Manufacture of Cottage Cheese Utilizing a pH-controlled, Whey-based Lactic Culture

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MANUFACTURE OF COTTAGE CHEESE UTILIZING A pH-CONTROLLED, WHEY-BASED LACTIC CULTURE

by

Yen-Luon Chen

A thesis submitted in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in
Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah
1975
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Yen Luon Chen
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ABSTRACT

Manufacture of Cottage Cheese Utilizing a pH-controlled, Whey-based Lactic Culture

by

Yen Luon Chen, Master of Science

Utah State University, 1975

Major Professor: Dr. Gary H. Richardson
Department: Nutrition and Food Sciences

Eleven strains of lactic cultures were used to determine their effectiveness in different bulk culture media for the manufacture of Cottage cheese. The acid phosphate-treated whey-based medium (APWM) made from Cottage cheese whey was found to be as effective as sweet phosphate-treated whey-based medium (PWM) made from Swiss cheese whey in supporting the development of lactic cultures. The relative activity of cultures in APWM with pH control was higher than phage inhibitory medium and nonfat dry milk without pH control. APWM was used successfully in the manufacture of Cottage cheese. Greater reliability and shorter cutting time resulted, and the product was comparable to those made with conventional media. More lactose was utilized when pH control was involved. More acid was also produced at a greater rate with an implied reduction in growth curve lag phase and an increase in maximum viable cells in the maximum stationary phase.

(80 pages)
INTRODUCTION

Although some whey from cheese-making is utilized in food and in making by-products, there are still considerable quantities of whey being wasted (68, 78, 85). There are approximately 7 percent total solids in whey with over 4 percent lactose as a potential energy source for lactic organisms (21, 78). Whey has been used as a bulk culture medium in cheese-making (78). Though it is low in cost, the advent of bacteriophage (phage) problems has necessitated the replacing of whey and milk culture media with protective phage inhibitory media (PIM). Bacteriophages, active against the streptococci during cheese-making, present one of the most important causes of slow acid production by lactic cultures (2, 51, 82). Whey itself is one of the major sources of phage (21, 52, 82). Therefore, whey has been discredited as a good lactic culture medium for bulk cultures. Cheng (10) successfully developed a sweet phosphate-treated whey-based medium (SPWM) from Cheddar cheese whey which should be less expensive than commercial PIM. However, the SPWM was found to be less stimulatory to the cultures during commercial trials than PIM. This problem was remedied by Richardson et al. (4, 5, 65) who combined lower levels of phosphate in SPWM with pH control. During control near pH 6 there was less soluble calcium in the medium for phage proliferation. With a pH-controlled system the lactic cultures grew rapidly because they were less inhibited by acid production and their own end products (69). Richardson (65) has shown that SPWM under pH control supports greater activity than nonfat dry milk (NDM) or PIM.
Acid whey (Cottage cheese whey) has a higher content of calcium and phosphate than sweet whey (Cheddar and Swiss cheese whey). The lactose content in acid whey is less than that of sweet whey (21, 38, 50, 78). Sweet whey has been evaluated using pH control with SPWM in the research cited. This research will evaluate acid phosphate-treated whey-based medium (APWM) made from Cottage cheese whey in conjunction with pH control in preparation of bulk culture for Cottage cheese manufacture.
REVIEW OF LITERATURE

Bacteriophage

Bacteriophages (phages) are submicroscopic, filterable microbes, which are only parasitic on bacteria. They possess only one type of nucleic acid, are reproduced from their own genetic material, and are unable to grow apart from host cells and to undergo binary fission (67). Most of the phages examined resemble tadpoles, with long tails attached to spherical, cylindrical, or polyhedral heads. In numerous instances the tail has proven to be a special organ for attachment to a bacterium, and very likely all phages have such an organ. Different phages range in size from approximately 0.1 micron in diameter to a minimum of about 20 m\(\mu\). They also show differences in their shape and structure. The lytic process of bacteriophage can approximately be divided into four stages: absorption, penetration, multiplication, and lysis. The phenomenon brings about inactivation and dissolution of the host cell. Susceptibility of some bacteria to phages and the wide distribution of phages in nature sometimes cause trouble in the food fermentation industry (1).

Bacteriophage problems in dairy industry

Lactic cultures that are unable to produce acid uniformly every day present an important problem to the dairy industry. Studies on the causes of slow acid production, or complete failure of a culture to produce acid, indicate that bacteriophages are often responsible
(2, 7, 51, 81, 82). In addition to disruption of careful time schedules in the cheese plants, production of insufficient lactic acid also causes undesirable products with such defects as off-odors, off-flavors, discoloration, gas blowing, putrefaction, toxin formation, etc. (17, 77).

Sources and important properties of lactic streptococcus bacteriophages

Sources. Since a phage proliferates only in the presence of the organisms susceptible to that phage, it is expected that phages will be found where the host bacteria occur. Therefore, the possible sources of phages for lactic streptococci might be the same as those of the organisms themselves; for example, milk, dairy products and by-products, dairy utensils and the environment of dairies. Milk used in cheese-making may be considered as a massive culture medium for lactic streptococci. Therefore, whey separated from the curd during cheese-making is also considered as a very important source of lactic streptococcus bacteriophage (21, 52, 82).

Morphology. Under the electron microscope, most phage strains active against lactic streptococci appear sperm-shaped. They have a total length of 180 to 280 µm, with the spherical heads measuring 60 to 90 µm in diameter, and the tails measuring 20 to 40 µm wide and 120 to 190 µm long (12, 17, 56). However, Williamson and Bertaud isolated one special lactic streptococcus bacteriophage having a spherical head 70 to 80 µm in diameter attached to a long flagellum-like tail 15 µm wide and 560 to 610 µm long (6, 83).
**Nutrition requirement.** Little is known of the nutrition requirements of various strains of lactic streptococcus bacteriophages. The requirement may be different than those of the host cell. Potassium phosphate, potassium chloride, sodium chloride, calcium chloride, magnesium sulfate, and sodium acetate promoted lysis of host cells according to their efficiency in promoting phage adsorption to the host cells (17). These may not be necessary nutrients for the phage but may be necessary for the adsorption or infection leading to lysis of host cells. Phages are unable to multiply in a calcium deficient medium in which their host bacteria grow well. Strontium, barium, or manganous ions will replace calcium but are less effective at an equivalent concentration. Using one step growth and single infected cell techniques the function of those ions appears to be in facilitating bacteriophage invasion (62). Maximum virus production occurred with 0.004 to 0.03 percent calcium but slight changes in calcium content affected the phage titer (6, 60, 61). Other nutrients such as tryptone, individual amino acids, purine and pyrimidine bases, vitamins, and electrolytes also have some influence on the adsorption or multiplication of the lactic streptococcus bacteriophage (6, 17).

**Effect of pH on phage.** The maximum and minimum pH levels at which bacteriophage proliferation occurred has varied for each strain studied. Bacteriophage proliferation usually failed to occur at a pH level at which the growth of their host organism was considerably inhibited. At pH 4, the lactic streptococci were not influenced by phage, or if infection occurred, it did not prevent cellular multiplication. At pH 5, some cells were probably infected, since incomplete
lysis occurred. Cells were completely lysis at pH 6, 7, and 8, but at pH 8 the latent period was extended. The optimum pH for bacteriophage proliferation was found to be approximately pH 6.5 to 7 (6, 11, 17, 55). Bacteriophages were rapidly destroyed at room temperature when the pH was above 11.8 and below 2.5. Therefore, the alkaline and acid detergents used in cleaning of dairy equipment seem of no great value in controlling the outbreak of bacteriophage infection since they don't produce these extremes in pH. They are ineffective if used at low temperatures (63).

**Phage reproduction at different temperatures.** Bacteriophages showed a wider diversity of reaction to temperature than their host organisms. Hunter (35) found that most phages for *Streptococcus cremoris* (optimum growth temperature at 30 C, with some inhibition at 37 C) developed more readily at 30 C than at 22 C. Some phage strains developed as well at 22 C as at 30 C. Others developed more readily at 37 C than at lower temperatures and still others were completely inhibited at 37 C.

**Latent period and burst size.** There are differences in latent period and burst size, resulting in differences in multiplication rate (6, 12, 17, 25, 43). Graham and Potter (25) studied the growth characteristics of three strains of lactic streptococcus bacteriophage each propagated on their host bacteria in two different media. They found that the latent periods ranged from 25 to 40 minutes, depending on virus strain and medium. Average burst size ranged from 21 to 129, depending on virus strain, medium, and method of determination. Babel (6) reported the latent period to vary from 40 to 90 minutes
at 30 °C, and the burst size from 21 to 77 phage particles among six combinations. By studying the action of different phages on one bacterial strain, it was claimed that latent period and burst size were characteristics of the phage and not of the bacteria.

Heat resistance. Phage strains for *Streptococcus lactis* and *Streptococcus cremoris* appear more resistant to heat than do their host cells (17, 80, 86). Phage at pH 8 was destroyed by holding at 100 °C for 5 sec; at 95 °C for 1 min; at 85 °C for 4 min; at 75 °C for 20 min; and at 65 °C for 30 min. At pH 6, phage was destroyed almost instantly at 100 °C; at 95 °C for 10 sec; at 85 °C for 1.5 min; at 75 °C for 15 min; and at 65 °C for 30 min (6). Whitehead and Hunter (80) reported that the thermal death points of lactic streptococcus bacteriophage were in the region of 70 to 75 °C for 30 min at pH 6.0. Therefore, they can not be destroyed under ordinary pasteurization conditions (79). Elliker (17) indicated the necessity of high pasteurization temperatures in preparation of milk for bulk culture in order to destroy lactic streptococcus bacteriophages. A minimum exposure of 82 to 88 °C for at least 30 min was recommended (17).

Destruction by chemical germicides and ultraviolet radiation. Chlorine compounds are the most effective and convenient agents for destruction of phage. Fine mists containing 0.003 to 0.02 percent chlorine usually gave satisfactory destruction of air-borne phage (6). In a 1:2 dilution of whey, 400 ppm chlorine destroyed phage in 15 sec at pH 5.0 and in 5 min at pH 6.5. Quaternary ammonium compounds and permanganate were also very effective in inactivating bacteriophage particles.
Ultraviolet energy can be used to destroy bacteriophage. However, the long time-exposures required at relatively short distances from the ultraviolet lamp, and also for destruction of dry phage, limited the use of ultraviolet lamps for destroying phage in dairy plants (6, 17).

**Nascent phenomenon.** Certain bacteriophages have been quite unusual in their action on certain strains of bacteria. They appeared able to lyse the mixtures of sensitive and resistant strains of bacteria, although the same bacteriophages appeared to have no effect on the resistant strains growing alone. Collins (12) indicated that inhibition of certain heterologous strains of bacteria by bacteriophages also occurred in the absence of their host bacteria when large numbers of bacteriophages were present. The homologous host, which was thought previously to be essential for the occurrence of the nascent phenomenon, appeared in this case only as a means of increasing the numbers of bacteriophage to a point where most of the heterologous bacteria would be attacked (6, 17).

**Practical control methods**

against bacteriophage

Several ways can be used to control the lysis of cultures used in producing fermented dairy products. It is often advantageous to use several strains of the same organism in dairy fermentation. Thus bacterial strains not sensitive to specific phages would be included in the culture and would be able to grow in the milk. Different culture strains could be rotated every day or every few days. In this manner strains of lactic cultures not sensitive to the special
bacteriophages would always be present in sufficient numbers (6, 12, 17, 31, 53).

Another method of preventing bacteriophage development is to transfer and incubate mother and bulk cultures in a separate room isolated from the rest of the dairy plant. Elaborate precautions have also been employed including sanitation of the room, maintaining positive air pressure and using specially constructed culture vessels. All cultures should be checked periodically for viability as determined by acid production (12, 17, 53, 77).

A medium which supports good growth of lactic cultures and prevents the development of bacteriophage is a desirable alternative control product. Media for this purpose such as Marstar (a PIM) are available (32).

The development of media for preventing bacteriophage activity

The knowledge that calcium ions are required for adsorption and subsequent proliferation of lactic streptococcus bacteriophage has been well established (1, 13, 60, 62, 71). Babel (7) described a calcium-deficient milk medium designated as phage resistant medium (PRM) in which calcium was removed by ion exchange and replaced by a nonessential ion. Bacteriophages were inhibited with this medium, but there was a concurrent inhibition of some strains of lactic streptococci. Thus, this medium could not always be successfully used. Hargrove et al. (29) initiated the addition of phosphate to a lactic starter milk medium for the purpose of binding the essential calcium,
thus preventing bacteriophage proliferation. Subsequently, they developed a procedure that successfully inhibited the bacteriophage tested. Their procedure indicated that phosphate treated milk became more effective as the pH of the phosphate buffer and milk was increased from pH 6.4 to 7.0. The best combination for phage inhibition, and economy was obtained when milk was treated with 1.7 percent orthophosphate salt at pH 6.6, followed by the addition of 0.3 percent pyrophosphate.

Kadis and Babel (39) added mixtures of phosphates to skim milk or reconstituted milk in order to determine their effectiveness for binding calcium ions in milk so that bacteriophages would not develop, and to determine the influence of phosphate on the growth of lactic cultures. They found that the simple addition of phosphate is inadequate to produce a suitable medium which inhibits streptococcus bacteriophage and still permits satisfactory growth of lactic cultures.

Zottola and Marth (87) developed dry product blends which were as easy to handle in cheese-factory operations as NDM. The product contained yeast extract as a stimulant and would inhibit proliferation of bacteriophage without concurrent inhibition of the host lactic streptococci. The dry products were prepared by combining mono- and di-basic orthophosphates with yeast extract, NDM, and a combination of NDM and electro-dialyzed whey (EDW). They found three combinations that would inhibit most or all of the indicated bacteriophages. Those combinations were: 1) a 3:2 mixture of NDM and EDW with a 2 percent orthophosphate concentration (reconstituted basis) consisting of a 1:1 ratio of Na$_2$HPO$_4$ and KH$_2$PO$_4$; 2) a 4:1 mixture of
NDM and EDW with a 2 percent orthophosphate concentration (reconstituted basis) consisting of 1:1 ratio of Na$_2$HPO$_4$ and NH$_4$H$_2$PO$_4$; 3) a 4:1 mixture of NDM and EDW with a 2 percent orthophosphate concentration (reconstituted basis) consisting of a 1:1 ratio of NH$_4$H$_2$PO$_4$ and (NH$_4$)$_2$HPO$_4$. All of the mixtures contained 0.32 percent yeast extract (reconstituted basis). The first two formulae inhibited five of six phages examined, whereas, the third effectively inhibited all six. None of the three mixtures was inhibitory to the host lactic streptococci.

Commercial products of PIM such as "Marstar" are available to the dairy industry. These media contain mixtures of phosphates, NDM, EDW powder, and other stimulatory substances. The ability of these media to inhibit the development of bacteriophage and support the growth of lactic cultures has been established (32).

The composition and usage of cheese whey

Dry sweet whey contains the following major components: 1 percent fat, 13 percent protein, 71 percent lactose, and 8 percent total ash. Dry acid whey contains .6 percent fat, 12.1 percent protein, 64.1 percent lactose, and 10.6 percent total ash. Sweet type whey has a higher pH, contains more total nitrogen, citrate, lactose and solids, but is lower in calcium, phosphorus, and ash than acid type whey (16, 21, 38, 50, 78). Acid whey is less useful than sweet whey because of the high lactic acid content which limits its usage as a food source for human consumption. Therefore, developing new outlets for the use of acid whey represents an important challenge (46).
The use of whey to propagate lactic acid bacteria

Since whey contains over 4 percent lactose as an energy source for lactic organisms, whey has been used as a bulk culture medium in cheese making (8, 33, 78). However, whey has been discredited as a relatively low cost bulk culture medium because of bacteriophage problems (21, 52, 82). Cheng (10) has successfully developed a less expensive bulk culture medium using a SPWM made from Cheddar cheese whey. This product was found to be less stimulatory to cultures during commercial trials when compared to PIM. Richardson et al. (4, 5, 65) remedied this problem using lower levels of phosphate in SPWM with pH control. At a system controlled near pH 6, there is less soluble calcium in the medium for phage proliferation. The pH-controlled system allowed better growth of lactic culture because they were less inhibited by acid production and their own end products (69). Richardson (65) has shown that SPWM under pH control supported greater starter activity than NDM or PIM, and he suggested utilizing SPWM under pH control to develop an improved lactic bulk culture system.

Properties of Streptococcus lactis and Streptococcus cremoris

Morphology. Cells of Streptococcus lactis are gram positive, ovoid in shape having diameters ranging from 0.5 to 1.0 µ. They occur mostly in pairs or short chains, and sometimes in long chains. Cells of Streptococcus cremoris are gram positive, spheres or ovoid in shape
having diameters range from 0.6 to 1.0 µ. They usually form long chains in milk but some cultures occur predominantly as pairs (67).

**Nutrient requirements.** *Streptococcus cremoris* and *Streptococcus lactis* are homofermentative, utilizing a number of carbohydrates with production chiefly of D-lactic acid. They generally follow the classical Embden-Meyerhof-Parnas (EMP) glycolytic scheme of carbohydrate metabolism. However, the hexose monophosphate shunt pathway also operates, indicating they can be facultatively homofermentative (49, 67, 69, 70, 76). Crude extracts of various tissues (e.g., liver fractions, yeast extract, pancease extract, corn steep liquor, etc.), and enzymatic hydrolysates of protein (e.g., tryptone, Bacto casamino acid, BBL polypeptone, gelatin, sodium caseinate, etc.) can stimulate suitable growth for lactic cultures (3, 18, 22, 42, 72, 74). The former contains peptides, nucleic acid derivatives, and the latter contains amino acids and peptides. These organic compounds stimulate or are essential for the growth of certain lactic bacteria (45, 73, 74). The addition of ammonium salts to media deficient in certain amino acids stimulated the growth of lactic acid bacteria. This indicates that ammonia can serve as the source of nitrogen when certain amino acid and nitrogenous compounds are unavailable in the medium (27, 73). Similarly, an external supply of carbon dioxide either stimulated, or was essential for, growth of several lactic acid bacteria when the medium was deficient in certain organic nutrients (e.g., certain amino acids). Under such conditions, carbon dioxide is utilized in the synthesis of those compounds, and must be
considered as an essential nutrient (73). Sodium acetate and sodium chloride could increase the number and size of colonies of lactic bacteria in solid media (18). Ferrous sulfate can prevent the accumulation of peroxide and increase the proteolytic activity of Streptococcus lactis, which results in faster growth and acid production (14, 24). Other nutrients such as acetic acid and certain vitamins or coenzymes (e.g., lipoic acid, riboflavin, thiamine), inorganic salts (e.g., calcium, potassium, manganese, phosphate, and magnesium) were also found to stimulate or to be essential for growth of certain lactic acid bacteria (73).

Effect of pH on lactic streptococci. The optimum pH for the growth of the lactic streptococci is around 5.5 to 6.5 (30, 47, 58, 59). Streptococcus cremoris will not grow at pH 9.2, while Streptococcus lactis will still grow at pH 9.2 but not at pH 9.6 (18, 67). Death of lactic streptococci begins at approximately pH 5. The results are consistent with either of two mechanisms of damage. Growth at low pH causes either direct inactivation of a number of enzymes, or loss of control of the differential rate of synthesis of individual enzymes. The rate of death (loss of enzymatic activity) decreases in proportion to the cells remaining at pH below 5 for any extended period of time. Consequently, the longer these cells are subjected to pH levels below 5, the longer is the lag time when the cultures were retransferred to fresh substrate (30, 69).

Effect of temperature on lactic streptococci. Streptococcus lactis grows at 10 C or below and at 40 C but not at 45 C. Streptococcus cremoris grows at 10 C and below but not at 40 C. Most
Streptococcus lactis strains grow at a more rapid rate when the temperature is at 30°C. Streptococcus cremoris will develop satisfactorily at 30°C but not at the higher temperatures (59, 67, 69).

**Effect of air, CO₂ and N₂ on lactic streptococci.** Although no precautions are usually taken to exclude oxygen in culturing lactic streptococci, their metabolism is basically anaerobic and the aeration involved in stirring or agitation could seriously inhibit their growth. The effect of O₂ on lactic streptococci is believed to be due to the accumulation of growth-inhibiting quantities of H₂O₂ in the medium (41, 59, 75). Addition of catalase to this aerated medium resulted in increased rates of acid production due to the destruction of metabolically produced peroxide. Ferrous sulfate seems more effective than catalase in this case because it not only destroys hydrogen peroxide in the medium but also increased the proteolytic activity of Streptococcus lactis, which resulted in faster growth and acid production (14, 24). The effect of stirring or agitation was counteracted also by bubbling a mixture of 5 percent CO₂ in N₂ or with N₂ only through the medium, and the lactic cultures grew satisfactorily with this treatment (40, 47, 59, 64).

**Preparation of concentrated lactic streptococcus starters**

Concentrated suspensions of starter cultures have rapidly found importance in the fermentation of food products. In the dairy industry, frozen concentrates of culture can be supplied in an active form to avoid regular transfer of cultures in the dairy plant. Thus the
possibility of bacteriophage contamination and proliferation is decreased. The control of pH in preparing culture concentrates is important for obtaining the largest number of cells possible that will actively grow and produce acid normally when transferred into milk. The optimum pH for producing concentrated cultures is near 6, the optimum pH for growth (47, 58, 72). Either ammonium hydroxide or sodium hydroxide can be used to control the pH of the medium. Maximum populations were increased about two fold with ammonium hydroxide as compared with sodium hydroxide (58). This may have been due to the stimulatory effect of \( \text{NH}_4^+ \) on the lactic cultures (27, 58, 73). Another factor in limiting growth may have been the increase of sodium lactate when sodium hydroxide was used (59, 64).

The time of harvesting cells from the concentrated cultures has not been critical. When controlling pH below 6 in the preparation of concentrated cultures, the cells could be harvested within 6 hr after reaching their maximum population without loss of activity. However, there was no benefit in this kind of overincubation. Since the inhibitory properties of the spent broth of the culture were not analyzed, it would be difficult to explain the mechanism of growth limitation (58). Pont and Holloway (59) indicated that the space requirement for the growth of lactic cultures is not restrictive and shortage of lactose does not appear to be a critical factor in whey medium fortified with tryptone and yeast extract. They believed that the increasing concentration of sodium lactate during fermentation was an important factor in limiting cell growth. Rahn et al.
(64) also found the fermentation rate of *Streptococcus lactis* under pH control to be retarded slightly by sodium lactate in concentration over 1 percent and to be reduced to less than half the normal rate in 4 percent sodium lactate. Rogers and Whittier (66) controlled hydrogen-ion concentration in a culture of *Streptococcus lactis* at pH 5.8 to 6.0 and found the concentration of undissociated lactic acid to be a principle, though not the only factor, in the limitation of growth and metabolism. Gilliland and Speck (23) identified D-leucine to be an autoinhibitor for lactic cultures when grown at pH 6.0. Growth limitation of lactic cultures seems to be the result of combined effects of several factors (58).

One important challenge is to find application for the great bulk of fluid acid whey that is not used in food products, either as a liquid or as a powder. Because of manufacturing and handling, there is an obvious economic advantage for using cottage cheese whey with pH control, phosphates, and stimulants over frozen concentrated starter culture medium. Our combination will be referred in this thesis as APWM to differentiate it from SPWM studied to date.
EXPERIMENTAL METHODS

Preparation of lactic acid producing bacteria

Eleven single strain lactic cultures were obtained from the Nutrition and Food Sciences Department, Utah State University. These cultures were *Streptococcus lactis* Sl#1 and Sl#2, and *Streptococcus cremoris* #1, #2, #3, Sc#3, Sc#4, Sc#5, KH, R, and AM2. The numbered cultures were originally obtained from DPL Culture Service, San Francisco, California. All cultures were maintained in sterilized NDM. They were transferred twice a week by using a one percent inoculum, incubated for over 10 hr (depending on the coagulation of the milk) at 30 C, and then stored at about 4 C.

Preparation and comparison of APWM and SPWM

Phosphate-treated whey-based media (PWM) including both APWM and SPWM were formulated by adding 0.32 parts Lactivate Concentrate (a proprietary blend provided by Dairyland Food Laboratories, Inc., Waukesha, Wisconsin), 0.75 parts of a 1/1 blend of ammonium mono- and di-hydrogen phosphates, and 0.015 parts of Bacto-brom cresol purple into 100 parts liquid whey. Since Cottage cheese whey was more acid, it was neutralized with 10.3 N NH₄OH to pH 6.3. Ten milliliter aliquots of PWM were transferred into test tubes, pasteurized at 88 to 90 C for 45 min, cooled to 21 to 30 C, and each
inoculated with 1 percent inoculum (5, 11, 29, 87). The inoculated tubes were incubated at 30 C for various time intervals, and the tubes were neutralized with 10.3 N NH\textsubscript{4}OH. Figures were made comparing total volume of NH\textsubscript{4}OH needed for neutralization versus time.

A pump (Sage Instruments, Model 341) fitted with 19 and 23 gauge needles\textsuperscript{1} and a 50 ml syringe was used to control the flow of neutralizer. A needle size change caused a change in the number of delivery drops/ml. A Vortex mixer was used to agitate the test tubes. This provided good mixing and incorporated air simulating bulk tank agitation conditions.

Comparison of culture activity in pH-controlled APWM with NDM and PIM media not under pH control

APWM was prepared as described in a previous paragraph. "Marstar," a popular commercial PIM was prepared according to the manufacturer's directions.\textsuperscript{2} The NDM medium was prepared by adding 10 percent NDM powder to distilled water. All reconstituted media were heated at 88 to 90 C for 45 min, cooled to 21 to 30 C, and inoculated with 1 percent inocula. APWM was neutralized with 10.3 N NH\textsubscript{4}OH to pH 6.3 before incubation.

\textsuperscript{1}A 23 gauge needle was used for PWM experiments under pH control. A 19 g needle was used in the experiment testing for final amount of acid production in APWM after 66 hr incubation not under pH control.

\textsuperscript{2}Instructions from Marschall Division, Miles Laboratories, Inc., Elkhart, Indiana.
The activities of cultures in different media were tested at different time intervals, using a modified procedure of Horrall and Elliker (34). Ten milliliter samples of 10 percent (w/v) NDM were sterilized at 250 F for 10 min. The milk samples were tempered to 37 C and inoculated with 0.3 ml of culture. At the end of 3.5 hr incubation, the net pH drop during incubation was determined, and graphs were plotted comparing net pH drop with culture age (hr). A pH drop of 0.4 would correspond to a Horrall-Elliker TA reading of 0.4 percent lactic acid. This would indicate an active culture.

**Activity test of starter culture in pasteurized skim milk**

Five percent starter was added to pasteurized skim milk in a 10 ml test tube, at 32 C (90 F). The time needed for surface coagulation was measured to simulate the AC endpoint method (20).

**Method of Cottage cheese manufacture**

Cottage cheese was manufactured from 8 to 16 lb quantities of skim milk using the following conditions (20, 21, 48, 54, 87):

1) Single strain lactic cultures were used exclusively.
2) A standard short set procedure involving an eight hr schedule was followed.
3) The curd was a medium cut.
4) The skim milk contained about 10 percent solids.
5) The curd was creamed using a homogenized 12.5 percent fat creaming mixture.
6) Bulk cultures were prepared in APWM, NDM and PIM media. APWM were under pH control. Cultures were incubated at 30°C following inoculation until optimum activity was reached.

7) Five percent of each ripened medium was added to cheese milk.
RESULTS AND DISCUSSION

Comparison of APWM and SPWM

Comparisons of growth of eleven lactic cultures were made on Cottage cheese whey (pH 4.40) and Swiss cheese whey (pH 6.25). One part of fresh Swiss cheese whey was pre-acidified to pH 4.45 by putting the whey into a 37 C incubator and allowing the growth of the lactic cultures originally in Swiss cheese whey to drop the pH. The pre-acidified Swiss cheese whey was also used for comparison. The whey samples were made into PWM by adding 0.32 parts Lactivate Concentrate, 0.75 parts phosphate blend, and 0.015 parts Bacto-brom cresol purple into 100 parts liquid whey. After formulation, the rehydrated APWM, SPWM, and preacidified SPWM had pH values of 5.5, 6.4, and 5.7 respectively. All media were adjusted to near pH 6.3 by using 10.3 N NH₄OH in 10 ml PWM samples (0.0297 ml/10 ml NH₄OH for APWM, and 0.0198 ml/10 ml for preacidified SPWM). Preparation of APWM would thus require approximately 0.68 kg more 28.5 to 30 percent NH₄OH per 100 gallons of culture than SPWM. One percent of each of eleven lactic cultures was inoculated into each medium. The acid produced during incubation at room temperature (20 C) within 66 hr was neutralized at various time intervals with 10.3 N NH₄OH to around pH 6.3 as indicated by Bacto-brom cresol purple. Figures 1 to 11 compare the drops of NH₄OH added for neutralization vs the time after inoculation.

The effectiveness of APWM was found to be almost as good as both SPWM and preacidified SPWM when the lactic cultures were in their
Figure 1. Comparison of the effectiveness of different whey-based media to support growth of cul #1 at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 2. Comparison of the effectiveness of different whey-based media to support growth of cul #2 at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 3. Comparison of the effectiveness of different whey-based media to support growth of cul #3 at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 4. Comparison of the effectiveness of different whey-based media to support growth of S1#1 at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 5. Comparison of the effectiveness of different whey-based media to support growth of S1#2 at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 6. Comparison of the effectiveness of different whey-based media to support growth of Sc#3 at 20 C. 1. SPWM 2. Preacidified SPWM 3. AFWM
Figure 7. Comparison of the effectiveness of different whey-based media to support growth of Sc#4 at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 8. Comparison of the effectiveness of different whey-based media to support growth of Sc#5 at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 9. Comparison of the effectiveness of different whey-based media to support growth of KH at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 10. Comparison of the effectiveness of different whey-based media to support growth of R at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
Figure 11. Comparison of the effectiveness of different whey-based media to support growth of AM$_2$ at 20 C. 1. SPWM 2. Preacidified SPWM 3. APWM
exponential growth phase. The average total drops of 10.3 N NH₄OH used in neutralizing acid production by those eleven cultures during 66 hr incubation were 44.2 drops for SPWM, 42.2 drops for preacidified SPWM, and 38.2 drops for APWM. APWM without pH control was also used. An average of 13.8 drops of 1.65 N NH₄OH was used in neutralizing the acid produced. From these results the lactose utilized by the lactic cultures was calculated:

Reagent grade ammonium hydroxide contained 28.5 to 30 percent or 29.3 percent NH₃. The specific gravity was 0.90 gm/ml. The weight of ammonia was thus 0.90 gm/ml x 0.293 x 1000 ml/liter = 264 gm/liter. The molar concentration of ammonium hydroxide was 264/17.032 = 15.5 M or 15.5 N. The concentrated solution was diluted with 50 percent distilled water. Therefore the final normality was 15.5/1.5 = 10.3 N. If 44.2 drops of diluted 10.3 N NH₄OH (100.9 drops per ml from the #23 gauge needle) were used throughout the completed fermentation of 10 ml SPWM then 44.2/100.9 x 10.3 meq/ml = 4.51 meq/10 ml culture or 451 meq/liter. With preacidified SPWM the average was 42.2 drops per 10 ml tube and therefore the value was 42.2/100.9 x 10.3 meq/ml = 4.31 meq/10 ml or 431 meq/liter. APWM was neutralized with 38.2 drops for 38.2/100.9 x 10.3 meq/ml = 3.90 meq/10 ml or 390 meq/liter. APWM without pH control was neutralized with 13.8 drops (52.4 drops per ml from the #19 gauge needle) or 13.8/52.4 x 1.65 meq/ml = 0.435 meq/10 ml 43.5 meq/liter.
The molarity of 4.9 percent lactose in sweet whey is 0.136 M \((49/360)\). Four moles of lactic acid can be produced from every mole of lactose. Thus the maximum theoretical lactic acid to be produced in whey at 4.9 percent lactose would be \(4 \times 0.136 \text{ M} = 0.544 \text{ M lactic acid, or 0.544 equiv/liter or 544 meq/liter.}\)

Sweet whey contains approximately 0.2 percent lactic acid whereas acid Cottage cheese whey would contain approximately 0.5 percent lactic acid. The molarity of lactose in Cottage cheese whey would thus calculate \((4.9 - 0.5)/36 = 0.122 \text{ M or 122 meq/liter.}\) Similarly, the maximum possible lactic acid moles produced from available lactose would be \(0.122 \times 4 = 0.488 \text{ M or 488 meq/liter.}\) The mean percent lactose utilized would be \(451/544 \times 100\% = 83\%\) in SPWM, \(431/544 \times 100\% = 79\%\) in preacidified SPWM, \(390/488 \times 100\% = 80\%\) in APWM with pH control, and \(43.5/488 \times 100\% = 8.9\%\) without pH control.

From these computations, approximately 80 percent of the lactose was consumed in APWM with pH control compared with 8.9 percent in APWM without pH control. All the lactose in the two other PWM was not utilized during the growth of the lactic acid bacteria (21).

Pont and Holloway (59) found the same results in pH-controlled fermentation of *Streptococcus lactis* C10 in whey medium fortified with tryptone and yeast extract. They indicated that at the end of the logarithmic phase in fermentation studied, the lactose concentration
had varied from 1.5 to 3.0 percent. Therefore, shortage of lactose in the whey medium did not appear to be a critical factor of growth limitation. The present system apparently consumed a larger percentage of lactose than found by Pont and Holloway. This was possibly due to the extensive incubation time. The fermentation was extended beyond that required to obtain maximum cell population. The reasons for cessation of growth are not quite clear. But only small differences could be detected in growth patterns among the cultures produced from APWM, SPWM, and preacidified SPWM indicating the acid end products and NH₄ lactate ions may be the most important inhibitors. Several other researchers reported that the phenomenon of growth limitation of lactic culture might be due to the production of several substances which inhibited the growth of lactic cultures when they reached a certain concentration (23, 58, 59, 64, 66).

Comparison of culture activity in pH-controlled APWM with NDM and PIM media not under pH control at 20°C

The activity of cultures in pH-controlled APWM was compared with those in NDM and PIM media not under pH control following inoculation of 1 percent lactic culture into 10 ml of reconstituted NDM. The activities of the cultures were tested every 3.5 hr from 8 to 29 hr, and at 40 hr.

The curves in Figures 12 to 22 show that the lactic cultures reached maximum activity slowly at room temperature (20°C). They did not reach their mean maximum activities until 15 hr after inoculation.
Figure 12. Comparison of culture #1 activities in different media at 20 C.
Figure 13. Comparison of culture #2 activities in different media at 20 C.
Figure 14. Comparison of culture #3 activities in different media at 20 C.
Figure 15. Comparison of culture S1#1 activities in different media at 20 C.
Figure 16. Comparison of culture Sl#2 activities in different media at 20 C.
Figure 17. Comparison of culture ScS#3 activities in different media at 20 C.
Figure 18. Comparison of culture Sc#4 activities in different media at 20 C.
Figure 19. Comparison of culture Sc#5 activities in different media at 20 C.
Figure 20. Comparison of culture KH activities in different media at 20 C.
Figure 21. Comparison of culture R activities in different media at 20 C.
Figure 22. Comparison of culture AM$_2$ activities in different media at 20 C.
The relative activity of every strain of culture in pH-controlled APWM was higher throughout the growth cycle than those in NDM and PIM media not under pH control.

**Culture activity test simulating Cottage cheese making conditions**

Five percent culture was added to 10 ml pasteurized skim milk in a test tube at 32°C. No rennet was incorporated. The time needed for surface coagulation was determined (20). Cultures in pH-controlled APWM produced shorter surface coagulation intervals (Table 1). Therefore, the cultures in pH-controlled APWM should develop shorter cutting times than those of NDM and PIM media when used in the manufacture of Cottage cheese.

Of those eleven cultures, S1#1, S1#2, Sc#3, Cul#3, and KH grew very slowly in pasteurized skim milk, and therefore they were eliminated in the following experiments.

**Comparison of culture activity in pH controlled APWM with NDM and PIM media not under pH control at 30°C**

Since the cultures developed slowly at 20°C, the activity of cultures incubated at 30°C was determined to establish a shorter incubation time for the bulk medium in the manufacture of Cottage cheese. The procedure was the same as previously mentioned at 20°C.

Most starter cultures in APWM and NDM media reached the highest activity during 10 to 12 hr incubation, but some lactic cultures grew very slowly in PIM. The highest activity obtained in PIM was
Table 1. Coagulation time of skim milk by cultures transferred from different media after incubation for 12 hr

<table>
<thead>
<tr>
<th>Cultures</th>
<th>APWM</th>
<th>NDM</th>
<th>PIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cul#1</td>
<td>3:00</td>
<td>5:00</td>
<td>8:00</td>
</tr>
<tr>
<td>Cul#2</td>
<td>3:00</td>
<td>5:30</td>
<td>4:45</td>
</tr>
<tr>
<td>Cul#3</td>
<td>6:30</td>
<td>7:30</td>
<td>&gt;10:00</td>
</tr>
<tr>
<td>S1#1</td>
<td>4:45</td>
<td>8:30</td>
<td>5:30</td>
</tr>
<tr>
<td>S1#2</td>
<td>&gt;10:00</td>
<td>&gt;10:00</td>
<td>&gt;10:00</td>
</tr>
<tr>
<td>Sc#3</td>
<td>6:30</td>
<td>7:15</td>
<td>&gt;10:00</td>
</tr>
<tr>
<td>Sc#4</td>
<td>4:30</td>
<td>6:15</td>
<td>7:45</td>
</tr>
<tr>
<td>Sc#5</td>
<td>3:30</td>
<td>5:30</td>
<td>&gt;10:00</td>
</tr>
<tr>
<td>KH</td>
<td>5:00</td>
<td>6:30</td>
<td>&gt;10:00</td>
</tr>
<tr>
<td>R</td>
<td>4:45</td>
<td>6:15</td>
<td>6:45</td>
</tr>
<tr>
<td>AM₂</td>
<td>3:45</td>
<td>4:45</td>
<td>4:15</td>
</tr>
</tbody>
</table>
reached over a wide range of 8 to 18 hr (Figures 23 to 28). Incubation at 30 C and 12 hr was therefore chosen when APWM was used as a bulk culture medium in the manufacture of Cottage cheese because it best fit the laboratory schedule. The previous work at 20 C established that alternative time-temperature relationships are practical depending upon manufacturing schedules.

The utilization of APWM under pH control in the manufacture of Cottage cheese

Four strains of Streptococcus cremoris (Cul#1, Cul#2, Sc#5, and KH) were chosen for manufacture of Cottage cheese. The pH controlled APWM and also NDM and PIM not under pH control media were used as bulk culture media. One percent of the above cultures were inoculated into these media, which were then held at 30 C for 12 hr. Five percent of each was then inoculated into 8 to 16 lb of pasteurized skim milk at 32 C.

Cottage cheese made with APWM as bulk culture medium had a shorter cutting time than both NDM and PIM media (Table 2). One reason for a shorter cutting time may be due to the pH control system protecting lactic cultures from a loss of their enzymatic activity at pH less than 5 (30, 69). Further evidence is presented in Figures 29 to 32 to establish that cultures using APWM showed less of a lag phase than other media when transferred into fresh skim milk. The pH-controlled APWM should also contain more viable cells because lactic cultures have a higher growth rate when the pH is controlled around
Figure 23. Comparison of culture #1 activities in different media at 30 C.
Figure 24. Comparison of culture #2 activities in different media at 30 C.
Figure 25. Comparison of culture Sc#4 activities in different media at 30 C.
Figure 26. Comparison of culture Sc#5 activities in different media at 30 C.
Figure 27. Comparison of culture R activities in different media at 30 °C.
Figure 28. Comparison of culture $\Delta M_2$ activities in different media at 30 C.
Table 2. The time and pH of cutting in the manufacture of Cottage cheese using cultures transferred from different bulk culture media

<table>
<thead>
<tr>
<th>Cultures</th>
<th>APWM (controlled)</th>
<th>Media NDM</th>
<th>PIM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>pH</td>
<td>Time</td>
</tr>
<tr>
<td>Cul#1</td>
<td>3:25</td>
<td>4.68</td>
<td>4:40</td>
</tr>
<tr>
<td>Cul#2</td>
<td>5:20</td>
<td>4.68</td>
<td>6:55</td>
</tr>
<tr>
<td>Sc#5</td>
<td>3:35</td>
<td>4.71</td>
<td>5:25</td>
</tr>
<tr>
<td>AM2</td>
<td>4:10</td>
<td>4.71</td>
<td>5:05</td>
</tr>
</tbody>
</table>

aDid not coagulate after 11 hr incubation.
Figure 29. Acid production rate in Cottage cheese skim milk by culture #1 after growth in NDM, PIM, and pH-controlled APWM for 12 hr at 30°C.
Figure 30. Acid production rate in Cottage cheese skim milk by culture #2 after growth in NDM, PIM, and pH-controlled APWM for 12 hr at 30 C.
Figure 31. Acid production rate in Cottage cheese skim milk by culture Sc#5 after growth in NDM, PIM, and pH-controlled APWM for 12 hr at 30 C.
Figure 32. Acid production rate in Cottage cheese skim milk by culture AM$_2$ after growth in NDM, PIM, and pH-controlled APWM for 12 hr at 30 C.
6 (47, 58, 59). One explanation why Sc#5 did not coagulate the skim milk during Cottage cheese manufacturing within 11 hours can be found in Table 1 and Figure 26, which indicated low starter activity after 12 hours incubation. Comparing the results of Cul#2 in Table 1 and 2 shows a great increase in coagulation time in Table 2 in all media. A possible reason for increased coagulation times may have been the presence of inhibitors in the skim milk.

A taste panel judged the products to be slightly acid in all products made. But, the Cottage cheese made with APWM showed the same quality as those products made with NDM and PIM media. Tables 1 to 2 and Figures 29 to 32 show some cultures with abnormally long cutting times. This problem may be due to the use of single strain cultures. Also some cultures may not be suitable in ordinary Cottage cheese manufacturing. Multiple strain cultures, selected to grow well together, should be studied to evaluate their significance in pH-controlled APWM.

There is less buffering capacity of APWM than SPWM when only 0.75 percent phosphates are incorporated. Studies are needed to establish if higher phosphate levels are required in APWM to assume protection from bacteriophage activity.
CONCLUSIONS

1. APWM with pH control was found almost as effective as SPWM and preacidified SPWM in supporting the development of lactic cultures in their exponential growth phase. Near the end of the growth cycle, both SPWM products allowed slightly more acid production as expected.

2. Approximately 80 percent of the lactose in pH-controlled APWM was consumed in 66 hr at 20°C as compared with 8.9 percent lactose consumption in APWM not under pH control.

3. The activity of cultures in pH-controlled APWM was higher throughout the growth cycle than in the cultures propagated in NDM and PIM media not under pH control.

4. APWM cultures could be used at 5 percent inocula in the manufacture of Cottage cheese with the advantages of greater reliability, economy, and shorter cutting time. The products were comparable to Cottage cheese made with NDM and PIM media.
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