5-1955

The Use of Lactic Acid in the Manufacture of Cheddar Cheese from Milk Containing an Antibiotic

Elmer George Jr.
Utah State University

THE USE OF LACTIC ACID IN THE MANUFACTURE OF CHEDDAR CHEESE FROM MILK CONTAINING AN ANTIBIOTIC

by

Elmer George Jr.

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Dairy Manufacturing

UTAH STATE AGRICULTURAL COLLEGE
Logan, Utah

1955

ACKNOWLEDGMENT

My sincere thanks are due to Professor A. J. Morris for the valuable suggestions that led to the selection of this thesis problem. I am also grateful to Professor A. J. Morris and Professor Paul B. Larsen for their assiatance in scoring the cheese.

I wish to thank Professor Royal A. Bagley for his help in obtaining the antibiotic used in this experiment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Importance of problem

The manufacture of cheddar cheese is greatly dependent on bacterial growth for acid production. The quality of cheese depends upon the type and extent of microbial activity.

An important function of the lactic fermenting bacteria is the production of acid resulting from cellular metabolism. If little or no acid is produced the resulting cheese will have an inferior body, flavor, and texture and may even cause the cheese to be used as grinders.

The major causes for inhibited lactic bacterial growth are poor starter handling procedures, antibiotics in milk coming from cows treated for mastitis, quaternary ammonium compounds used in plant sanitation, and bacteriophage.

The occurance of bacteriophage contamination and the increased use of quaternary ammonium compounds and antibiotics have caused a serious problem in cheese manufacturing.

Purpose of project

The purpose of this project is to determine if cheddar cheese, comparable to normal cheese, can be made from slow or non-acid milk with the use of added lactic acid.

Lactic acid will be used for the purpose of substituting for the acid that is normally produced by bacterial metabolism in the manufacture of cheddar cheese.

REVIEW OF LITERATURE

Acid production appears to be the important function of the culture in cheddar cheese making according to Miller and Elliker (21), and Wilster and Price (34). Streptococcus lactis is the first species to bring about an important change with the transformation of lactose to lactic acid. The production of acid begins after the starter is added to the milk and continues as long as lactose is available for fermentation.

The effects of acid in cheese making

The following are five effects that lactic acid has in the manufacture and ripening of cheddar cheese according to Wilster and Price (34), and Hastings et al. (11).

Acid favors coagulation of milk. The coagulation of milk for cheddar cheese is a sweet or rennet coagulation. The activity of rennet is influenced by the reaction of the medium. A more rapid coagulation of milk with rennet is then obtained with an increase in acid. Acid reacts with dicalcium phosphate to form a soluable calcium salt, monocalcium phosphate, which is necessary for the coagulation of the casein by rennet.

Acid favors expulsion of whey. The proper expulsion of whey is necessary to reduce the moisture content of cheese to the required standards. When the moisture content is too high the cheese may have off-flavors and a defective body.

The rapid production of acid aids in expelling the whey by causing contraction of the curd.

Acid favors fusion of the curd particles. It is necessary that the curd particles mat together and form a compact mass. The fusion of these curd particles is favored by the developed acid. Hastings et al. (11) report that a crumbly body usually results when there is insufficient acid to fuse the curd particles together.

Acid exerts a protective action against putrifactive bacteria. Putrifactive bacteria of various types are often present in cheese but they are prevented from growing largely because of acid that is present.

Inhibition of acid production in milk by antibiotics

Antibiotics may be responsible for slow or cessation of acid production. The work of Ruehe (28) showed that penicillin was in milk from cows treated for mastitis with penicillin.

Such antibiotics as aureomycin, penicillin, and streptomycin used for the treatment of mastitis in dairy cattle have remained in the milk for several milkings after treatment, according to Doan (6).

Hunter (12) reported that in some cases, as a result of herd treatment for mastitis with antibiotics, there were sufficient antibiotics in the mixed herd milk to inhibit bacterial growth.

Concentrations of antibiotics in milk that inhibit acid production

The work of Katznelson and Hood (16) in an experiment

with various antibiotics found penicillin to be the most active substances with aureomycin and subtilin equal in their ability for complete inhibition of acid production. The results of Katznelson and Hood are given in table 1.

Table 1. Influence of six antibiotics on acid production in milk by a mixed strain starter culture.

Antibiotic	Reciprocal of dilution necessary for: Complete inhibition	No inhibition
Penicillin	3,300,000	166,000,000
Aureomycin	1,000,000	20,000,000
Streptomycin	500,000	20,000,000
Chloromycetin	100,000	5,000,000
Subtilin	1,000,000	100,000,000
Bacitracin	100,000	20,000,000

Doan (6) reported that almost complete inhibition of acid production was caused by the presence of 0.1 units of penicillin per ml. of milk.

In work done by Krienke (17) it was found that at incubation temperatures of 70° F. and 95° F. there was little acid produced at the end of 18 hours and seven hours respectively when the milk contained 0.0005 mg. of aureomycin hydrochloride per ml. of milk. This milk had been pasteurized at 143° F. for 30 minutes.

Inactivation of antibiotics in milk

Doan (6), Hunter (12), and Katnelson and Hood (16) reported that ordinary pasteurization has no effect on any of the antibiotics studied. Doan (6) further studied the effect

of autoclaving on milk containing penicillin found that the potency of penicillin was only reduced to a detectable degree.

Doan (6) and Ruehe (28) reported the use of the enzyme penicillinase as a positive antidote for penicillin but they found that the cost was prohibitive for practical use.

Doan (6) also reported that oxidizing agents such as copper, and reducing agents such as cysteine and ascorbic acid had no effects on the antibiotics studied.

Detection of antibiotics in milk

Stoltz and Hankinson (30) report the use of a modified Scharer field test for the detection of several antibiotics in milk including penicillin and aureomycin. Churchill et al. (5) investigating this modified phosphatase test for the detection of antibiotics in milk found that the test can not be depended upon to indicate the presence of antibiotics. Churchill et al. (5) found that the modified Scharer field test for phosphatase indicated the variation of phosphatase content of milk from different cows instead of the presence of antibiotics.

No simple method for the detection of antibiotics in milk was found by Ruehe (28), but he suggested that suspected milk could be checked by heating 10 ml. samples in test tubes to 175° F. and incubating with 1 ml. of starter. A coagulum would form in 10 hours or less if no antibiotics were present.

Hunter (12) found that in cheese made from milk having sufficient amounts of penicillin to retard the rate of acid production the curd was 'short' and 'chippy', giving the appear-

ance of insufficient cooking. Cheese three months old made from milk containing 0-10 penicillin units per ml. had a weak pasty body. Cheese made from milk containing 0.15 units per ml. had a weak, pasty, and fermented body.

Mattick et al. (20) reported that there are several methods for the detection of antibiotics in milk but they require a great deal of time and do not always detect the lower concentrations which would prove to inhibit fermentative processes.

Mattick et al. (20) did describe a method used for the quantitative estimation of penicillin in milk based upon the ability of this antibiotic to inhibit the production of nitrite from nitrate by an active growing culture of Micrococcus pyrogenes var. aureus. The decreased production of nitrite, as compared with the control, was measured colormeterically. The test, according to Mattick et al. (20) is accurate for concentrations less than 1.0 Oxford unit of penicillin per ml., but the percision of the test slowly diminishes in concentrations higher than 1.0 Oxford unit per ml.

It was found by Mattick that 0.5 Oxford unit of penicillin resulted in complete inhibition of acid production and as little as 0.1 Oxford unit per ml. caused partial inhibition.

Mattick concluded that the test is applicable for the critical range of penicillin in milk (0-0.5 Oxford unit per ml.). The test can be completed in two hours.

Du Bois and Dibblee (7) concluded that the presence of

quaternary ammonium compounds had no effect on the bacterial counts of raw or pasteurized milk at concentrations from 40 ppm. to 2,200 ppm. He observed that concentrations higher than 2,000 ppm. inhibited growth of gram positive acid producing organisms but not that of gram negative type.

Mull and Fouts (24) reported that quaternary ammonium compounds would have to be in low quality milk in concentrations of 200 to 250 ppm. to bring about significant decreases in bacterial counts.

Moore (23) found that 25 ppm. of quaternary ammonium compounds in milk caused partial inhibition and 50 to 75 ppm. caused complete inhibition of lactic acid starter organisms. Barber et al. (3) reported some inhibition of Streptococcus lactis at 100° F. in reconstituted skim milk containing as little as 10 ppm. added quaternary ammonium compounds.

A study conducted by Miller and Elliker (21) on the effect of three different quaternary ammonium compounds on acid development in lactic acid starter found that 50 ppm. of quaternary ammonium compounds in milk caused almost complete inhibition.

A mixed commercial culture of Streptococcus lactis, L. lactis, and Streptococcus thermophilis was slightly inhibited by each of the quaternary ammonium compounds. The inhibition of acid development was nearly complete in all cultures with 25 to 30 ppm. of quaternary ammonium compounds in the milk when the organisms were incubated at their maxium growth temperatures. At normal incubation temperatures,

50 ppm. of the quaternary ammonium compounds in milk nearly effected complete inhibition.

In the same study (21) triplicate lots of cheddar cheese were made with milk containing 0, 5, and 10 ppm. of alkyl diethyl benzyl ammonium chloride. Commercial lactic cultures were used for these trials. Table 2 shows that the milling time was delayed 15 minutes with the presence of five ppm. and 45 to 60 minutes by 10 ppm. of quaternary ammonium compounds in the manufacture of cheddar cheese.

The results of Miller and Elliker (21) suggest the necessity of employing farm and plant sanitizing procedures that would avoid contamination of milk with inhibitory concentrations of quaternary ammonium compounds.

Detection of quaternary ammonium compounds in milk

Various tests have been recommended to determine the concentration of quaternary ammonium compounds in water solutions but few have been recommended for milk.

Miller and Elliker (21) modified the eosin-indicator method for determining quaternary ammonium compound concentrations and were able to detect as little as five ppm. The modified eosin-indicator method (22) is based on the extraction and precipitation of quaternary ammonium compounds in a tetrachloroethane-acetone-eosin indicator solution. The method has proven suitable for determining concentrations in milk of quaternary ammonium compound preparations commonly used in dairy sanitation procedures.

Table 2. Effect of added quaternary ammonium compound in cheese milk on acid development during manufacture of cheddar cheese.

	Time	Change in per cent titratable acidity with the following concentrations of QAC:			
		Control 0 ppm.	QAC 5 ppm.	Control 0 ppm.	QAC 10 ppm.
	(hr.:min.)	(%)	(%)	(%)	(%)
Received milk	0:00	0.17	0.17	0.17	0.17
Added starter	0:15	0.18	0.18	0.17	0.17
Added rennet	0:15	0.19	0.19	0.18	0.18
Cut curd	1:45	0.12	0.12	0.13	0.13
Began cooking	2:00	0.12	0.12	0.13	0.13
Steam off	2:30	0.14	0.14	0.14	0.14
Drained whey	3:30	0.16	0.16	0.17	0.16
Packed curd	3:45	0.21	0.21	0.23	0.18
Cheddared	4:00	0.27	0.26	0.30	0.23
	4:15	0.34	0.31	0.40	0.27
	4:30	0.39	0.37	0.45	0.30
	4:45	0.44	0.42	0.52*	0.33
	5:00	0.52*	0.48*		0.36
	5:15		0.52*		0.40
	5:30				0.48
	5:45				0.54*

*Milling time.

Effect of bacteriophage on acid production in milk

The work of Smith et al. (29) showed that bacteriophage was the primary cause of slow acid production. From 87 whey and product samples from 32 Oregon dairy plants 40 were found to contain bacteriophage active against one or more of 18 single strain starters. Out of 23 cases of serious starter failures, 21 were traced to bacteriophage.

In a similar study (29) filtrates from various cheddar, blue mold, and cottage cheese, and buttermilk samples, 65 out of 99 samples were found to contain heat labile inhibitory agents. Smith et al. (29) concluded that most of the 65 samples owed their inhibitory effects to bacteriophage.

Babel (2) reported that the presence of bacteriophage in cheese starters caused almost a complete cession of acid production and was the most common cause of dead starters.

Jordan (15) and Burrows reported that bacteriophage is a filterable virus that caused the lysis of the bacterial cell resulting in the stoppage of acid production or metabolic processes.

In further studies by Jordan and Burrows (15) it was generally agreed that the multiplication of bacteriophage requires the presence of young, active, growing, and metabolizing bacteria in nonnutritious solutions.

Source of bacteriophage

Whitehead and Hunter (32), John and Katznelson (16), and Anderson et al. (1) all agreed that the major source of

bacteriophage was from the separator. Droplets of infected whey may easily gain access to the starter milk and a small whey infection of one day may result in a starter failure on the following day.

Factors effecting the reaction of bacteriophage on bacteria

Bacteriophage does not react the same on all bacteria but is influenced by several factors which produce different results.

Nourishment. Gardner (8) reported that bacterial multiplication is necessary for the regeneration of bacteriophage. If Streptococcus lactis organisms are suspended in water or in any other fluid that does not offer the nourishment necessary for growth, the strongest bacteriophage has no appreciable effect on the bacterial cell. The condition is different if a little meat broth is added to the mixture; reaction of the bacteriophage upon the organisms begins immediately. Gardner (8) further reported that if a small fully active dose of bacteriophage is introduced into two suspensions of a sensitive bacterium in fresh broth, one being thin and the other very dense, the former will show the typical regeneration and lysis by the phage, while nothing at all can be seen to happen in the latter. Gardner (8) believes that there was plenty of nourishment for the small number of bacteria in the first, so they multiplied freely and were subject to attack from the bacteriophage; but in the second suspension, the first stages of growth of the very numerous cells exausted the medium, and the culture failed to reach the stage of unrestricted multiplication which was necessary for production

of bacteriophage.

Single strain starters. In New Zealand, Whitehead and Hunter (32) found that the use of a single strain starter to make cheese has been accompanied with the occasional failure of the starter to develop acid. The same difficulty was experienced in England (32) so the policy of adding four per cent starter was practiced. The addition of the large amount of starter made it difficult to make cheese of good body and texture and long keeping quality.

Parmelee and his associates from the United States (26) concluded that single strain organisms were destroyed completely when incubated in the presence of bacteriophage from four to five hours at 30° C.

Whitehead and Hunter (32) in 1937 incubated bacteriophaged Streptococcus lactis cultures and noticed that some of the organisms remained alive. A selection of colonies grown from the resistant ones gave lysogenic variants which were either weak or strong acid producers. The resistant strain, according to Jordan and Burrows (15), may be unaltered in its immunological character and behavior toward other bacteriophage.

Multiple strain starters. To overcome the difficulty of slow acid production multiple strain starters were used. During an outbreak of bacteriophage in Iowa in 1947 Babel (2) reported that of 15 different cultures of Streptococcus lactis one culture was resistant to all of the bacteriophage types isolated to date. This one resistant culture produced

acid very slowly making it unsuitable for a single strain culture. Babel (2) further stated that multiple strain cultures may fail as completely as single strain cultures to produce acid. In an experiment he also stated that after running a test on the effects of bacteriophage on single and multiple strain starters that the presence of bacteriophage resulted in almost complete cessation of acid production when either single or multiple strain cultures were used.

Fixation. Gardner (8) emphasized that if a quanity of bacteriophage is added to a sensitive bacterial suspension and the mixture is permitted to stand for ½ of a hour and then centrifuged, the clear liquid will be found to have lost nearly all its lytic power. Gardner stated that the experiment showed the bacteriophage had attached itself to the bacteria and had been removed from the liquid. The bacteria fixed the susceptible bacteriophage whether the bacteria was living or dead but the lysis followed only in living cultures. Gardner (8) further states (p. 86),

> A race of bacteria that is insensitive to a particular bacteriophage has no fixing power for it. Further, if a phage has only feeble action on a certain bacterium, a large number of cells are needed to fix it, whereas a strong phage needs far fewer. There can be little doubt that this is because the 'weakness' of a phage in respect to a particular culture is an expression of the small proportion of the cells on which it can act, for when the fixing cells are few, a large amount of culture will be needed to effect complete fixation. On the contrary, 'strength' means affinity for all the cells; wherefore a relatively small amount of culture will fix a strong phage completely.

Bacteriophage inhibitors

The use of chemicals, ultraviolet light, and acid are considered as inhibitors of bacteriophage.

Acidity. Prouty (27) conducted a test to find the effect of acidity and storage on bacteriophage. A susceptible culture of bacteriophage was added at the rate of 1 per cent to sterile non-infected whey filtrates in which the natural acidity had been allowed to develop to 0.1%, 0.5%, and 0.9%. The samples were then stored at 3° C. for a period of 29 days. At intervals the bacteriophage titre was determined. The results showed that the destruction of the bacteriophage was brought about if the acidity during the storage remained above 0.5%. However, if the acidity during storage remained below 0.5% there appeared to be little loss of strength of the bacteriophage.

Ultraviolet light. Greene and Babel (9) reported that ultraviolet light inactivated bacteriophage. Factors such as decreased output of bulbs, increased distance from bulb, bacteriophage in a thick film, decreased the effectiveness of irradiation by ultraviolet light.

Greene and Babel (9) said (p. 514),

> The long time necessary to destroy bacteriophage by ultraviolet light at relatively short distances from the lamp and increased resistance of dry bacteriophage to ultraviolet light appears to make this procedure of doubtful value for the destruction of bacteriophage in commercial plants experiencing difficulty with bacteriophage.

Whitehead and Hunter (32) also agreed with Green and Babel (9) on the effectiveness of ultraviolet light on bacteriophage.

Heat. Streptococcus lactis bacteriophage is reported by Hunter and Whitehead (13) as being destroyed by heat treatment at 30° C. to 70° C. at pH 6.0.

Chemical disinfectants. The essential features of any disinfectants to be used in the destruction of bacteriophage are the absence of deletrious influences on dairy produce and the absence of poisonous effects on animals and humans. The second essential feature of a disinfectant is rapidity of action. This second feature is important because there is a development of bacteriophage and regularly there is a constant reinfection of the surroundings.

Disinfection experiments on bacteriophage differ from those on bacteria in that the bacteria can be filtered out of its growth medium but bacteriophage can not be filtered out but remains associated with large amounts of protein. Hunter and Whitehead (13) reported that the proteins obviously modify the influence of chemicals on the bacteriophage.

Hunter and Whitehead (13) conducted an experiment on the effect of chemicals on eight different strains of bacteriophage. The bacteriophage types were all of the same order of strength, giving titers of 10^{-7} to 10^{-8} and were all propagated on susceptible organisms growing in samples coming from one batch of milk.

The temperature at which the following experiment was carried out was room temperature. Equal volumes of phage and chemical solutions of various strengths were mixed together in sterile tubes. After the end of the specified time a

standard loop full of the mixture was transferred to the sterilized milk inoculated with the Streptococcus lactis organisms. The inoculated milk was incubated for five hours at 37° C., then overnight at 22° C. Surviving bacteriophage was indicated by the presence of coagulated milk after the incubation periods.

The results obtained with seven chemicals acting on bacteriophage which attacked Streptococcus lactis organisms are given in table 3, p. 17. The results show that active chlorine and permanganate had greater killing power in comparison with all the other disinfectants used. Hydrogen peroxide and formaldhyde had sufficient disinfecting power to merit consideration but they were not suitable for use under dairy conditions.

Use of edible acid in making curd for process cheese

Under U. S. patent number 2,325,217 Beers (4) describes the use of edible acid in making process cheese. The cheese making process involved few changes as compared with the regular procedure of making cheddar cheese up to the time of dipping.

The temperature of the curd and whey mixture up to dipping was maintained at 107° F. When the acidity had risen to 0.155 per cent, two thirds of the whey was drained from the vat. After the curd had properly firmed and had reached the pH values of 5.8 to 6.0 the remainder of the whey was removed and a aqueous solution of edible acid was added to curd. Beers (4) recommends 27 pounds of edible acid in 720 pounds

Table 3. Power of disinfectants in destroying bacteriophage. (13, p. 64)

Disinfectant	Final concentration in phage-disinfectant mixture %	Time required for complete destruction
Hypochlorite (available chlorine)	0.05	Less than 1 minute
$K Mn O_4$	0.25	Less than 1 minute
	0.05	Between 1 and 5 minutes
	0.025	Not in 2 days
H_2O_2	3.0	Between 15 and 60 minutes
	2.5	Between 1 and 24 hours
	0.5	Between 1 and 24 hours
H C H O	5.0	Between 5 and 30 minutes
	2.5	Between 30 and 60 minutes
	1.0	Between 1 and 24 hours
$Hg Cl_2$	2.5	Between 1 and 24 hours
	1.0	Between 2 and 3 days
	0.5	Not in 14 days
Alcohol	90.0	Between 3 and 4 days
	85.0	Between 3 and 4 days
	80.0	Between 2 and 3 days
	75.0	Between 3 and 4 days
	70.0	Not in 6 days
Phenol	2.5	Not in 14 days

of water for 10,000 pounds of whole milk containing 3.5 per cent fat. The acid was disolved in water at 55° F. There was no further heating of the curd and acid mixture. Lactic, acetic, and propionic acid may be used according to Beers (4).

Beers further reports (4) that best results are obtained when the curd is allowed to drain overnight. The cheese particles failed to mat together as in regular cheese making. After the curd was dipped it was ready to be processed into process cheese.

According to Beers (4) the processing temperature may be higher than 160° F. as compared with ordinary cheese processing which uses a temperature of 150° F.

Upon examination of five pound loaves of the processed cheese, according to Beers (4), an improvement of flavor occured during storage, whereas no such improvement was regularly observed in ordinary process cheese.

PROCEDURE

The manufacture of cheddar cheese

Preliminary work on this experiment was concerning the optimum time at which acid could be added as an emergency method during the manufacture of cheese from slow or non-acid milk. Lactic acid was added at the following cheese manufacturing steps: one half of the acid to the milk before setting, with the remainder of the acid added to the curd and whey; to the curd and one third of the whey just prior to packing; to the milled curd prior to salting; and to the milled curd after salting. Sufficient acid was added to adjust the pH of the cheese to approximately 5.4.

The supply of milk for this experiment was obtained from non-antibiotic treated cows at the Utah State Agricultural College dairy farm. Prior to the manufacture of the cheese, the milk was pasteurized at 143° F. and held overnight.

Lactic fermenting cultures of Streptococcus lactis, Leuconostoc citrovorum, and Leuconostoc dextranicum obtained from a commercial laboratory was the source of the starter used. The mother starter was transferred in sterile milk and incubated for 15-16 hours at 70° F. before transferring to bulk starter. The bulk starters were also incubated at 70° F. for 15-16 hours before adding to the milk for cheese making. The average titratable acidity of the bulk starter after incubation was 0.8 per cent.

Two ounces of cheese color was added to each 1000 pounds

of milk.

Three ounces of rennet were added to vat A and vat B for each 1000 pounds of milk. The amount of rennet added to vat C was reduced due to the fact that lactic acid was added to the milk which accelerated the rennet coagulation.

Nine lots of cheese were made with three vats in each lot. Vat A was designated as the control vat in which normal cheddar cheese was made according to the time-schedule method outlined and illustrated in figure 1. The time-schedule method is described by the United States Department of Agriculture, circular no. 880 (19).

The same procedure was followed in vat B except one ppm. of antibiotic was added to the milk after pasteurization.

Vat C was treated the same as vat B except lactic acid was added to the milk before the addition of rennet. The milk of lots one to six inclusive was acidified to a pH 5.4 to 5.5. Lots seven, eight, and nine were acidified to a pH of 5.75 to 6.0. Table 4 shows the different amounts of lactic acid added to the nine lots of milk in vat C.

The antibiotic used in this experiment to inactivate the lactic fermenting bacteria is sold under the registered trade mark of Polycitic. It is a tetracycline HCl crystalline compound active against both gram-positive and gram-negative bacteria, Clostridia, and Rickettsia, This antibiotic is relatively new on the market and it is believed to have great value in the treatment of mastitis in cows.

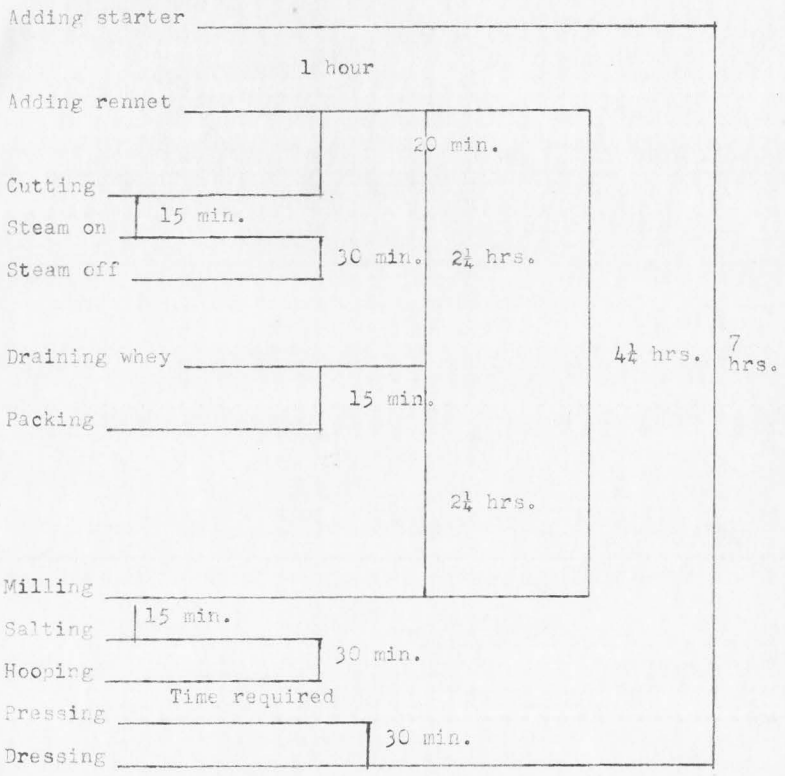

Figure 1. USDA time-schedule method for making cheddar cheese. (18, p. 16)

Table 4. Amounts of lactic acid added to 240 pounds of milk in vat C for cheese making.

Lot no.	Milliliters of 85% lactic acid in 10 liters of water
1	285
2	310
3	280
4	280
5	280
6	280
7	175
8	125
9	180

The analysis of cheddar cheese

The official method of the Association of Official Agricultural Chemists (18) was the method used in determining the moisture content of the cheese.

The Babcock test for fat in cheese according to Van Slyke (31) was the method used in determining the fat content.

The Beckman glass electrode pH meter was used in determining the pH of the cheese.

When the cheese was one week old, it was judged by two experienced judges for flavor, body, texture, and color. The cheese was scored according to the American Dairy Science Association score card as described by Nelson and Trout (25, 231).

RESULTS AND DISCUSSION

The manufacture of cheddar cheese

The results of the preliminary work concerning the use of added lactic acid in slow or non-acid milk showed that when the acid was added to the curd after cutting, the curd failed to mat together. The outside of the curd particles developed a slime layer which seemed to inhibit the fusion of the curd. When the acid was added to the curd before salting, the curd particles would mat together. However when the salt was added the curd broke down into the original curd particle, with the development of a slime layer on the outside of the curd. Further preliminary trials indicated that the cheese had good matting quality when the acid was added to the milk prior to the addition of rennet. Subsequent trials were thus based upon the addition of acid at this period.

The acid development during the manufacturing process of the cheese from vat A, B, and C are given in tables five, six, and seven respectively.

The results of vat A show the normal development of acid in cheese made from antibiotic free milk. The differences in titratable acidity and pH values of the nine lots of normal cheese at milling may be due to the differences in starter activity.

The cheese in vat B from milk containing an antibiotic showed no increase in acid during the making procedure but there was a drop in pH of the cheese between milling and after

Table 5. The acid development during the manufacturing process of normal cheese

Lot number	Whey at cutting		Whey at dipping		Whey at milling		Cheese 1 wk. old
	Tit. acidity	pH	Tit. acidity	pH	Tit. acidity	pH	pH
1.	.115	6.4	.14	6.2	.39	5.65	5.6
2.	.12	6.45	.13	6.25	.32	5.8	5.6
3.	.11	6.5	.13	6.3	.44	5.7	5.6
4.	.11	6.45	.13	6.25	.40	5.75	5.25
5.	.11	6.5	.135	6.2	.58	5.5	5.3
6.	.11	6.45	.14	6.25	-	5.55	5.2
7.	.11	6.45	.135	6.2	.40	5.5	5.2
8.	.11	6.45	.135	6.2	-	5.5	5.2
9.	.105	6.5	.13	6.3	.86	5.1	4.9

Table 6. The acid development during the manufacturing process of cheese made from milk containing an antibiotic.

Lot number	Whey at cutting		Whey at dipping		Whey at milling		Cheese 1 wk. old
	Tit. acidity	pH	Tit. acidity	pH	Tit. acidity	pH	pH
1.	.11	6.45	.115	6.45	.12	6.45	6.05
2.	.105	6.5	.105	6.5	.105	6.5	6.3
3.	.11	6.5	.11	6.5	.11	6.5	6.2
4.	.105	6.55	.105	6.5	.105	6.5	6.2
5.	.105	6.55	.105	6.5	.105	6.5	6.2
6.	.10	6.55	.10	6.5	.10	6.5	6.2
7.	.105	6.5	.11	6.5	.11	6.5	6.25
8.	.105	6.55	.105	6.5	.105	6.5	6.1
9.	.105	6.55	.105	6.5	.105	6.5	6.1

Table 7. The acid developed during the manufacturing process of cheese made from milk containing an antibiotic and added acid.

Lot number	Ml. of 85% lactic acid in 10 liter of water	After adding acid		Whey at cutting		Whey at dipping		Whey at milling		Cheese 1 wk. old
		Tit. acidity	pH	Tit. acidity	pH	Tit. acidity	pH	Tit. acidity	pH	pH
1.	285	.35	5.45	.29	5.45	.28	5.45	.28	5.5	5.65
2.	310	.38	5.4	.27	5.45	.28	-	-	5.5	5.7
3.	280	.35	5.5	.27	5.55	.28	5.55	-	5.55	5.75
4.	285	.37	5.5	.29	5.5	.28	5.5	.28	5.55	5.65
5.	285	.37	5.5	.27	5.5	.27	5.5	-	5.5	5.6
6.	280	.36	5.5	.27	5.5	.27	5.5	-	5.5	5.7
7.	175	.32	5.75	.22	5.8	.22	5.8	.22	5.8	5.8
8.	125	.27	6.0	.19	6.0	.19	6.0	-	6.0	5.95
9.	180	.30	5.75	.21	5.75	.21	5.75	-	5.75	5.8

the cheese was one week old. This drop in pH ranged from .2 to .4.

All of the lots of cheese made in vat B appeared to be insufficiently cooked. The curd was crumbly and did not have a close body or meaty texture after the end of the cheddaring process.

The emergency type cheese made in vat C from milk containing an antibiotic showed no increase in acid until the lactic acid was added. The titratable acidity and pH of the whey remained almost constant throughout the making process. The six lots of cheese with a pH of 5.45 to 5.5 had a softer, closer body as compared with lots seven, eight, and nine. The pH of lots seven and nine was 5.75, with eight having a pH of 6.0. The latter three lots were more comparable to normal cheese in body and texture during the cheddaring process.

A 10 per cent yield of cheese was obtained from vat A. The yield of cheese in vat B was slightly higher than in vat A because of the higher moisture content in the cheese from vat B. Lots one to six inclusive from vat C showed a slightly lower yield in cheese as compared to vat A. This lower yield is due to excessive fat losses in the whey. Lots seven, eight, and nine from vat C had a similar yield to vat A.

The analysis of cheddar cheese

Table 8 shows that the moisture content of all the cheese made in vat A was below 39 per cent.

The cheese made from milk treated with antibiotics in vat B was uniformely high in moisture. The lowest moisture

test was 44.4 per cent and the highest was 46.4 per cent. The results for each lot will be found in table 8.

The first six lots of the cheese made in vat C, with milk containing an antibiotic and added acid, had a similar high moisture test of 45.1 to 45.6. The milk in these first six lots were acidified to pH 5.45 to 5.5. When the milk was acidified to pH 5.75, as in lots seven and nine, the moisture content was reduced to 40.7 and 41.1 respectively.

Table 9 shows that cheese made from normal milk in vat A was above the cheddar cheese standards of not less than 50 per cent fat on the dry weight basis.

The cheese made in vat B made from milk containing an antibiotic generally had a lower fat test in comparison with vat A. This is to be expected in cheese with a higher moisture content.

The fat test in the first six lots of cheese made in vat C with milk containing an antibiotic and added acid, showed the lowest fat tests of all the cheese made. A whey sample from six lots tested 3.0 per cent fat while the whey from lots seven, eight, and nine tested only 0.5 per cent.

The cheese was scored for body and flavor one week after it was made. Tables 10, 11, and 12 gives the score of the cheese.

Lots one, two, and nine of the cheese made from normal milk in vat A were judged as having no criticisms except some mechanical openings. Lots three, four, five, six, seven, and eight were inferior in quality. Contamination of milk or starter caused some sweet fermentation which resulted in cheese

Table 8. Moisture analysis of cheese made from normal milk, milk containing an antibiotic, and milk containing an antibiotic and added acid.

Lot number	Normal cheese	Antibiotic cheese	Antibiotic cheese plus various amounts of added lactic acid
	%	%	%
1.	38.5	44.4	45.6
2.	38.6	45.2	45.2
3.	38.0	45.4	45.1
4.	38.9	44.8	45.5
5.	37.7	45.4	45.3
6.	38.5	46.4	45.2
7.	38.4	44.8	40.7
8.	38.8	45.6	41.6
9.	37.0	44.5	41.1

Table 9. Fat analysis of cheese made from normal milk, milk containing an antibiotic, and milk containing an antibiotic and added acid.

Lot number	Normal cheese	Antibiotic cheese	Antibiotic cheese plus various amounts of added lactic acid
	%	%	%
1.	51.2	49.5	47.4
2.	51.3	50.2	46.8
3.	51.5	50.2	46.5
4.	51.5	49.6	47.7
5.	52.5	51.3	45.5
6.	52.8	50.4	46.5
7.	52.8	51.6	51.4
8.	52.3	50.6	53.0
9.	50.8	51.4	52.6

% moisture on dry basis

Table 10. Score of cheese made from normal milk

Lot number	Flavor criticism	Flavor score	Body & texture criticism	Body & texture score	Color criticism	Color score
1.	None	40.0	None	29.5	None	10
2.	None	40.0	Open	29.0	None	10
3.	Unclean	37.5	Pin holes	27.0	None	10
4.	Unclean	37.5	Pin holes	27.0	None	10
5.	Fermented	38.0	Pin holes	27.5	None	10
6.	Fermented	38.0	Open	29.0	None	10
7.	Bitter	38.0	Pin holes	28.0	None	10
8.	Fermented	38.5	Pin holes	28.0	None	10
9.	None	40.0	Open	29.0	None	10

Table 11. Score of cheese made from milk containing an antibiotic.

Lot number	Flavor criticism	Flavor score	Body & texture criticism	Body & texture score	Color criticism	Color score
1.	Flat	39.0	Corky & open	27.0	None	10
2.	Flat	39.0	Corky & open	28.0	None	10
3.	Fermented	38.5	Corky & open	28.5	None	10
4.	Fermented	38.5	Corky & open	28.5	None	10
5.	Flat	39.0	Corky & open	28.5	None	10
6.	Flat	39.0	Corky & open	28.5	None	10
7.	Flat	39.0	Corky & open	28.5	None	10
8.	Flat	39.0	Corky & open	28.5	None	10
9.	Flat	39.0	Corky & open	28.5	None	10

Table 12. Score of cheese made from milk containing an antibiotic and added lactic acid

Lot number	Flavor criticism	Flavor score	Body & texture criticism	Body & texture score	Color criticism	Color score
1.	flat	39.5	Weak	29.0	None	10.0
2.	None	40.0	Weak	29.0	None	10.0
3.	None	40.0	Open weak	28.5	Mottled	9.0
4.	None	40.0	Open weak	28.5	Mottled	9.0
5.	Flat	39.0	Open weak	28.5	Mottled	9.0
6.	None	40.0	Open weak	28.5	Mottled	9.0
7.	None	40.0	None	29.5	None	10.0
8.	Flat	39.0	Corky	29.0	None	10.0
9.	None	40.0	Open	29.0	None	10.0

with a lower score.

The main criticism of the cheese made from milk in vat B containing an antibiotic was a flat flavor. The body and texture was corky and open in all nine lots. The color of the cheese was lighter than vats A and C.

The cheese made from milk in vat C containing an antibiotic and added acid had the highest flavor score in comparison with the other two vats of cheese. It was noted that in lots one through six the body was weak. Lots three, four, five, and six were mottled. All the cheese made in vat C seemed to give a more pronounced ripened flavor.

It is hoped that the impression will not be conveyed that best results can be obtained in cheese making by adding an antibiotic to the milk and then using an edible acid to get the desired flavor and body of cheddar cheese.

CONCLUSIONS

The addition of lactic acid to slow or inactive milk, prior to setting with rennet, offers a possible way of making good quality cheddar cheese. The same milk made into cheese without added acid is more inferior in quality. However, the limited results of this experiment point out the need for more research work to be done on this emergency method of making cheese with added acid.

The emergency method of adding acid to milk for cheese making may minimize the effects of bacteriophage, quaternary ammonium compounds, and antibiotic inactivation of the lactic fermenting bacteria.

The best results were obtained, in this experiment, when the milk for cheese was acidified to pH 5.75. The cheese made from milk acidified to pH 6.0 was comparable in quality but lacked flavor. When the milk was acidified to pH 5.45 and 5.5 the cheese had a higher moisture content, lower fat test, weak body, and mottled color.

In preliminary trials the addition of acid to the curd after cutting caused slimeness on the outside of the curd particles. This condition of the curd resulted in failure to fuse or press together.

The analysis of the cheese, made by the emergency method of adding acid, showed that the cheese manufactured from milk acidified to pH 5.75 resulted in superior quality, but was too

high in moisture to meet the cheddar cheese standards. The temperature of cooking may possibly be increased to make cheese with a legal moisture content of 39 per cent or below. The cheese made from acidified milk met the minimum standards of not less than 50 per cent fat on the moisture-free basis.

The practical use of added acid in cheese making depends upon the detection of slow or inactive growth of the lactic fermenting bacteria.

SUMMARY

Nine lots of milk with three vats in each lot were made into cheddar cheese. Vat A of each contained mormal milk; an antibiotic was added to vat B; and an antibiotic and varing amounts of edible acid were added to vat C. The time schedule method for making cheddar cheese was followed in each of the three vats.

The cheese was scored for flavor, body, and texture, and color one week after manufacture. The moisture and fat tests of the cheese were also determined at this time.

The limited results show that it is possible to make cheese of superior quality with the emergency method of adding lactic acid to inactive milk.

LITERATURE CITED

(1) Anderson, E. M. et al. The problem of bacteriophage in cheese making. Jour. of Dairy Research (13):58-71. 1942.

(2) Babel, F. J. Studies on the susceptibility of certain cheese cultures to the action of bacteriophage. Jour. of Dairy Science (30):507-516. 1947.

(3) Barber, F. W. et al. The effects of quaternary ammonium compounds on lactic starter cultures. Society of American Bact. Abstracts of papers, 49th. general meeting: p. 56. 1949.

(4) Beers, William O. Manufacture of process cheese. United States Patent Office, Patent number 2,325,217. July 27, 1943.

(5) Churchill, E. S. et al. A report on the modified phosphatase method of detecting antibiotic substances in milk. Milk Plant Monthly (4):28-31. 1951.

(6) Doan, F. J. Antibiotics in milk. Milk Plant Monthly 39 (9):28-30. September, 1952.

(7) Du Bois, A. S. and Dibblee, D. D. The influence of surface active cationic germicides on the bacterial population of milk. Jour. of Milk and Food Tech. 9:260-268. 1946.

(8) Gardner, A. D. Microbes and ultramicrobes. New York: The Dial Press, 1931.

(9) Greene, G. I. and Babel, F. J. Effects of ultraviolet irrediation on bacteriophage active against Streptococcus lactis. Jour. of Dairy Science (31):509-515. 1948.

(10) Harper, W. J. et al. Quaternary test-sensitive method for testing concentrations of quaternary type germicides. Soap and San. Chem. 24:159-160. 1948.

(11) Hastings, E. G. et al. Studies on the factors conderned in the ripening of cheddar cheese. University of Wisc. Agric. Exp. Sta. Research Bul. 25. Madison, Wisc. 1912.

(12) Hunter, G. J. Penicillin and the reductase test. Jour. of Dairy Research 16(2):149-151. October, 1949.

(13) Hunter, G. J. and Whitehead H. R. The action of chemical desinfectants on bacteriophage for the lactic Streptococci. Jour. of Dairy Research (11):52-66. 1940.

(14) Johns, C. K. and Katznelson, H. Studies on bacteriophage in relation to cheddar cheese. Canadian Jour. or Research (19):48-58. 1941.

(15) Jordan, E. D. and Burrows, W. Textbook of Bacteriology. 4th. ed. Philadelphia: W. B. Saunders, 1947.

(16) Katznelson, H. and Hood, E. G. Influence of Penicillin and other antibiotics on Lactic Streptococci in starter culture used in cheddar cheese making. Jour. of Dairy Science 32(11):961. November, 1949.

(17) Krienke, W. A. Effects of various 'drugs' in milk from mastitus treated cows. The Milk Dealer 39(7):50. April, 1950.

(18) Liepper, Henry A. et al. Official and tentative methods of analysis of the association of Official Agricultural Chemists. 6th. ed. Washington D. C.: Association of Official Agricultural Chemists, 1945.

(19) Lochre, Harry R. et al. Making American Cheddar cheese of uniformly good quality from pasteurized milk. U. S. D. A. Circular No. 880. 1951.

(20) Mattick, L. R. et al. A quantitative procedure for the determination of inhibitory substances in milk-Penicillin. Jour. of Dairy Science 38(8):829-834. August, 1955.

(21) Miller, D. D. and Elliker, P. R. Effect of quaternary ammonium compounds on the activity of lactic acid starter bacteria in milk and cheese. Jour. of Dairy Science 34: 279-286. 1951.

(22) Miller, D. D. and Elliker, P. R. A rapid method of testing for quaternary ammonium compounds in milk. Jour. of Dairy Science 34:273-278. 1951.

(23) Moore, A. V. The effects of quaternary ammonium sanitizers on the quality of milk. Southern Dairy Prod. ucts Jour. 44(5):62-64. 1948.

(24) Mull, L. E. and Fouts, E. L. Some observations on the use of Roccal. Jour. of Food Tech. 10:102-104. 1947.

(25) Nelson, John A. and Trout, M. G. Judging Dairy Products. 3rd. ed. Milwaukee, Wisc.: Olsen Publishing Co., 1951.

(26) Parmelee, C. E. et al. The bacteriophage of cheese cultures. Nat. Butter and Cheese Jour.:28-30. 1950.

(27) Prouty, C. C. Inactivation of bacteriophage of the lactic acid Streptococci at high and low pH levels. Jour. of Milk and Food Tech.:329-331. 1950.

(28) Ruehe, A. A. Penicillin and other starter inhibitors. Milk Plant Monthly 39(3):36. March, 1950.

(29) Smith, V. M. et al. Importance of bacteriophage as a cause of slow starters. Jour. of Milk and Food Tech.: 15: 1952.

(30) Stoltz, E. I. and Hankinson, D. J. A test for antibiotics in milk. American Milk Review 12(12):60-63. Dec. 1950.

(31) Van Slyke, L. L. and Price, W. V. Cheese. 2nd. ed. Rev. and enlarged. New York: Orange Judd Pub. Co. Inc., 1952

(32) Whitehead, H. R. and Hunter, G. J. Bacteriophage infection in cheese manufacturing. Jour. of Dairy Research (14):64, 80. 1945.

(33) Wilson, J. B. Report on quaternary ammonium compounds in food. Jour. of Official Agric. Chemists 29:311-327. 1946.

(34) Wilster, C. M. and Price, F. Preparation of starter for cheese, butter-milk, and butter. Oregon State College Agricultural Experiment Station, Station Bul. 379, Corvallis, Oregon. Dec., 1940.