection in hundreds of raw milk samples studied from natural flora lactics in raw milk. Finally, tests were conducted using M-16 BCP agar for differentiation of S. lactic, S. cremoris, and S. diacetylactis to determine if domination of either stock strain (UC77 and UL13) occurred in regular milk coagulation trials, and if one of the stock cultures carried active phage against the other. Results indicated UC77 at a TPC of 1×10^8 and UL13 at 8.8×10^7 as expected. Therefore, concern for phage was eliminated from this study.

CONCLUSIONS

1. There was no significant difference between Manufacturing grade and Grade A milk in the ability to support lactic culture growth, though there were differences in somatic cell counts and acid degree values in the two milk supplies.

2. For this study, acid degree values (ADV) and somatic cell counts were not useful in predicting the ability of cultures to grow in milk. It was recognized that the ADV and somatic cell counts were important for determining milk quality, but the values were so low that these factors became unimportant.

3. The Horrall-Elliker activity test (31) provided the most sensitive method to predict culture performance in cheese milk. This test could be made even more sensitive by using rennet whey from its milk counterpart in the test.

4. Whey carried over the inhibitory character of the milk from which it was prepared.

5. Pasteurization improved the culture performance in cheese milk.

6. Pasteurization coupled with a vacuum treatment equivalent to 13" Hg for 4 sec improved the milk's ability to support lactic culture growth over pasteurization alone. This treatment had the greatest effect upon optimizing culture performance and establishing uniformity of culture activity over the one year study.

7. Maintenance of one year make records helped cheese makers to anticipate those months of the year when culture inocula adjustments must be made.

8. Variations in the ability of milk to support culture growth were not strictly identifiable by seasons. The cold winter months (December, January, February, and March) indicated poor culture performance months, although February of 1980 showed average culture performance. April, May, June, July, and August, the warmer months, showed good culture performance. September was a transition month and for 1979 showed poor culture performance. October and November showed exceptionally good culture performance months.

RECOMMENDATIONS

A. Run phage, antibiotic, somatic cell counts, and culture sensitivity tests on cheese milk.

   1. Culture sensitivity tests or activity tests should prove more sensitive if rennet whey from the milk is used.

B. Insure good pasteurization and where possible vacuum treat milk to be used for cheese making.

C. Carry accurate cheese make records. Graph the previous year's starter inoculum additions for each culture. Refer to those graphs to anticipate possible starter culture slowdown according to the seasonal variation of milk.

   1. Organize the inoculation rotation period of cultures such that starters demonstrating high performance and activity can be utilized during months of anticipated slowdown due to inferior milk quality.

   2. Refer to the previous data contained within this research when problems do arise. Attempt to isolate the inhibitory problem of a seasonal milk period and correct it (i.e., vacuum treat the milk, lower somatic cell counts, prevent and monitor rancidity, observe for heat labile inhibitory factors, etc.).

3. Plants using whey-based media should refer to the whey analysis data research to anticipate culture performance in whey throughout the year and add stimulants accordingly.

D. Economical savings can be realized by the cheese plant operator by anticipation of culture slowdowns in milk or whey during the year and corrective measures implemented beforehand.

E. There was nothing found in this study to suggest that whey-based media could not be used in plants using either Grade A or Manufacturing grade milk.

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APPENDICES

Appendix I

Procedure for Correlation of Vacuum Treated Milk at Plant A (Laboratory Method) to Industrial Vacuum Treatment at Plant B

Principle

From the Kinetic Theory of Gases by R.D. Present (McGraw Hill, 1958. p. 22), we are able to estimate the amount of water vapor removed from Plant B given the temperature, inches of Mercury, and total holding time (in sec) for Plant A at a specified temperature and inches of Mercury.

Calculations

At equalibrium (saturated pressure) the rate at which molecules leave equals the rate at which molecules enter

$$= \frac{n\,\bar{c}}{4}$$

If P < Psat, rate of leaving > rate of entering. For non-equalibrium conditions, the net rate of evaporation is estimated as

$$\left(\frac{n\,\bar{c}}{4}\right)_{sat} - \left(\frac{n\,\bar{c}}{4}\right)_{\substack{\text{actual}\\ \text{vapor}\\ \text{pressure}}}$$ where

n = vapor molecule density at saturated vapor equalibrium or at vacuum

$\bar{c}$ = mean speed of molecules

$$= \left(\frac{8\,KT}{\pi\,m}\right)^{1/2}$$ where

m = mass of molecule
T = absolute temperature
K = Boltzmans constant

$$\left(\frac{n\,\bar{c}}{4}\right)_{sat} - \left(\frac{n\,\bar{c}}{4}\right)_{vac} (4\ \text{sec}) = \left(\frac{n\,\bar{c}}{4}\right)_{sat} - \left(\frac{n\,\bar{c}}{4}\right)_{vac} (X\ \text{sec})$$

Appendix I
(Continued)

$$\frac{\text{Plant B}}{(72C \text{ at } 13 \text{ in. Hg for } 4 \text{ sec})} = \frac{\text{Plant A}}{(65C \text{ at } 16 \text{ in. Hg for } X \text{ sec})}$$

Results

Calculation of X yields 5.7 sec. In an attempt to satisfy the increase surface area and efficiency of industrial vacuum treatment (Plant B), the factor was multiplied 10 times yielding (10 x 5.7 sec) 1 min at 65C and 16 inches of Mercury for the laboratory method of Plant A.

Appendix II

Analysis of Variance on Milk - pH Measurements

Source	Degrees of Freedom	Mean Square	F Ratio
Dairy	1	.2083333E-03	.17
Milk (Treatment)	3	.2710694E-01	22.67**
Dairy x Milk	3	.1034722E-02	.87
Error A	8	.1194792E-02	-
Month	11	.1596402E-01	16.79**
Error B	11	.9509470E-03	-
Dairy x Month	11	.1891629E-01	27.86**
Milk x Month	33	.4553535E-02	6.71**
Dairy x Milk x Month	33	.3324495E-02	4.90**
Error C	77	.6789773E-03	-
Total	191	.4191579E-02	-

*Significant at $\alpha = .05$

**Significant at $\alpha = .01$

Appendix III

Analysis of Variance on Milk - TA Measurements

Source	Degrees of Freedom	Mean Square	F Ratio
Dairy	1	.1463021E-02	36.48**
Milk (Treatment)	3	.9699653E-03	24.19**
Dairy x Milk	3	.6963542E-03	1.74
Error A	8	.4010417E-04	-
Month	11	.1433097E-02	133.92**
Error B	11	.1074811E-04	-
Dairy x Month	11	.7925663E-03	23.38**
Milk x Month	33	.5366319E-03	15.83**
Dairy x Milk x Month	33	.3501420E-03	10.32**
Error C	77	.3390828E-04	-
Total	191	.3311927E-03	-

*Significant at $\alpha = .05$

**Significant at $\alpha = .01$

Appendix IV

Analysis of Variance on Whey - Meq. NaOH

Source	Degrees of Freedom	Mean Square	F Ratio
Dairy	1	.9375E-05	.0056
Whey	3	.9081597E-02	5.4638
Error A	3	.1662153E-02	-
Month	11	.5182102E-02	14.10**
Dairy x Month	11	.8298295E-03	2.2586
Whey x Month	33	.9232639E-03	2.513**
Error B	33	.3674558E-03	-
Total	95	.1483849E-02	-

*Significant at $\alpha = .05$

**Significant at $\alpha = .01$