EVALUATION OF COMMERCIAL BUTTERMILK AND PRE-ACIDIFIED CULTURED BUTTERMILK BY ORGANOLEPTIC AND GAS CHROMATOGRAPHIC ANALYSES

by

HAROLD A. ROBERTS

B. S., Kansas State University, 1959

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy & Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Approved by:

Major Professor

TABLE OF CONTENTS

INTRODUCTION

The dairy product commonly known as buttermilk has changed considerably during the last century. Originally buttermilk was that portion of the cream left after most of the fat was removed during the buttermaking process. Today most, if not all, buttermilk available for consumption is made with the aid of selected bacteria known as lactic starter cultures. A primary reason for this change was the problem of producing a high quality and uniform product from cream of varying quality.

The consumer has shown an increased interest in cultured dairy products. Cottage cheese, cultured sour cream, and cultured sour cream products such as chip dips have shown an increase in consumer consumption. With more public awareness of cultured products, their food values, and their various uses in food preparation, the manufacturer must maintain or improve the quality of his products.

A description by Nelson and Trout (50) of the lactic culture flavor is that "cultures should have a pleasing, bouquet flavor resulting from the blend of a clean, delicate, somewhat aromatic odor and a pronounced though clean acid taste." With the methods used today in the manufacturing of cultured buttermilk, this description could apply to desirable buttermilk.

There has been much investigation on lactic starter cultures used in cultured buttermilk. There also has been some study on the direct acidification process with added synthetic flavor compounds. Little work has been published on direct acidification plus the use of lactic starter cultures in developing a desirable buttermilk. Lactic starter

cultures are often difficult to maintain in proper balance through repeated culture transfers, and problems of contamination and bacteriophage are always present. The primary disadvantage of the direct acidification process is poor body and texture of the finished buttermilk.

The objectives of the research reported in this manuscript were to determine quality standards for buttermilk and to determine if buttermilk could be improved or be more consistent in quality by using a combination of cultures and direct acidification. Gas chromatographic analysis was used to determine certain volatile chemical components and their concentrations in buttermilk samples and organoleptic evaluation was used as an indication of how these components affected flavor and aroma. This study was accomplished by evaluating and comparing commercial cultured buttermilk, cultured buttermilk produced for control samples, and buttermilk produced using direct acidification and lactic starter cultures. The time required to coagulate the buttermilk using the combined culture and direct acidification process as compared to the control cultured buttermilk also was considered.

REVIEW OF LITERATURE

Certain chemical components that affect flavor and aroma are necessary to produce a quality buttermilk. There is a definite relationship between the lactic starter cultures used and production of the chemical components.

Starter Organisms Used in the Production of Cultured Buttermilk

Lactic cultures or starters are used to produce a number of different cultured products. The organisms vary depending upon which product is desired. Harmon (27) listed four general functions of a lactic starter culture: (a) forms acid to induce coagulation, (b) facilitates expulsion of whey in cheese making, (c) produces organic compounds that are associated with desirable flavor, and (d) inhibits undesirable contaminating organisms. To produce a culture that meets the above functions which in turn will yield a cultured buttermilk of desired acid, flavor, and aroma, a single strain or a group of different lactic organisms may be used. Lindsay (37) classified lactic starter culture bacteria into three general groups. One produces lactic acid from lactose, the second is an associative type which ferments citric acid producing desirable flavor and aroma components and the third type is referred to as dual purpose as it ferments both lactose and citrates.

In the production of cultured buttermilk, the organisms used are Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetilactis, Leuconostoc citrovorum, and Leuconostoc dextranicum. The type of bacteria used to ferment lactose and produce lactic acid are the streptococci which consist of S. lactis and S. cremoris. The bacteria that ferment the citrate salts in milk to the desired flavor and aroma components are L. dextranicum and L. citrovorum. S. diacetilactis ferments lactose and citrates to lactic acid and volatile chemical components.

Harmon (27) stated that a desirable lactic culture consists of 90% streptococci strains and 10% leuconostoc strains to produce the desired acid, flavor, and aroma. The blending of the different types of bacteria and the proportion in which they are blended varies as is evident by the large number of stock cultures available on the commercial market.

Lactic starter cultures may consist of single species of organisms or mixed strains of two or more species. One or more strains of S. lactis or S. cremoris is used in manufacturing hard cheese such as Cheddar and Monterey since lactic acid is the primary component desired. The general purpose culture used in cultured buttermilk is made up of the streptococci and leuconostoc organisms mentioned above in any combination of the numerous strains of these organisms. S. diacetilactis may be added with the above cultures or used alone to give an additional flavor to the cultured buttermilk. Elliker (21) stated that S. diacetilactis also produced a large amount of carbon dioxide as well as other desirable components.

The activity of cultures was reported by Elliker (21) with regard to how rapidly acid was produced in the cultured buttermilk. He observed the activity of single and mixed strain lactic starter cultures and found that some lactic streptococci produce acid at a rapid rate while others produced acid very slowly. He discussed the various factors that affect the activity of the lactic cultures such as enzymes, amino acids, antibiotics, bacteriophage, and the genetic makeup of the different strains of lactic streptococci. Vincent (55) reported on

different methods used to stimulate starter activity by the use of proteolysate.

Collins (15) observed that different strains of S. lactis and different strains of S. cremoris differed mainly in the rate of acid production, in the amount of nisin produced, and in bacteriophage sensitivity. He reported a need for a fast acid producing strain that is low in nisin and highly resistant to bacteriophage.

Chemical Components of Cultured Buttermilk

Lactic cultures containing the desired blend of bacterial species produce a desirable flavor through the fermentation process. Babel (4) stated that the principal chemical components found in cultured buttermilk were diacetyl, volatile acids, carbon dioxide, and lactic acid. Other workers (14, 28, 39, 48, 53) listed other compounds including ethyl alcohol, acetaldehyde, acetone, methyl sulfide, and acetylmethylcarbinol in addition to the volatile acids consisting of acetic acid, propionic acid, formic acid, and butyric acid. Harper (28) reported that some chemical components may be present in such small amounts that they may be undetected by most analytical methods. There have been a number of theories reported (11, 20, 45) for the breakdown of milk constituents in the fermentation process to produce the desired flavor of cultured buttermilk.

Reports by several workers (25, 37) indicated that there was little agreement on the breakdown or metabolism of citric acid to 2,3-butylene glycol. The most recent theory reported by Lindsay (37) is summarized

in Fig. 1. In this metabolic pathway, enzymes are important in the different reactions in the metabolism of citric acid.

Citric acid --------→ Oxaloacetic acid + Acetic acid

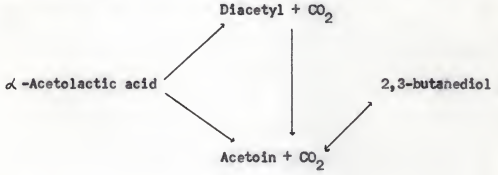
Oxaloacetic acid --------→ Pyruvic acid + CO_2

2 Pyruvic acid + 2 TPP --------→ 2 Acetaldehyde • TPP + $2CO_2$

Acetaldehyde • TPP --------→ Acetaldehyde - TPP

Acetaldehyde • TPP + Acetaldehyde --------→ Acetoin + TPP

Acetaldehyde • TPP + Pyruvic acid --------→ α-Acetolactic acid + TPP



TPP = Thiamine pyrophosphate

Fig. 1. Metabolic pathway of citric acid by S. diacetilactis (Lindsay)

Lactic acid. Lactic acid is produced in the fermentation of lactose primarily by S. lactis and S. cremoris. The amount produced is measured as developed acidity in determining titratable acidity. Lactic acid is stable and usually does not decrease once it is produced. Although lactic acid affects the flavor of cultured buttermilk, it does not contribute to aroma. The rate of production varies depending upon the species of the organism in the culture as reported by Hammer and Babel (25).

Volatile acids. In the 1920's, Hammer et al. (16, 24, 26) reported on total volatile acids produced by starter organisms. The method used was steam distillation and the total volatile acids were measured by titration with NaOH. It was found that an increase in total volatile acids accompanied the increase in total acidity and that this increase in total volatile acids was greatest in the later stages of the ripening period. The amount of total volatile acids also varied depending upon the lactic culture used. Acetic acid and propionic acid were found to be the two main acids in total volatile acids with acetic acid being in the greater amount. Acetic acid influences flavor and aroma by having a distinct acid characteristic. Acetic acid was produced by associate organisms and propionic acid was produced by S. lactis and S. cremoris organisms. Later reports by Chou (14) indicated that valeric acid was also present in buttermilk.

Hempenius and Liska (29) reported that the amount of acetic acid recovered by steam distillation and gas chromatographic analysis increased as the fat content increased. His conclusion was based on a study in which a known amount of acetic acid was added to milk with different fat contents.

Acetaldehyde. A number of workers (10, 32, 33, 38, 40) reported that acetaldehyde was produced in cultured buttermilk by the action of bacteria, especially S. lactis, S. cremoris, and S. diacetilactis. A green flavor was noted in the lactic starter culture and in the cultured buttermilk and was associated with the type of the organism and age of the products. Keenan and co-workers (33) made a study of the production of acetaldehyde by single-strain lactic streptococci and

found that all cultures produced a green flavor. They found that the production of acetaldehyde and the corresponding increase in microbial population showed a relationship with the above three organisms. Keenan et al. (33) and Bassette et al. (5) concluded that during the period of incubation, a reduction of acetaldehyde to ethanol could be expected with S. lactis and S. cremoris cultures but there was no change in the acetaldehyde concentration with S. diacetilactis.

Bills and Day (10) also observed that organisms that produced lower amounts of ethanol retained higher amounts of acetaldehyde. An increase in ethanol was reported when acetaldehyde was added to cultures. This process of the breakdown of acetaldehyde to ethanol by organisms used in the production of buttermilk and other cultured products is very important in desirable flavor development.

Lindsay et al. (40) discussed the diacetyl-acetaldehyde ratio where a ratio lower than 3.2:1 produced a green flavor and a ratio of 5.5:1 produced a harsh flavor. They considered a 4:1 ratio ideal although Keenan et al. (31) found that it was not necessary to have an exact 4:1 ratio to have a good flavor. From these studies, the conclusion was that a small concentration of acetaldehyde is necessary in the culture to arrive at a full flavored product. It is generally felt that there is no way commercially to remove acetaldehyde from a green flavored culture to improve the flavor although with the use of L. citrovorum, a reduction was noted due to the metabolic change of acetaldehyde to ethanol.

Methyl sulfide. Day et al. (18) reported that dimethyl sulfide could improve the flavor of butter by reducing the harsh diacetyl flavor. The work by Day et al. (18) and Reddy et al. (52) indicated that methyl sulfide affects the flavor of dairy products and that it was associated with feed flavor in the milk supply. However, it was reported by Toan et al. (54) that methyl sulfide also was produced by Aerobacter aerogenes.

Acetone. Acetone is a chemical compound produced by the change of acetic acid and butyric acid by special types of bacteria. In its pure state, acetone is a colorless liquid with a pleasant odor. Leviton and Marth (35) proposed a general pathway for conversion of acetic acid to acetoacetic acid to acetone with the aid of certain enzymes.

Ethanol. Bills and Day (10) indicated that there was a difference in ethanol production between strains within a species of lactic streptococci but that each strain was consistent in its production of ethanol as well as acetaldehyde. Leuconostocs also were able to produce ethanol by reducing acetaldehyde. Ethanol, in its normal concentration produced by lactic organisms, had very little effect on the flavor of buttermilk due to a very high flavor threshold value. However, the esterification of ethanol and short chain fatty acids yielded a flavor that is detectable in low concentrations.

A comparison of concentrations of acetaldehyde and ethanol in lactic cultures made by Bills and Day (10) showed that the concentration of ethanol generally increased as acetaldehyde decreased over a long

incubation period. As the incubation temperature increased, both components increased in concentration except when S. cremoris was used.

In cheese studies, Bills and co-workers (11) found a relationship between the level of ethanol and fruity cheese. They considered that excessive ethanol might have a direct effect on the esterification of free fatty acids and result in the change of the level of ethyl esters.

Diacetyl. Diacetyl, which also may be referred to as biacetyl, is volatile and has a definite yellowish color. Numerous studies have been made and the conclusion is that diacetyl is one of the most important flavor components in cultured buttermilk. Babel (4) found a definite relationship between flavor and the concentration of diacetyl. He observed that the amount of diacetyl in buttermilk varied between 1.75 and 2.5 ppm with an average of 2 ppm. If the diacetyl was much over 2.5 ppm, a harsh flavor developed. A relationship existed between the amount of diacetyl and the acidity of the cultured buttermilk; as the titratable acidity increased, the diacetyl concentration increased.

Bennett et al. (9) reported on the flavor threshold value of diacetyl in skimmilk at various pH's. He indicated that an average diacetyl threshold in skimmilk with a pH 6.8 was 0.01 ppm and in skimmilk with a pH 5.0, the threshold value was 0.20 ppm.

Acetoin. Acetylmethylcarbinol or acetoin is a product derived from the metabolism of citric acid by lactic organisms in cultured products. The associated bacteria or the citrate fermenters are primarily responsible for the production of acetoin. Hammer and

Babel (25) and Lindsay (37) reported that acetoin in its pure form is odorless and flavorless and that it is important only in its direct relationship to the diacetyl concentration of cultured products. Acetoin is a liquid but it polymerizes to a solid especially at low temperature. The general view is that acetoin is produced by reducing the diacetyl enzymatically and that -acetolactic acid is the precursor of both diacetyl and acetoin.

Both diacetyl and acetoin are considered to be unstable components in lactic cultures, reducing to 2,3-butylene glycol (2,3-butanediol). 2,3-butylene glycol is of no importance in the flavor or aroma of buttermilk because it is odorless and flavorless.

Carbon dioxide. Carbon dioxide is produced by fermentation of citric acid and is important in developing an ideal buttermilk flavor. Reports by Babel (4) stated that the agitation or working of cultured buttermilk caused the release of carbon dioxide and possibly a flat flavor defect. Many processing techniques used today include the addition of citric acid or sodium citrate to the milk to increase the production of carbon dioxide. Carbon dioxide is not desired except in a very small amount in cottage cheese making as it will cause floating curd.

Titratable Acidity and pH of Cultured Products

In cultured products, titratable acidity and pH are used as measures of the lactic acid produced. It is known that a change in serum solids will change the titratable acidity and pH; as serum solids

increase, there is an increase in titratable acidity. Wilkowske (56) reported that a relationship existed between titratable acidity, pH, and the serum solids content of cultured buttermilk during the fermentation process. He developed a good quality cultured buttermilk with 11% serum solids, a titratable acidity of 0.90 to 0.95% and a pH of 4.5 to 4.4.

Manufacturing Methods Used in Production of Commercial Cultured Buttermilk

A study of cultured buttermilk practices in Ohio reported by Kristoffersen and Gould (34) indicated that a wide variety of manufacturing practices existed. They found the program of purchasing lactic cultures and the preparation of cultures and bulk starters also varied among manufacturers. They stated that this wide variation in manufacturing practices could be the reason for the inconsistencies in quality of cultured buttermilk and that one procedure seemed no better than another.

Other reports (1, 12) also indicated that a problem existed in the manufacturing of a consistently high quality cultured buttermilk. There have been no great changes noted in commercial processing techniques in the past thirty years even though there is greater knowledge of cultures and culture handling. A study by Bingham et al. (12) introduced a new processing technique by altering the heat treatment of the milk. The selection of the lactic culture was very important in this process since some cultures used in the vat method of processing would

not produce the desired body and flavor due to the altered heat treatment of the milk.

Since there are various methods used in producing cultured buttermilk, no one procedure can be called standard. In general, fresh milk containing 1 to 2% fat and 9 to 10% solid-not-fat is used. The milk is pasteurized at 82 C to 88 C for 30 to 45 min by the vat method or 88 C to 90 C by high-temperature-short-time. The milk is then cooled to 21 C and a 1 to 1.5% inoculation of a good buttermilk starter culture is made and followed by agitation for 10 to 15 min. The buttermilk is incubated at 21 C for 14 to 16 hours until approximately an 0.80% titratable acidity is reached and then it is cooled to 7 C or below as it is agitated.

Factors Affecting Cultured Buttermilk Flavor

Elliker (21) has suggested several ways to enhance the flavor of buttermilk by the use of selected organisms. S. diacetilactis has been used in lactic starter cultures but due to the large amount of CO_2 it produces, the trend has been away from this organism. L. citrovorum has been used extensively in recent years as an addition to commercial lactic cultures. Elliker also reported a method using the direct acidification process with L. citrovorum. Lindsay et al. (41) also reported methods of improving the flavor of lactic cultures by the use of different strains of S. diacetilactis and L. citrovorum but a lack of uniformity in the desired flavor existed.

Other factors affecting flavor include acidity caused by too high incubation temperature and/or too long incubation time. A flat

flavor or lack of acid development is caused by low solids milk, too low incubation temperature, insufficient starter culture, or too short incubation time. Dilution of buttermilk with milk may also affect the flavor to a point that a flat defect is noted. Bacterial contamination, enzymatic action, poor equipment, and improper handling are also factors affecting the flavor of buttermilk.

Surveys on Quality of Commercial Cultured Buttermilk

A recent study by Keenan and his co-workers (31) on the quality of commercial cultured buttermilk showed a wide variation in flavor score and the amounts of diacetyl, acetaldehyde, and volatile acids present. The variation in these flavor components indicated that it was difficult to produce a consistent product using lactic cultures. Most of the samples evaluated lacked the well-balanced flavor expected from cultured buttermilk and several samples had off flavors. In an Ohio study (34), some of the flavor defects reported included green, flat, unclean, rancid, oxidized and yeasty.

Keenan et al. (31) and Nageotte (49) evaluated cultured buttermilk organoleptically by assigning numerical scores of 31 to 40 with 40 indicating no criticisms. This scale is generally similar to those used in evaluating other dairy products. The types of defects and their intensity determined the score which was given to each sample. Chou (14) and Olson (51) reported the use of good, fair, and poor in the organoleptic evaluation of cultured buttermilk and other cultured products.

There appears to be no standard procedure used in the method of reporting the organoleptic properties of buttermilk. There is also a lack of standardization and uniformity in evaluating buttermilk due to a wide variance in individual preferences and lack of knowledge of the various defects in buttermilk.

Application of Gas Chromatographic Analysis to Volatile Chemical Components

In recent years, more sensitive procedures for the analysis of volatile chemical components affecting flavor have been developed. Recently, gas chromatographic analysis has been used to determine the components in food products both qualitatively and quantitatively. Workers at Oregon State (36, 47) have developed a method of distilling the volatile flavor components from the samples and analyzing them by gas chromatography. Bassette et al. (7) developed a method of determining volatiles, including those affecting flavor, by head space sampling and gas chromatographic analysis. Other work done by Bassette et al. (6, 8, 54) indicated that pure cultures of organisms inoculated into milk produced different chromatographic patterns that could lead to the characterization of bacteria.

A method of Hempenius and Liska (29) for determining volatile acids in cultured products by distillation and separation of acetic acid by gas chromatographic analysis has been described recently. It was suggested that propionic, butyric, valeric, and isovaleric acids present in buttermilk could be determined by this method.

Gas chromatography was used by Chou (14) to separate individual volatile components from cultured buttermilk. Components affecting flavor were determined using paper chromatography, gas chromatography and infra-red spectroscopy. Flavor quality was compared with the number of components per chromatogram. A good cultured buttermilk sample averaged 17 gas chromatographic peaks, a fair sample 11 and a poor sample 9 peaks. A relationship between the number of volatile chemical components and flavor was indicated but this relationship was questioned due to the wide flavor variation among samples. Commercial cultured buttermilk was analyzed by gas chromatography and a maximum of twenty-eight components were found among individual samples.

Direct Acidification of Dairy Products

Lindsay and co-workers (41) reported that 2 ppm diacetyl, 0.5 ppm acetaldehyde, 1250 ppm acetic acid and 25 ppb dimethyl sulfide added to acidified milk medium yielded a product that was similar in aroma to lactic cultured products. Work with direct acidification of dairy products has been reported by a number of workers (2, 9, 13, 19, 21, 22, 42, 43, 46, 55) in recent years. Acidified cottage cheese is produced for consumption in a limited area but acidified cream has been accepted as a replacement for cultured sour cream since 1962. Day (17) reported that the chief problem with direct acidification and added flavor was not the flavor itself but the body produced in this process.

Little (42, 43) reported on the direct acidification process in the manufacturing of sour cream by adding lactic acid and culture

distillate. His findings also were supported by other studies that a lack of a desirable body is the most criticized defect in directly acidified products, especially sour cream. This defect also may be influenced by the varied sources of milk as well as differences in the ratio of certain salts in milk.

Buttermilk is not commonly manufactured by a direct acidification process although Gerson (22) reports that the Nopco Chemical Company[1] has developed such a process. An erratic body characteristic of poor viscosity was noted in direct acidification products described by Little (43).

Olson (2) reported that direct acidification has been used in experiments with Italian pizza cheese, cottage cheese, and blue cheese. In this direct acidification process, only acid and rennet were added directly to the milk. If any additional flavor is desired, the components necessary must be added at a later point in the manufacturing process.

Vincent (55) used hydrochloric acid to acidify skimmilk to pH 5.8 to 5.9 for cottage cheese before adding the lactic starter culture. A softer curd was noted and a decrease in coagulation time was obtained. There was no preference in flavor and texture between commercial cottage cheese and pre-acidified cottage cheese in many cases. In another study by Boddicker et al. (13), HCl also was used for direct acidification of skimmilk before adding lactic cultures in the production of cottage cheese.

---

[1] Nopco Chemical Company, Newark, New Jersey.

Bennett et al. (9) and Deane and Thomas (19) reported the use of lactic acid and glucono-delta-lactone for acidification in the study of diacetyl and other volatile components in milk. Bennett found the flavor threshold of diacetyl in skimmilk varied according to the pH of the skimmilk. The lower the pH, the higher the threshold value.

Use of Imitation and Synthetic Flavors in Cultured Products

In the report by Deane and Thomas (19), it was indicated that a combination of gluco-delta-lactone and citric acid produced sour cream that coagulated in four hours and produced a flavor with no noticeable astringent characteristics. A starter distillate was added to the cream which resulted in a typical aroma and flavor of cultured cream.

Lindsay and his co-workers (41) reported that a prepared butter culture flavor concentrate contained diacetyl, acetaldehyde, dimethyl sulfide, acetic acid, and lactic acid. This concentrate was reported to be similar in flavor to that of a natural butter culture. Equal flavor preference was given to the natural and the artificially flavored buttermilk, sour cream, and butter. A buttermilk flavor stabilizer is available from a commercial source (3) to improve the flavor of buttermilk, yogurt, and other cultured products.

Harper (28) concluded that it is almost impossible to add all the flavor components found in cultured products back in their exact proportions to duplicate flavor characteristics.

EXPERIMENTAL PROCEDURES

Buttermilk samples were either prepared in the University Dairy or obtained from retail sources. In addition, data were obtained on commercial samples obtained in a buttermilk clinic held at Kansas State University. All samples were evaluated organoleptically and analyzed by gas chromatography for certain volatile chemical components. They were also analyzed for total volatile acidity, titratable acidity and pH.

Source and Propagation of Buttermilk Cultures

Five commercial mixed species lactic starter cultures and one single species lactic starter culture recommended for cultured buttermilk were obtained in powdered form from two national lactic starter culture supply houses. The initial propagation of these cultures was accomplished by inoculating whole milk which had been autoclaved for 10 min at 15 lb pressure. The inoculated milk was then incubated at 21 C. Samples remained in the incubator until a firm coagulation was noted, then cooled in ice water. After the initial propagation, all mother cultures were carried in sterile litmus milk and transferred at regular intervals.

Intermediate cultures were prepared by inoculating 100 ml autoclaved whole milk with a 1% inoculum from the litmus milk cultures and incubated at 21 C. In the preparation of the bulk starter, 500 ml whole milk was steamed for 1 hour. A 1% inoculum again was used and the milk incubated at 21 C. All cultures were placed in ice water after removal from the incubator.

Preparation of Cultured Buttermilk

All milk used in the experimental buttermilk study was obtained from the University Dairy processing facilities. Pasteurized whole milk (3.5% butterfat) and pasteurized fortified (2% solids) skimmilk were mixed, then sodium chloride and stabilizer ("Sta-Rit", Germantown) were added making the composition of this mixture 2% butterfat, 9% serum solids, 0.08% sodium chloride, and 0.05% stabilizer. Milk was repasteurized in a 50 gal processing vat at a temperature of 85 C with a holding time of 30 min and then cooled to 21 C. Twenty-five pound portions of this prepared milk were placed into each of two 5 gal stainless steel milk cans for preparation of buttermilk control samples. The remainder of the milk in the vat was cooled to 7 C and again, two 25 lb portions were placed in stainless steel cans to be used in the preparation of pre-acidified cultured buttermilk. Milk cans were used in all cases except the coagulation rate study in which smaller amounts of milk were required.

Samples were inoculated with 1% lactic starter culture and incubated at 21 C until coagulation occurred, then removed and cooled in ice water. Different lactic starter cultures were used to inoculate the milk described above. After sufficient incubation, these samples were used in comparison with commercial buttermilk samples and pre-acidified cultured buttermilk samples by organoleptic evaluation, gas chromatographic analysis, total volatile acidity, titratable acidity, and pH.

Preparation of Pre-Acidified Cultured Buttermilk

A commercial grade concentrated HCl was diluted to 5% with sterile distilled water and held refrigerated at 7 C until ready to be used. All milk that was acidified was cooled to at least 7 C then 5% HCl was slowly added and the milk was agitated vigorously during the acidification process. Enough HCl was added to lower the pH of the milk to 5.2.

Sterilized distilled water was added to control milk equal to the amount of HCl required to acidify milk used in preparing the pre-acidified cultured buttermilk. This was done to prevent any effect on the control samples due to the dilution factor in the pre-acidified cultured buttermilk samples. The acidified milk was then warmed by placing the container of milk into a warm water bath and agitated continuously until a temperature of 21 C was reached. The inoculation and incubation of these samples were performed in the same manner as described in the preparation of cultured buttermilk.

Organoleptic Evaluation

Three phases of this work, the commercial cultured buttermilk study, the pre-acidified cultured buttermilk study, and the commercial cultured buttermilk versus the pre-acidified cultured buttermilk study required the evaluation of flavor and aroma by a selected panel consisting of five experienced judges.

For organoleptic evaluation, all samples were transferred from the original containers to 100 ml glass stoppered Erlenmeyer flasks. The flasks and stoppers were previously sterilized in an oven at 90 C for 1 hour to remove any odors that might be present.

A one to seven hedonic scoring system was used in the evaluation of the samples. The descriptive scale ranged from one or "like very much" to seven or "dislike very much". Each judge was asked also to check defects and add comments. Different evaluation cards were used for aroma and flavor but the scale was the same in both cases although different defects were listed for each. Score cards used for scoring these samples are shown in Appendix Figures 5 and 6.

Samples were prepared, coded, and refrigerated until ready for examination. The judges knew in all cases that duplicate samples were used but these were randomly placed.

Analyses for Volatile Chemical Components in Buttermilk by Gas Chromatography

The instruments and procedures used in this work were described by Loney (44) using modifications of earlier work by Bassette et al. (7) and Toan et al. (54).

Apparatus. Two instruments were used in the separation of volatile components and were designated "A" and "B". The "A" instrument consisted of an Aerograph model 600-B with a 1.05 mv Brown-Honeywell recorder. The "B" instrument was a model 550-B Aerograph with a 1.00 mv Brown-Honeywell recorder. Both instruments were equipped with hydrogen flame ionization detectors. Identical columns were used in both instruments; 3.05 m by 0.318 cm stainless steel column packed with 20% Carbowax on 60/80 mesh, HMDS treated chromosorb P. Nitrogen was used as the carrier gas with operating conditions for the two instruments as follows:

	Instrument A	Instrument B
Column temperature (°C)	100	100
Injection temperature (°C)	192	192
Nitrogen flow (ml/min)	14.1	16.3
Hydrogen flow (ml/min)	24.4	26.0
Oxygen flow (ml/min)	120	110
Chart speed (cm/min)	0.85	0.85

Other apparatus included 15 x 52 mm, 5 ml serum vials with self sealing rubber caps; a Hamilton no. 1001, 1 ml gas tight syringe with a 25 gauge needle 5.08 cm long; and a Fisher clinical mechanical shaker adjusted to operate at 275 to 285 oscillations per minute. The reagents included ACS grade anhydrous sodium sulfate, ACS grade anhydrous mercuric chloride and solutions of acidic and basic hydroxylamine as described by Bassette et al. (7).

Procedure. The method used in analyzing buttermilk samples was by head space gas. Two ml buttermilk was measured into a serum vial containing 1.2 g sodium sulfate. The vial was sealed with a serum cap and placed in a hot water bath for 2 min at 60 C. The vial then was removed from the water bath and mixed on the shaker for 5 min. After mixing, a clean serum cap was placed on the vial and it was again placed in the 60 C water bath for 8 min. One ml head space gas was withdrawn from the vial by inserting the syringe needle through the cap being careful not to contaminate the needle with the mixture in the vial. The 1 ml head space gas was then injected into the chromatograph. All chromatographic analyses in this investigation were made in duplicate.

Chromatographic peak times were recorded in minutes starting at the time the gas was injected into the chromatograph. Acetone with a retention time of 4.0 min was used for instrument standardization. The peak heights recorded were measured by taking the distance from the base line to the tip of the peak. This total height was then multiplied by the attenuation factor to give the total peak height.

The total peak height was corrected daily for any instrument sensitivity changes. This was done by measuring the total peak height produced by 1 ppm acetone and dividing this value into 1600 (an arbitrary value established for 1 ppm acetone). The results gave the adjusted acetone factor. The total peak height for each compound was then multiplied by the adjusted acetone factor resulting in the adjusted total peak height.

Identification of chromatographic peaks was made by comparison of these peaks with those of the retention times of known compounds. Bassette et al. (7) identified sulfides, carbonyls, and esters by eliminating these components from the head space gas. He found that esters and carbonyl peaks were eliminated by using basic hydroxylamine. Acidic hydroxylamine treatment of samples resulted only in the removal of the carbonyl compounds. Sulfides were removed by treating the samples with mercuric chloride before removing the head space gas for analysis. Alcohol peaks were removed by boric acid on the column reaction technique as described by Ikeda et al. (30).

Preparation of standard curves. Standard curves were determined statistically for the concentrations in ppm of different components analyzed by gas chromatography. At least five different concentrations

were prepared for each compound. Analytical grade acetaldehyde, acetone, diacetyl, and ethanol were employed. Acetoin was obtained from a commercial company.[2] Acetoin was distilled through fractional distillation column to purify it before making dilutions.

Determination of Titratable Acidity and pH

Titratable acidity was determined on each sample using the procedure for cream as described by Goss (23). One-tenth N NaOH was used to titrate the sample using phenolphthalein as an indicator. A Beckman pH meter was used to determine the pH of each sample.

Total Volatile Acidity by Steam Distillation

Apparatus. Micro-Kjeldahl equipment was used to distill total volatile acids from buttermilk in place of the equipment described by Hammer et al. (16, 24, 26). Distilled water was used in the steam generator at all times.

Procedure. Twenty-five grams of well mixed buttermilk was weighed into the 100 ml digestion flask. One ml concentrated H_3PO_4 was added to the weighed buttermilk and mixed thoroughly. Three drops of antifoam agent[3] was used to reduce any excessive foaming that might occur in the distillation process.

---

[2] Acetoin from Rare and Fine Chemicals - Plainview, N. Y.

[3] Dow Corning Food Grade Silicone Defoamer FG 10 Emulsion - Midland, Michigan.

Heat was applied to the steam generator and some steam was expelled from the equipment before placing the digestion flask under the steam outlet. The operation was adjusted to collect 100 ml distillate in approximately 30 min. A 100 ml graduated cylinder, placed in ice water, was used to collect the distillate.

The distillate was transferred to a 200 ml Erlenmeyer flask and titrated with 0.05 N NaOH in a semi-micro buret with phenolphthalein used as the indicator. The results were expressed as ml of 0.05 N NaOH required to neutralize the first 100 ml of distillate obtained from a 25 g sample. Distilled water was steam distilled through the system before and between each sample tested.

Evaluation of Commercial Buttermilk

Kansas State University clinic study. A single session clinic in the Department of Dairy and Poultry Science was conducted to evaluate commercial buttermilk from a number of Kansas processors. Two quarts from the same batch were required for the clinic. One quart was used for laboratory and official evaluation and the other quart was used for the organoleptic evaluation by the clinic participants.

Buttermilk samples were prepared for organoleptic evaluation by five qualified judges and the clinic participants ranked the samples for aroma and flavor. A scale of good, fair, and poor was used for this evaluation and unusual flavor and aroma defects were noted by each judge using the evaluation sheet shown in Fig. 2.

Titratable acidity was determined on each sample as described earlier. Each sample also was analyzed by gas chromatography for

DAIRY INDUSTRY CONFERENCE
Kansas State University
March 15 & 16, 1967

Buttermilk Clinic

Name _____

Address _____

_____

Cultured Buttermilk Evaluation

Sample No.	Flavor				Body and Texture				Rating		
	Good	Fair	Poor	Criticism	Good	Fair	Poor	Criticism	Good	Fair	Poor

CRITICISMS:

FLAVOR

Flat, Cheesy, Coarse
Green (undeveloped),
High acid, Metallic,
Yeasty, Rancid, Unclean

BODY AND TEXTURE

Curdy, Gassy, Lumpy, Ropy,
Thin body, Too thick, Chalky,
Wheyed off.

Fig. 2. Kansas State University buttermilk clinic evaluation sheet.

volatile chemical components. The concentrations of the different volatile chemical components were not computed in this study since the interest was the identity of volatile chemical components and the organoleptic evaluation of the buttermilk.

Other commercial buttermilk evaluation. In order to provide additional data, other commercial cultured buttermilk samples were obtained from supermarkets, except for two which were obtained from dairies out of their cooler stock. All samples were obtained in one quart containers. The tests used for evaluating these were organoleptic evaluation by the panel, volatile chemical component analyses by gas chromatography, and titratable acidity.

Volatile chemical components in commercial milk samples. This study was performed to determine the volatile chemical components present in commercial whole milk and skimmilk samples chosen at random since these are the basic products used in making cultured buttermilk. This gave a basis for determining the development of volatile chemical components present from the breakdown of citric acid in the fermentation of lactic starter cultures. The skimmilk and whole milk were examined only for components by gas chromatographic analysis and no attempt was made to evaluate flavor or acidity of these samples.

Commercial milk and skimmilk samples were obtained from supermarkets in one quart and one-half gallon containers. All samples were purchased shortly before testing and refrigerated until used.

Rate of Coagulation of Pre-Acidified Milk by Lactic Starter Cultures

This phase of the study was performed to determine if there was any time difference in coagulation or acidity increase using the pre-acidification method as compared with standard cultural procedures. All milk was prepared by previously described methods. Six cultures selected for use in the preparation of the experimental buttermilk samples were used in this experiment. The samples were examined for pH and titratable acidity, and were observed for coagulation at regular intervals. The time of coagulation was recorded, then samples were removed from the incubator and placed in ice water. Samples were again checked for pH and titratable acidity at the end of a 24 hour period except for two which were tested at 26 hours.

RESULTS AND DISCUSSION

In this investigation, comparisons were made between pre-acidified cultured buttermilk and standard cultured buttermilk using different lactic starter cultures. Gas chromatographic analysis, organoleptic evaluation, total volatile acidity, titratable acidity, and pH were all used as a basis of comparison. A study of the coagulation time was made to determine any time savings in this pre-acidified process.

Quality and Some Chemical Characteristics of Commercial Buttermilk

Evaluations of commercial buttermilk were made in a buttermilk clinic held at Kansas State University in March, 1967, and on commercial

samples subsequently obtained in retail outlets. With clinic samples, results of the gas chromatographic analysis were expressed only in adjusted peak heights for the volatile chemical components. In later buttermilk studies, the concentrations of these components were expressed in parts per million (ppm).

Kansas State University Clinic. The flavor rating of the eighteen commercial buttermilk samples showed a wide range of flavor quality (Tables 1 and 2). The official judges rated only three of the samples good and half of the total poor (Table 1). The remainder were considered fair. There was also a wide variety of flavor defects among the different samples as indicated by the criticisms, with high acid and green being the most common.

The same samples evaluated by clinic participants (Table 2) received flavor ratings similar to those of the official judges with most of the samples being rated fair or poor. Not only was there considerable variation in flavor defects indicated among samples but individual samples were criticized for a wide variety of defects by the different participants. No correlation between titratable acidity and flavor ranking could be determined.

Some volatile chemical components present in the buttermilk samples as determined by gas chromatographic analysis are shown in Table 3 and Fig. 3. Figure 3 shows a typical chromatogram of a cultured buttermilk sample. Each component present in the buttermilk is represented by a peak produced at a time specific for that component. The value reported for each sample is the adjusted peak height for each component as shown on the chromatogram and is an indication of

Table 1. Evaluation of commercial buttermilk samples by official judges at the Kansas State University clinic - March, 1967.

Sample	Titratable acidity	Flavor rating[a]	Flavor criticisms
1	0.76	P	unnatural
2	0.85	P+	green, unclean
3	0.87	P	high acid, foreign
4	0.89	F	off flavor
5	0.97	G-	sl. high acid
6	0.87	G-	sl. high acid
7	0.86	P+	green, flat, unclean
8	0.88	G-	high acid
9	0.75	F	high salt, green, cooked
10	0.74	F-	cabbage, cheesy
11	0.90	P	rancid
12	0.80	P	salty, oxidized
13	0.83	F	high acid, green
14	0.87	F-	high acid, green
15	0.94	F+	high acid, cabbage
16	0.84	P+	foreign, flat
17	0.88	P	cooked, stale, high acid
18	0.83	P+	green, high acid

[a] G-Good, F-Fair, P-Poor - consensus of judges.

Table 2. Evaluation of commercial buttermilk samples by participants at the Kansas State University clinic - March, 1967.

Sample	Flavor rating[a]	Flavor criticisms
1	F	flat, high acid, low solids, metallic, salty, yeasty
2	P	green, high bacteria, metallic, rancid
3	P+	bitter, cheesy, high acid, low acid, rancid, unclean, yeasty
4	F	flat, high acid, green, salty, yeasty
5	G-	coarse, green, high acid
6	G-	green, high acid, mild, unclean
7	P+	flat, green, high acid, metallic, unclean
8	F	flat, green, high acid, salty
9	G-	acid, flat, rancid, undeveloped
10	F-	flat, green, high acid, mild, metallic, unclean
11	P	green, metallic, rancid, unclean
12	P	cheesy, flat, green, high acid, metallic, rancid, salty
13	F	cheesy, green, high acid, low acid, rancid, unclean
14	F	cheesy, coarse, flat, green, high acid, unclean, yeasty
15	F	cheesy, flat, green, high acid, low acid, metallic
16	P+	acid, flat, green, metallic, unclean
17	F	cheesy, flat, metallic, unclean, undeveloped
18	P	cheesy, flat, green, metallic, unclean

[a] G-Good, F-Fair, P-Poor - consensus of judges.

Table 3. Some volatile chemical components and adjusted peak heights determined by gas chromatographic analysis on commercial buttermilk samples at the Kansas State University clinic - March, 1967.

Sample	Adjusted peak height				
	Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin
1	106	2585	1653	431	99
2	5697	1969	1917	844	259
3	97	3131	4190	325	69
4	64	2063	2040	881	180
5	109	2012	2085	786	263
6	45	1748	1980	1552	172
7	511	963	2045	559	84
8	40	2625	2256	1025	69
9	60	2154	2045	650	96
10	52	1832	2040	1437	110
11	43	1994	1414	649	93
12	42	1503	1530	769	127
13	104	2394	3269	789	81
14	60	1094	1301	1002	121
15	147	1820	1748	1249	268
16	36	1623	1410	491	85
17	85	1499	1641	928	76
18	4755	1829	1884	823	174

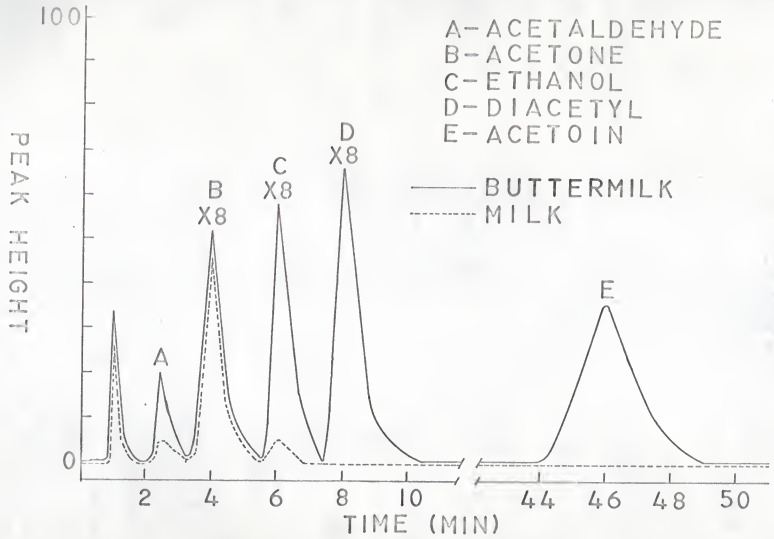


Fig. 3. Gas chromatographic peak heights of volatile chemical components of a typical cultured buttermilk sample (attenuation factor of X4 used in all cases unless indicated).

concentration. There was considerable variation in the amount of each component among the different samples. With few exceptions, there was no consistent relationship between the peak heights of individual components and the organoleptic quality of the buttermilk. However, a high peak height for acetaldehyde was definitely associated with what was criticized by the official judges as a green flavor (Table 1). Although samples rated good had a relatively high peak height for diacetyl, many of the poorer samples also showed equal value. There appeared to be little relationship between acetoin peak heights and flavor. With the exception of one sample (No. 3) which had the highest acetone and ethanol peak and was rated poor with a foreign flavor, there appeared to be no consistent relationship between the amounts of these constituents and flavor. With respect to acetone, it might be noted that the component is present in the initial milk (Table 6) and was not greatly influenced by fermentation.

This clinic study showed that much of the commercial buttermilk in Kansas was of poor flavor quality. It was also evident the organoleptic evaluation of buttermilk lacks the standardization that exists in judging certain other dairy products and that accurate evaluation is extremely difficult. This was emphasized particularly by the various concepts of flavor quality shown by inexperienced judges. Also, although certain volatile chemical components are recognized to be important in flavor of cultured products, little consistent relationship was found in the clinic samples.

Commercial buttermilk evaluation. Results of organoleptic evaluation of the 13 commercial cultured buttermilk samples obtained from

supermarkets are shown in Table 4. A panel of five experienced judges scored these samples and an average of their scores was recorded. The flavor scores had a slightly wider range and were ranked lower than the aroma scores. It appears that the results of the organoleptic evaluation of these samples were similar to those in the Kansas State University clinic. Only one sample was comparable to a good rating in flavor evaluation with a score of 2.75. As indicated in the clinic samples and again confirmed in this study, no correlation between titratable acidity or pH could be found with flavor ranking and flavor criticisms.

Table 5 shows the total concentration in parts per million of five volatile chemical components of the cultured buttermilk as determined by gas chromatographic analysis. As in the clinic study, there was marked variation in the concentration of each component among the samples. The flavor and the chemical concentration of these samples did not always relate. However, sample C (Appendix Table 20) was criticized for a cheesy defect in both flavor and aroma by several judges and this could be related to a high ethanol concentration. No pattern could be detected in the acetone concentration as affecting the flavor and aroma. The ratio of diacetyl to acetaldehyde varied from 13.2:1 to as high as 1613.7:1 but at no time did a judge criticize the flavor as being harsh as reported by Lindsay (40) when in his studies a ratio of 5.5:1 or higher resulted in a harsh flavor or aroma. Furthermore, there were no correlations between the acetaldehyde-diacetyl ratio and the flavor and aroma scores in the group of buttermilk samples.

Table 4. Evaluation of commercial buttermilk.

Sample	Aroma score[a]	Flavor score[a]	Titratable acidity	pH
A	2.75	4.25	0.83	4.5
B	3.0	3.6	0.85	4.3
C	3.75	5.25	0.97	4.3
D	2.4	4.1	0.88	4.2
E	3.0	3.9	0.89	4.2
F	3.5	3.75	0.93	4.2
G	3.25	2.75	0.91	4.2
H	2.9	4.9	0.90	4.2
I	3.75	4.0	0.90	4.3
J	3.5	3.9	0.91	4.1
K	3.9	5.0	0.92	4.1
L	4.4	5.25	0.89	4.3
M	4.5	3.5	0.94	4.2

[a] 1-7 hedonic scale; 1-Like very much, 7-Dislike very much. Average score of duplicate samples.

Table 5. Some volatile chemical components in commercial buttermilk samples.

Sample	Concentration ppm[a]					Ratio
	Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
A	0.106	1.869	15.468	6.937	105.338	65.4:1
B	0.003	1.242	14.626	4.841	103.609	1613.7:1
C	0.017	1.534	32.784	3.061	60.721	180.1:1
D	0.051	1.585	16.592	2.806	120.374	55.0:1
E	0.054	0.535	9.802	6.085	117.868	112.7:1
F	0.032	1.823	18.121	3.976	182.493	124.3:1
G	0.098	1.874	19.410	3.491	121.209	35.6:1
H	0.035	1.686	21.970	2.853	129.648	81.5:1
I	0.050	2.058	18.147	6.240	88.632	124.8:1
J	0.077	2.396	31.116	9.748	76.102	126.6:1
K	0.111	1.684	18.586	1.467	148.029	13.2:1
L	0.056	1.932	22.815	2.780	134.571	49.6:1
M	0.091	1.943	20.882	2.910	177.898	32.0:1

[a] Parts per million - average of duplicate samples.

Although the judges in this series were more experienced than the clinic participants in the Kansas State University study, there was still a wide range in flavor and aroma scoring and in the types of defects criticized. This again indicated the wide variation in individual preference and the lack of standardization in judging buttermilk quality. The samples of commercial buttermilk obtained at random could not be considered quality products.

Evaluation of milk for volatile chemical components. Six skimmilk samples and six whole milk samples were analyzed by gas chromatography to determine the volatile chemical components present and the concentration of each. Since all commercial cultured buttermilk is made with either skimmilk or whole milk, it was necessary to know the components present before inoculation. This gave an indication of the changes that took place in the fermentation process regarding the concentration of the five components detected by gas chromatographic analysis. The results are presented in Table 6.

The volatile chemical components in the skimmilk and in the whole milk varied in concentration among the samples. No relationship among the components of the individual samples could be determined. An average of the component concentration in the six samples was made to permit a more effective comparison with the component concentrations in buttermilk.

Table 6. Some volatile chemical components of commercial skimmilk and whole milk.

Sample	Concentration ppm[a]					
	Acetaldehyde		Acetone		Ethanol	
1	skim	whole	skim	whole	skim	whole
1	0.010	0.006	1.943	1.950	2.430	3.348
2	0.000	0.000	1.974	2.102	12.204	7.316
3	0.000	0.009	0.598	0.565	0.394	0.386
4	0.004	0.010	1.876	1.439	8.624	4.630
5	0.006	0.000	1.475	1.499	2.403	2.699
6	0.007	0.000	1.553	1.319	1.238	1.162
Av.	0.005	0.004	1.570	1.479	4.549	3.257

[a] Parts per million - average of duplicate samples.

Evaluation of Pre-Acidified Cultured Buttermilk

Comparison of pre-acidified cultured buttermilk with cultured buttermilk. Six different lactic starter cultures were used in this study. Buttermilk was prepared with and without supplementary acidification. Evaluations were made the day samples were removed from the incubator and again after a four-day storage period. Cultures 2, 3, 7, 8, and 10 were commercial lactic starter cultures consisting of streptococcus and leuconostoc species and culture 9 was the single species S. diacetilactis culture.

The milk used in this phase of study varied in titratable acidity from 0.17 to 0.20 and in pH from 6.4 to 6.7. These values are higher than those found in regular milk due to the addition of solids-not-fat. The titratable acidity (Table 7) of the buttermilk on the fourth day of storage was greater than on the first day in both the control and pre-acidified samples. Generally on both days, the acidified samples had less titratable acidity than their corresponding control samples, ranging in arithmetic mean from 0.66 to 0.97 in the acidified sample and from 0.85 to 0.98 in the control, although in some cases, this was reversed for unexplained reasons. There was no significant variation in the pH of the samples as indicated in Table 7.

Most of the samples were given a more desirable aroma score than flavor score by the panel members as indicated in Table 8. With the scoring method used in this study, a lower score is indicative of a better flavor or aroma. The aroma of pre-acidified samples using the single species lactic starter culture was preferred over the pre-acidified samples using the mixed species lactic starter cultures in both the first day and the fourth day evaluations. Generally, the aroma score was improved in the pre-acidified samples the fourth day over the first day.

The flavor score was shown to be a more reliable evaluation of the buttermilk than the aroma score as indicated by the criticism and comments of the panel members (Appendix Table 22 and 23). The fourth day samples generally had a preferred flavor score than the first day samples and were criticized less frequently for a green defect. The

Table 7. Titratable acidity and pH of cultured buttermilk with and without pre-acidification.[a]

Lactic culture No.	Titratable acidity		pH	
	A[b]	B[c]	A[b]	B[c]
2 Control	0.92 ± 0.08	0.97 ± 0.07	4.53 ± 0.11	4.47 ± 0.21
2 Acidified	0.82 ± 0.16	0.91 ± 0.10	4.40 ± 0.10	4.30 ± 0.10
3 Control	0.85 ± 0.00	0.90 ± 0.06	4.57 ± 0.06	4.47 ± 0.16
3 Acidified	0.77 ± 0.08	0.86 ± 0.05	4.50 ± 0.10	4.43 ± 0.06
7 Control	0.93 ± 0.03	0.95 ± 0.03	4.43 ± 0.16	4.45 ± 0.09
7 Acidified	0.83 ± 0.16	0.92 ± 0.06	4.47 ± 0.25	4.23 ± 0.11
8 Control	0.90 ± 0.00	0.99 ± 0.05	4.53 ± 0.06	4.40 ± 0.00
8 Acidified	0.79 ± 0.12	0.94 ± 0.07	4.50 ± 0.10	4.35 ± 0.09
9 Control	0.95 ± 0.05	0.98 ± 0.03	4.63 ± 0.11	4.52 ± 0.11
9 Acidified	0.89 ± 0.06	0.97 ± 0.08	4.57 ± 0.11	4.47 ± 0.16
10 Control	0.86 ± 0.03	0.95 ± 0.05	4.53 ± 0.06	4.68 ± 0.11
10 Acidified	0.87 ± 0.06	0.93 ± 0.05	4.40 ± 0.27	4.42 ± 0.23

[a] Arithmetic mean of three trials with standard deviation indicated.

[b] Samples tested same day as removed from incubator.

[c] Samples tested after four days storage.

Table 8. Aroma and flavor evaluation of cultured buttermilk with and without pre-acidification.[a]

Lactic culture No.	Aroma score[b]		Flavor score[b]	
	A[c]	B[d]	A[c]	B[d]
2 Control	4.27 ± 0.90	3.53 ± 0.31	4.57 ± 0.93	4.33 ± 0.81
2 Acidified	3.47 ± 0.42	3.30 ± 0.82	4.00 ± 0.87	4.00 ± 0.92
3 Control	3.60 ± 0.79	3.20 ± 0.44	4.33 ± 0.50	3.67 ± 1.12
3 Acidified	3.43 ± 0.42	3.67 ± 0.16	4.30 ± 0.69	3.70 ± 0.76
7 Control	2.90 ± 0.72	3.07 ± 0.23	4.37 ± 0.06	3.70 ± 0.35
7 Acidified	3.23 ± 0.16	3.40 ± 0.48	3.40 ± 0.87	3.27 ± 0.64
8 Control	3.13 ± 0.16	2.87 ± 0.35	4.30 ± 1.10	3.77 ± 0.84
8 Acidified	3.00 ± 0.36	2.83 ± 0.32	3.60 ± 0.79	3.70 ± 0.35
9 Control	3.23 ± 0.06	2.60 ± 0.40	3.37 ± 0.75	3.87 ± 0.29
9 Acidified	2.97 ± 0.16	2.80 ± 0.20	3.37 ± 1.40	3.67 ± 0.16
10 Control	3.23 ± 0.29	2.73 ± 0.16	3.67 ± 0.50	3.13 ± 0.38
10 Acidified	3.33 ± 0.59	2.90 ± 0.00	3.17 ± 0.32	3.40 ± 0.20

[a] Arithmetic mean of three trials with standard deviation indicated.

[b] 1-7 hedonic scale; 1-Like very much, 7-Dislike very much.

[c] Sample evaluated same day as removed from incubator.

[d] Sample evaluated after four days storage.

flavor was improved in the buttermilk using the single species culture on the first day. In general, the pre-acidified samples were preferred over the control samples on both days according to the organoleptic evaluation. There was some correlation between a lower titratable acidity and a preferred flavor score for the pre-acidified samples.

A variation in the concentration of different components was noted in the control and pre-acidified buttermilk samples. The analyses by gas chromatography of buttermilk using the six cultures are summarized in Tables 9 to 14. The concentration of acetaldehyde in the samples was lower on the fourth day than the first day which agrees with the general pattern in the breakdown of citric acid during the fermentation process. In many cases, the pre-acidified samples had less acetaldehyde than the control which was confirmed by a decrease in the number of green flavor criticisms in the organoleptic evaluation (Appendix Tables 28-33). The amount of acetone in the initial milk and the cultured samples indicated that this compound is not produced in the normal fermentation process. The importance of acetone in the flavor or aroma evaluation of the buttermilk could not be determined because of its high threshold level.

The amount of ethanol, diacetyl, and acetoin was directly related to the amount of acetaldehyde present in the samples. The amount of ethanol increased in most cases from the first day to the fourth day as would be expected in the metabolism of citric acid; the few exceptions could not be explained. The amount of acetoin increased in most fourth day samples as would also be expected although there was a

Table 9. Some chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 2)

Lactic culture no.	Trial[b]	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
2 Control	1-A	4.633	0.536	20.711	0.513	30.996	0.1:1
	1-B	0.177	0.588	23.422	0.755	46.867	4.3:1
	2-A	3.689	0.497	23.405	5.481	327.530	1.5:1
	2-B	0.306	0.497	20.942	2.122	246.505	6.9:1
	3-A	12.028	0.432	12.448	3.526	279.082	0.3:1
	3-B	2.527	0.407	22.712	1.324	277.411	0.5:1
	Av. - A	6.783	0.488	18.855	3.173	212.536	0.5:1
	Av. - B	1.003	0.497	22.359	1.400	190.261	1.4:1
2 Acidified	1-A	2.611	0.413	3.740	0.585	34.337	0.2:1
	1-B	1.833	0.518	6.879	0.682	36.843	0.4:1
	2-A	0.266	0.491	12.473	6.619	289.941	24.9:1
	2-B	0.247	0.505	10.651	3.127	201.398	12.7:1
	3-A	1.580	0.284	1.892	1.669	78.608	1.1:1
	3-B	1.003	0.322	5.527	2.976	207.246	3.0:1
	Av. - A	1.486	0.396	6.035	2.957	134.295	19.9:1
	Av. - B	1.028	0.448	7.686	2.262	148.496	2.2:1

[a] Parts per million - average duplicate samples.

[b] Three trials with two samples each (A and B); A - sample tested same day as removed from incubator. B - sample tested after four days storage.

Table 10. Some chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 3)

Lactic culture no.	Trial[b]	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
3 Control	1-A	0.128	0.297	3.765	0.888	28.490	6.9:1
	1-B	0.086	0.353	6.947	1.100	28.490	12.8:1
	2-A	0.321	0.848	7.101	1.396	48.537	4.3:1
	2-B	0.088	0.778	9.180	1.711	126.221	19.4:1
	3-A	0.091	0.378	7.530	1.528	48.467	16.8:1
	3-B	0.109	0.388	10.188	1.474	59.955	13.5:1
	Av. - A	0.180	0.508	6.132	1.271	41.831	7.1:1
	Av. - B	0.094	0.506	8.772	1.428	71.555	15.2:1
3 Acidified	1-A	0.119	0.281	2.833	1.251	35.172	10.5:1
	1-B	0.093	0.331	4.877	1.747	38.514	18.8:1
	2-A	0.092	0.719	1.926	1.306	49.373	14.2:1
	2-B	0.067	0.698	2.456	2.280	104.503	34.0:1
	3-A	0.083	0.341	1.145	1.197	44.638	14.4:1
	3-B	0.093	0.334	2.279	2.624	66.848	28.2:1
	Av. - A	0.098	0.447	1.968	1.251	43.061	12.8:1
	Av. - B	0.084	0.454	3.204	2.217	69.955	26.4:1

[a] Parts per million - average duplicate samples.

[b] Three trials with two samples each (A and B); A - sample tested same day as removed from incubator, B - sample tested after four days storage.

Table 11. Some chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 7)

Lactic culture no.	Trial[b]	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
7 Control	1-A	0.969	0.419	4.852	7.478	112.856	7.7:1
	1-B	0.269	0.475	9.411	3.823	302.470	14.2:1
	2-A	1.792	0.390	5.365	19.913	219.775	11.1:1
	2-B	0.162	0.397	7.272	2.806	284.929	17.3:1
	3-A	0.605	0.413	9.924	5.886	228.128	9.7:1
	3-B	0.254	0.464	10.155	1.889	288.270	7.4:1
	Av. - A	1.122	0.407	6.714	11.092	186.919	9.9:1
	Av. - B	0.228	0.445	8.946	2.839	291.889	12.5:1
7 Acidified	1-A	1.016	0.342	1.832	2.292	61.902	2.3:1
	1-B	0.229	0.383	4.749	7.557	361.777	33.0:1
	2-A	0.346	0.325	2.739	5.118	124.550	14.8:1
	2-B	0.180	0.306	3.799	5.414	254.023	30.1:1
	3-A	0.364	0.323	4.150	8.367	166.316	23.0:1
	3-B	0.189	0.339	5.237	2.752	368.460	14.6:1
	Av. - A	0.575	0.330	2.907	5.259	117.589	9.1:1
	Av. - B	0.199	0.343	4.595	5.241	328.087	26.3:1

[a] Parts per million - average duplicate samples.

[b] Three trials with two samples each (A and B); A - sample tested same day as removed from incubator, B - sample tested after four days storage.

Table 12. Some chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 8)

Lactic culture no.	Trial[b]	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
8 Control	1-A	4.929	0.914	12.702	15.776	198.576	3.2:1
	1-B	0.291	0.839	17.753	3.394	111.268	11.7:1
	2-A	2.161	0.394	11.190	12.333	102.843	5.7:1
	2-B	0.193	0.414	17.753	3.773	110.502	19.5:1
	3-A	1.174	0.347	12.217	8.331	173.833	7.1:1
	3-B	1.960	0.426	19.334	3.750	96.150	1.9:1
	Av. - A	2.755	0.552	12.036	12.147	158.451	4.4:1
	Av. - B	0.815	0.560	18.280	3.639	105.973	4.5:1
8 Acidified	1-A	0.976	0.739	2.847	4.983	85.994	5.1:1
	1-B	0.185	0.718	9.688	5.555	103.609	30.0:1
	2-A	0.157	0.309	2.213	2.889	59.189	18.4:1
	2-B	0.134	0.356	7.930	4.351	86.760	32.5:1
	3-A	0.173	0.345	4.886	10.141	140.421	58.6:1
	3-B	0.164	0.393	14.244	4.525	65.243	27.6:1
	Av. - A	0.435	0.464	3.315	6.004	95.201	13.8:1
	Av. - B	0.161	0.489	11.621	4.810	85.204	29.9:1

[a] Parts per million - average duplicate samples.

[b] Three trials with two samples each (A and B); A - sample tested same day as removed from incubator, B - sample tested after four days storage.

Table 13. Some chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 9)

Lactic culture no.	Trial[b]	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
9 Control	1-A	3.623	0.296	12.691	6.633	147.263	1.8:1
	1-B	0.224	0.292	23.515	4.038	81.399	18.0:1
	2-A	3.172	0.597	12.350	5.549	81.399	1.7:1
	2-B	0.176	0.479	15.828	3.984	81.399	22.6:1
	3-A	1.142	0.337	10.433	5.369	66.848	4.7:1
	3-B	0.141	0.356	15.283	2.269	40.043	16.1:1
	Av. - A	2.646	0.410	11.825	5.850	98.503	2.2:1
	Av. - B	0.180	0.376	18.209	3.430	67.614	19.1:1
9 Acidified	1-A	0.244	0.288	12.213	8.992	169.473	36.9:1
	1-B	0.169	0.268	15.917	4.652	58.423	27.5:1
	2-A	1.587	0.468	3.425	3.424	56.892	2.2:1
	2-B	0.179	0.417	6.918	4.779	102.077	26.7:1
	3-A	0.170	0.386	3.859	4.345	58.423	25.6:1
	3-B	0.087	0.356	6.862	1.582	35.448	18.2:1
	Av. - A	0.667	0.381	6.499	5.587	94.929	8.4:1
	Av. - B	0.145	0.347	9.899	3.671	65.316	25.3:1

[a] Parts per million - average duplicate samples.

[b] Three trials with two samples each (A and B); A - sample tested same day as removed from incubator, B - sample tested after four days storage.

Table 14. Some chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 10)

Lactic culture no.	Trial[b]	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
10 Control	1-A	1.025	0.393	7.775	0.583	35.448	0.6:1
	1-B	0.221	0.447	15.083	3.009	71.443	13.6:1
	2-A	0.179	0.463	18.687	2.106	46.170	11.8:1
	2-B	0.233	0.460	15.139	1.968	66.082	8.4:1
	3-A	0.134	0.359	13.781	1.438	41.574	10.7:1
	3-B	0.269	0.414	16.618	1.534	43.106	5.7:1
	Av. - A	0.446	0.405	13.414	1.376	41.064	3.1:1
	Av. - B	0.241	0.440	15.613	2.170	60.210	9.0:1
10 Acidified	1-A	0.435	0.380	1.834	0.872	36.979	2.0:1
	1-B	0.169	0.356	7.263	4.357	69.911	25.8:1
	2-A	0.119	0.434	5.761	4.020	49.233	33.8:1
	2-B	0.202	0.426	10.767	2.636	58.423	13.0:1
	3-A	0.091	0.362	8.008	4.068	61.487	44.7:1
	3-B	0.294	0.378	22.770	2.708	59.189	9.2:1
	Av. - A	0.215	0.392	5.201	2.987	49.233	13.9:1
	Av. - B	0.222	0.387	13.800	3.234	62.508	14.6:1

[a] Parts per million - average duplicate samples.

[b] Three trials with two samples each (A and B); A - sample tested same day as removed from incubator, B - sample tested after four days storage.

decrease in a few samples indicating a further reduction to 2,3-butylene glycol at the end of four days storage. The 2,3-butylene glycol could not be identified with the chromatograph used in this study but the breakdown of citric acid indicates that this component is the final product. There was no diacetyl and acetoin in the milk used for preparation of buttermilk. The amount of ethanol in the milk was very small (Table 6) and did not influence the final concentration in the buttermilk samples. The ethanol concentration varied only slightly among the buttermilk samples that were made using the same culture. The pre-acidified samples were consistently lower than the control samples in the concentration of ethanol present. This indicates that ethanol is related more to the culture used than to the processing technique and the age of the product. It is believed that ethanol is not significant in flavor evaluation until it reaches a very high concentration.

The concentration of volatile chemical components was least with culture 3 (Table 10) which suggested that there were fewer leuconostoc organisms present. From the results of organoleptic evaluation and gas chromatographic analysis, there appeared to be no significant difference using the S. diacetilactis culture (No. 9) and the commercial mixed lactic starter cultures (Nos. 2, 3, 7, 8, and 10) in preparing buttermilk.

Comparison of pre-acidified cultured buttermilk with commercial buttermilk. This phase of study was to compare pre-acidified cultured buttermilk, normal cultured buttermilk (control), and commercial buttermilk samples. Two cultures were used in this experiment; culture 7 was a mixed commercial lactic starter culture and culture 9 was S. diacetilactis.

Culture 7 was chosen from five available mixed commercial lactic starter cultures as it contained a high concentration of flavor components in addition to giving a preferred flavor score in the organoleptic evaluation. Culture 9 was selected as it was the only single species lactic starter culture among six cultures used in the preparation of the experimental buttermilk in the previous study. The control buttermilk sample and the pre-acidified buttermilk samples prepared with these cultures were compared in eight different trials with different brands of commerical buttermilk obtained in several areas in Kansas.

In the organoleptic evaluation of the control buttermilk and the pre-acidified buttermilk samples, the most common defects mentioned by the panel were lacking aroma (Appendix Table 34) and high acid flavor (Appendix Table 35). The hedonic aroma and flavor scores summarized in Table 15 show very little difference between the control buttermilk and pre-acidified buttermilk samples although the aroma of the pre-acidified samples was preferred over the control samples by a small margin. The flavor scores for the control and pre-acidified samples also were close and there was no preference shown as with aroma. The titratable acidity and pH of the pre-acidified buttermilk varied little from the control buttermilk. The total volatile acids are an influencing factor in the flavor and aroma of buttermilk.

The concentration in ppm of the volatile chemical components present are summarized in Tables 16 and 17. The average acetaldehyde concentration indicated that the control sample using culture 7 produced more acetaldehyde than the pre-acidified sample using culture 7 by approximately 44.5%. The results also indicated that the control sample using culture 9 produced

Table 15. Organoleptic evaluation, titratable acidity, pH, and total volatile acids of cultured buttermilk with and without pre-acidification.[a]

Lactic culture no.	Aroma score[b]	Flavor score[b]	Titratable acidity	pH	Volatile acids[c]
7 Control	2.85 ± 0.24	2.78 ± 0.35	0.97 ± 0.03	4.29 ± 0.06	4.60 ± 0.21
7 Acidified	2.73 ± 0.10	2.95 ± 0.35	0.98 ± 0.02	4.15 ± 0.06	4.64 ± 0.46
9 Control	3.05 ± 0.30	3.05 ± 0.19	0.99 ± 0.07	4.23 ± 0.06	4.91 ± 0.33
9 Acidified	3.00 ± 0.25	2.68 ± 0.41	1.02 ± 0.07	4.15 ± 0.06	4.82 ± 0.66

[a] Arithmetic mean of four trials with standard deviation indicated.

[b] 1-7 hedonic scale; 1 - Like very much, 7 - Dislike very much.

[c] Expressed in ml of 0.05 N NaOH.

Table 16. Some volatile chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 7)

Lactic culture no.	Trial	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
7 Control	1	0.100	0.357	12.570	3.166	178.010	31.7:1
	2	0.121	0.376	10.583	3.482	139.605	28.8:1
	3	0.103	0.471	10.262	3.331	177.132	32.3:1
	4	0.154	0.361	10.313	5.307	151.093	34.5:1
	Av.	0.120	0.391	10.932	3.822	161.460	31.9:1
7 Acidified	1	0.063	0.288	8.954	3.707	210.830	58.8:1
	2	0.064	0.294	9.398	2.754	191.375	43.0:1
	3	0.060	0.486	7.610	3.846	199.728	64.1:1
	4	0.144	0.246	5.424	5.263	280.753	36.5:1
	Av.	0.083	0.329	7.847	3.893	220.672	46.9:1

[a] Parts per million - average duplicate samples.

Table 17. Some volatile chemical components of cultured buttermilk with and without pre-acidification.

(Culture no. 9)

Lactic culture no.	Trial	Concentration ppm[a]					Ratio
		Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
9 Control	1	0.136	0.468	18.681	2.783	86.126	20.5:1
	2	0.053	0.373	20.350	1.646	61.487	31.1:1
	3	0.093	0.335	22.832	2.331	82.931	25.1:1
	4	0.172	0.264	20.113	2.567	63.784	14.9:1
	Av.	0.114	0.360	20.494	2.332	73.582	20.5:1
9 Acidified	1	0.071	0.440	12.684	2.567	62.253	36.2:1
	2	0.070	0.421	17.202	1.583	36.843	22.6:1
	3	0.068	0.360	13.529	1.988	43.526	29.2:1
	4	0.151	0.272	11.179	3.446	109.515	22.8:1
	Av.	0.090	0.373	13.649	2.396	63.034	26.6:1

[a] Parts per million - average duplicate samples.

more acetaldehyde than the pre-acidified sample using culture 9 by approximately 26.5%. Acetone varied very little among individual samples and between the control and acidified samples. Although acetone is not produced by any chemical change during the fermentation process, a slight variation was noted. This variation could be due to the addition of HCl and distilled water to the milk in the preparation of the samples.

Diacetyl was one of the more important flavor components produced during the fermentation process and varied only slightly between the control and pre-acidified samples. No significant pattern could be detected although the buttermilk using culture 7 produced a greater amount of diacetyl than did the buttermilk with culture 9 in most cases. Since acetoin is odorless and tasteless, its only importance is in its relationship to the amount of diacetyl since diacetyl is the precursor of acetoin. In all cases, the amount of acetoin was directly related to the amount of diacetyl present.

Sixteen commercial buttermilk samples from eight individual companies (two samples each) in Kansas were used as comparison with the pre-acidified and control buttermilk samples. The results on these commercial samples are summarized in Tables 18 and 19. In Sample A, the high concentration of acetaldehyde with the low diacetyl-acetaldehyde ratio of 0.6:1 and 2.8:1 (Table 19) and the green flavor defect reported by the panel confirmed the report by Lindsay et al. (40) that green flavor corresponds to a ratio of 3.2:1 or lower. Sample A-2 had a lower acetaldehyde concentration but a high diacetyl concentration indicating a greater

Table 18. Organoleptic evaluation, titratable acidity, pH and total volatile acids of commercial buttermilk.

Sample[a]	Aroma[b]	Flavor[b]	Titratable acidity	pH	Volatile acids[c]
A-1	4.8	5.8	0.90	4.4	4.08
A-2	4.3	4.5	0.92	4.5	4.13
B-1	5.4	5.5	0.93	4.3	4.08
B-2	3.2	3.7	0.97	4.3	5.48
C-1	4.3	4.8	0.98	4.3	5.20
C-2	4.4	5.0	1.01	4.2	5.83
D-1	2.9	3.4	0.92	4.2	4.58
D-2	4.3	3.0	1.00	4.3	5.12
E-1	3.6	4.3	0.95	4.3	5.54
E-2	4.2	4.8	1.00	4.2	5.12
F-1	3.4	3.6	0.88	4.2	2.11
F-2	3.7	4.6	0.87	4.2	2.44
G-1	4.8	6.4	0.92	4.4	5.09
G-2	3.6	3.8	0.91	4.35	4.86
H-1	3.1	3.4	0.97	4.25	3.87
H-2	3.8	3.3	0.97	4.25	4.60

[a] Letters designate companies and corresponding numbers the samples for each company.

[b] 1-7 hedonic scale; 1 - Like very much, 7 - Dislike very much.

[c] Expressed in ml of 0.05 N NaOH.

Table 19. Some volatile chemical components present in commercial buttermilk.

Sample[b]	Concentration ppm[a]					Ratio
	Acetaldehyde	Acetone	Ethanol	Diacetyl	Acetoin	Diacetyl:Acetaldehyde
A-1	7.468	1.823	15.043	4.257	362.613	0.6:1
A-2	2.241	1.460	15.724	6.324	258.313	2.8:1
B-1	0.099	1.905	34.160	3.067	192.449	31.0:1
B-2	0.080	2.104	22.680	4.134	160.283	51.7:1
C-1	0.036	2.122	18.647	3.280	59.396	91.1:1
C-2	0.006	1.832	20.409	0.950	36.213	158.3:1
D-1	0.016	2.016	18.383	6.166	161.049	385.4:1
D-2	0.043	1.855	22.909	2.983	233.140	69.4:1
E-1	0.087	2.360	13.927	2.754	61.902	31.7:1
E-2	0.043	2.205	15.023	3.724	142.068	86.6:1
F-1	0.140	2.086	14.749	6.011	113.691	42.9:1
F-2	0.168	2.358	15.167	6.417	85.291	38.2:1
G-1	0.017	1.991	15.580	4.779	96.716	281.1:1
G-2	0.067	2.452	23.388	5.388	169.657	80.4:1
H-1	0.047	1.611	19.079	4.565	275.741	97.1:1
H-2	0.059	1.588	17.750	5.347	205.469	90.6:1

[a] Parts per million - average duplicate samples.

[b] Letters designate companies and corresponding numbers the samples for each company.

breakdown of components, although there was less acetoin as compared to Sample A-1. The high acetaldehyde, low diacetyl, and high acetoin of Sample A-1 does not correspond to the accepted theory of the pathway of citric acid metabolism.

A higher ethanol concentration was noted in Sample B-1 than in all others which could be related to the cheesy defect indicated by the panel. Samples D-1, F-1, and F-2 had a much higher concentration of diacetyl but less acetoin suggesting an incomplete breakdown of citric acid in the fermentation process.

Sample G-1 was criticized for being rancid by three of the five judges, but this defect could not be traced to any single component or any combination of components by gas chromatographic analysis. A high diacetyl-acetaldehyde ratio of 281.1:1 for this sample was noted, but since D-1 also had a high ratio and was not criticized for rancidity, no relationship could be determined.

As in previous tests, the titratable acidity and the pH (Table 18) showed little relationship to the organoleptic evaluation. Volatile acids showed even less influence on the flavor and aroma in this series of tests so no conclusion could be made.

The results of organoleptic evaluation (Tables 15 and 18) showed that both the control buttermilk and the pre-acidified buttermilk varied less in aroma and flavor than the commercial buttermilk. It also was noted that the pre-acidified samples were preferred in both aroma and flavor over most commercial samples. In many cases, the aroma was preferred over flavor in both the pre-acidified buttermilk and commercial buttermilk.

Less variation in volatile chemical components, titratable acidity, pH, and volatile acids (Tables 16, 17, and 19) were noted in the experimental samples. Processing techniques of the commercial buttermilk were not known and no attempt was made to determine the age of the samples.

There was no significant preference for one lactic starter culture over the other used in the production of buttermilk in this series. However, there was some preference for the pre-acidified buttermilk over the control buttermilk using the same culture. The experimentally produced buttermilk in this study was more uniform in organoleptic evaluation and in the concentration of volatile chemical components as analyzed by gas chromatography than the commercial cultured buttermilk.

Effect of Pre-Acidification on Rate of Coagulation by Lactic Starter Cultures

Figures 4 and 5 indicate the changes in the titratable acidity and pH in the control buttermilk and pre-acidified cultured buttermilk during incubation. Six commercial lactic starter cultures consisting of five mixed strains of at least two species of organisms and one culture consisting of a single species (S. diacetilactis) were used for this study. The average time required for coagulation of the pre-acidified buttermilk using the five mixed cultures was shortened by 2 1/2 to 4 hours. In the pre-acidified samples with S. diacetilactis coagulation time was shortened 1/2 hour. The milk used in all the tests had an initial titratable acidity of 0.20%. This appeared rather high but the added solids could cause the increase.

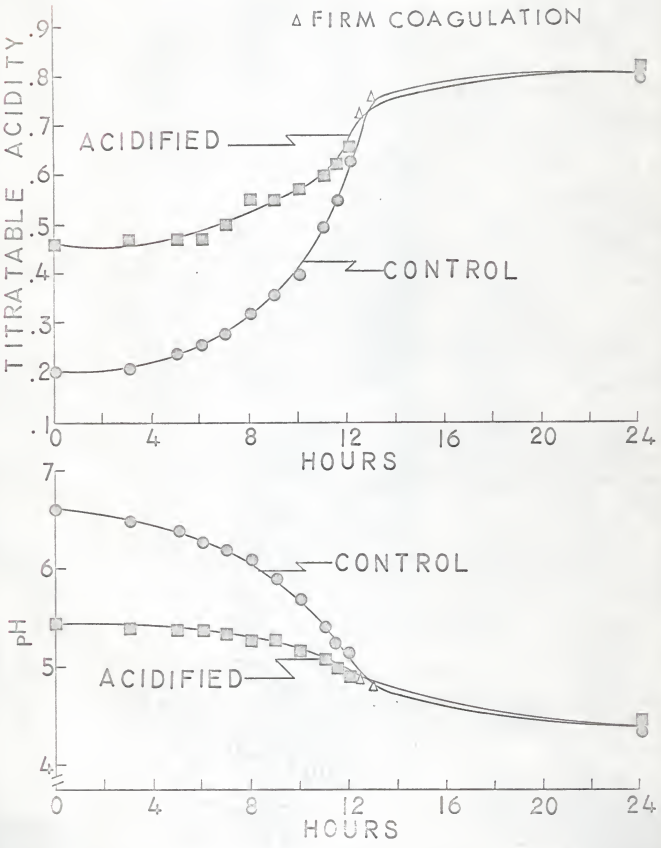


Fig. 4. Titratable acidity and pH changes during coagulation of pre-acidified cultured buttermilk with culture 9.

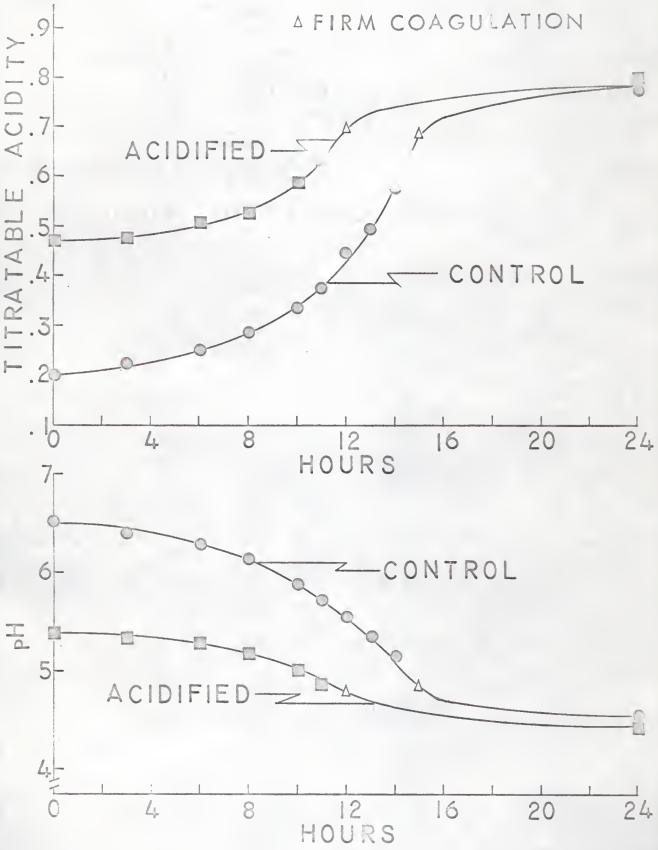


Fig. 5. Average titratable acidity and pH changes during coagulation of pre-acidified cultured buttermilk with cultures 2, 3, 7, 8, and 10.

Figures 4 and 5 show that a greater amount of acid was produced by the control buttermilk at the early stages of incubation than by the pre-acidified buttermilk. However, after about 7 hours incubation, the rate of increase in acid was about the same. The time required to coagulate the control buttermilk varied from 13 to 17 hours. The pre-acidified buttermilk varied from 11 1/2 to 14 hours in the time required to coagulate the samples. The change in acidity was very small during the remainder of the 24 to 26 hour holding time.

The pH decreased during the time required for coagulation from an average of 5.48 to 4.71 in the pre-acidified buttermilk and from 6.58 to 4.78 in the control buttermilk. There also was a small decrease in these values at the conclusion of the holding time.

Although there was a saving of time, it may not be considered significant in the total buttermilk production time. However, the control buttermilk using mixed commercial lactic starter cultures appeared to have a greater effect on the coagulation time than the sample using the S. diacetilactis culture.

SUMMARY AND CONCLUSIONS

Organoleptic evaluation of buttermilk was found to be much less consistent than with other dairy products. There appeared to be a wider range of flavor preference in buttermilk than in other dairy products in addition to a lack of recognition of flavor and aroma defects. This was especially noticeable in the scoring of commercial cultured buttermilk samples by participants of the Kansas State

University clinic. In many cases, the individual realized there was a defect in the buttermilk but was unable to identify that defect. Even with the experienced judges, there was a variation in buttermilk preference and identification of defects although this was not as pronounced as with the inexperienced judges participating in the clinic.

The commercial cultured buttermilk samples were not as consistent in quality as the experimental samples produced under more controlled conditions. Variations in processing techniques may be responsible for the lack of consistency in commercial cultured buttermilk. In many cases, there were no noticeable differences in the body and texture between the commercial buttermilk samples and the experimental samples.

Gas chromatographic analysis was performed on all buttermilk samples, both commercial and experimental, to determine the concentration of volatile chemical components which affect flavor and aroma. Commercial whole milk and skimmilk samples were examined by gas chromatographic analysis to determine the volatile chemical components present before inoculation.

Of the various components indicated by gas chromatographic analysis, the diacetyl and acetaldehyde concentrations of the buttermilk samples varied greatly and generally could be related to the organoleptic evaluations. These components are important in the flavor development of buttermilk although there was no noticeable relationship between the diacetyl-acetaldehyde ratio and a harsh flavor defect. The green

defect was noted and in several instances was related to a high acetaldehyde concentration which confirmed a previous report that a diacetyl-acetaldehyde ratio of 3.2:1 or less was a cause of this defect.

In the studies of buttermilk produced by the normal culturing procedure and the buttermilk produced by using the pre-acidified culturing procedure, the lactic starter cultures were shown to have a greater influence on the organoleptic quality of the buttermilk than the methods of production. A variation was noted in the concentration of each volatile chemical component for each sample. The titratable acidity and pH appeared to be related more to the lactic starter cultures used than to the processing methods. However, the pre-acidified cultured buttermilk samples were often lower in final titratable acidity and were sometimes criticized by the judges as being flat or lacking flavor and aroma. Nevertheless, it appeared that the panelists preferred a flat or milder flavor product and showed a definite preference for the pre-acidified cultured buttermilk.

There was a decrease in coagulation time using a pre-acidified culture procedure but it was not significant in the total buttermilk production time. Additional study using increased inoculum and a variation of temperature to further decrease the coagulation time of the pre-acidified cultured buttermilk appears to be warranted.

The wide variation in quality of commercial buttermilk in this study indicates a definite need for a more consistent product. Using

pre-acidification in combination with lactic starter cultures in the production of buttermilk, a more consistent product was obtained with a more uniform production of volatile chemical components and a product that was preferred over commercial cultured buttermilk by organoleptic evaluation.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. T. J. Claydon, Department of Dairy and Poultry Science, for his direction and encouragement during this course of study and for his assistance and guidance in the preparation of this manuscript.

Grateful appreciation is also expressed to Dr. Richard Bassette, Department of Dairy and Poultry Science, for his valuable suggestions during this investigation.

Thanks are extended to the members of the Dairy and Poultry Science Department who served as panel members for the organoleptic evaluation and to those who offered their assistance and cooperation during this study.

Appreciation is also given Mrs. Viola Reese for her help and technical assistance in laboratory analyses.

LITERATURE CITED

(1) Angevine, N. C.
Manufacturing a quality buttermilk. Cultured Dairy Products Journal, 2(1):4-6. 1967.

(2) Anonymous.
Continuous cheesemaking becoming a reality. Southern Dairy Products Journal, 81(5):25pp. 1967.

(3) Anonymous.
Buttermilk flavor stabilizer developed by Meyer-Blanke Company, Southern Dairy Products Journal, 82(2):73. 1968.

(4) Babel, F. J.
Techniques for cultured products. J. Dairy Sci., 50:431-433. 1967.

(5) Bassette, R., Bawdon, R. E., and Claydon, T. J.
Production of volatile materials in milk by some species of bacteria. J. Dairy Sci., 50:167-171. 1967.

(6) Bassette, R. and Claydon, T. J.
Characterization of some bacteria by gas chromatographic analysis of head space vapors from milk cultures. (Abstract) J. Dairy Sci., 48:775. 1965.

(7) Bassette, R., Ozeris, S., and Whitnah, C. H.
Gas chromatographic analysis of head space gas of dilute aqueous solutions. Anal. Chem., 34:1540-1543. 1962.

(8) Bawdon, R. E. and Bassette, R.
Differentiation of Escherichia coli and Aerobacter aerogenes by gas liquid chromatography. J. Dairy Sci., 49:624-627. 1966.

(9) Bennett, G., Liska, B. J., and Hempenius, W. L.
Effect of other flavor components on the perception of diacetyl in fermented dairy products. J. Food Sci., 30:35-37. 1965.

(10) Bills, D. D. and Day, E. A.
Dehydrogenase activity of lactic streptococci. J. Dairy Sci., 49:1473-1477. 1966.

(11) Bills, D. D., Morgan, M. E., Libbey, L. M., and Day, E. A.
Identification of compounds responsible for fruity flavor defect of experimental cheddar cheese. J. Dairy Sci., 48:1168-1173. 1965.

(12) Bingham, R. J., Moore, D. J., and Gregory, M. E.
Innovations in cultured buttermilk processing techniques. Southern Dairy Products Journal, 82(2):38-44. 1968.

(13) Boddicker, D. D., Baker, R. J., and Stoll, W. F.
Comparison of two cultures for use in preacidified skimmilk for cottage cheese manufacture. 62nd annual meeting of ADSA, Ithaca, N. Y. 1967.

(14) Chou, T. C.
The chemical nature of the characteristic flavor of cultured buttermilk. Ph.D. Dissertation. Ohio State University. 1962.

(15) Collins, E. B.
Culture identity and selection. J. Dairy Sci., 45:1263-1266. 1962.

(16) Cordes, W. A. and Hammer, B. W.
The relation between the volatile and total acidity in starters in cultures of S. lacticus. Iowa State Agr. Exp. Sta. Bul. 66. 1921.

(17) Day, E. A.
Personal interview. Manhattan, Kansas. 1968.

(18) Day, E. A., Lindsay, R. C., and Forss, D. A.
Dimethyl sulfide and the flavor of butter. J. Dairy Sci., 47:197-198. 1964.

(19) Deane, D. D. and Thomas, W. R.
Use of chemical compounds to replace lactic cultures in the manufacture of sour cream. (Abstract) J. Dairy Sci., 47:684. 1964.

(20) Eakle, D. E.
Flavor for cultured products. The Milk Dealer, 52(9):48pp. 1963.

(21) Elliker, P. R.
Activity, flavor production of butter, cheese cultures. Manufactured Milk Products Journal, 54(2):14-19. 1963.

(22) Gerson, M. F.
Modified dairy products: fight them or join. Southern Dairy Products Journal, 81(2):12pp. 1967.

(23) Goss, E. F.
Techniques of dairy plant testing. Ames, Iowa: The Iowa State College Press, 170pp. 1953.

(24) Hammer, B. W.
Volatile acid production of S. lacticus and the organisms associated with it in starters. Iowa State Agr. Exp. Sta. Bul. 63. 1920.

(25) Hammer, B. W. and Babel, F. J.
Dairy bacteriology. 4th ed. New York: John Wiley & Sons, 376pp. 1957.

(26) Hammer, B. W. and Sherwood, F. F.
The volatile acids produced by starters and by organisms isolated from them. Iowa State Agr. Exp. Sta. Bul. 80. 1923.

(27) Harmon, L. G.
The function of lactic cultures in fermented milk products. Cultured Dairy Products Jour., 3(1):3-4. 1967.

(28) Harper, W. J.
Chemistry of cheese flavors. J. Dairy Sci., 42:207-213. 1959.

(29) Hempenius, W. L. and Liska, B. J.
Method for determining volatile acids in cultured dairy products. J. Dairy Sci., 51:221-222. 1968.

(30) Ikeda, R. M., Simmons, D. E., and Grossman, J. D.
Removal of alcohol from complex mixtures during gas chromatography. Anal. Chem., 36:2188-2189. 1964.

(31) Keenan, T. W., Bodyfelt, F. W., and Lindsay, R. C.
Quality of commercial buttermilks. J. Dairy Sci., 51:226-227. 1968.

(32) Keenan, T. W. and Lindsay, R. C.
Removal of green flavor from ripened butter cultures. J. Dairy Sci., 49:1563-1565. 1966.

(33) Keenan, T. W., Lindsay, R. C., Morgan, M. E., and Day, E. A.
Acetaldehyde production by single-strain lactic streptococci. J. Dairy Sci., 49:10-14. 1966.

(34) Kristoffersen, T. and Gould, I. A.
Manufacturing practices for cultured buttermilk in Ohio dairy plants. J. Dairy Sci., 49:690-693. 1966.

(35) Leviton, A. and Marth, E. H.
Fundamentals of dairy chemistry. Westport, Conn.: The Avi Publishing Co., 673pp. 1965.

(36) Libbey, L. M., Bills, D. D., and Day, E. A.
A technique for the study of lipid-soluble food flavor volatiles. J. Food Sci., 28:329-333. 1963.

(37) Lindsay, R. C.
Chemistry and physiology of flavors. Westport, Conn.: The Avi Publishing Co., 315pp. 1967.

(38) Lindsay, R. C. and Day, E. A.
Rapid quantitative method for determination of acetaldehyde in lactic starter cultures. J. Dairy Sci., 48:665-669. 1965.

(39) Lindsay, R. C., Day, E. A., and Sandine, W. E.
Volatile flavor components of butter culture. (Abstract) J. Dairy Sci., 47:662. 1964.

(40) Lindsay, R. C., Day, E. A., and Sandine, W. E.
Green flavor defect in lactic starter cultures. J. Dairy Sci., 48:863-869. 1965.

(41) Lindsay, R. C., Day, E. A., and Sandine, W. E.
Preparation and evaluation of butter culture flavor concentrates. J. Dairy Sci., 50:25-31. 1967.

(42) Little, L.
The direct acid process for manufacturing sour cream. The Milk Dealer, 52(10):28pp. 1963.

(43) Little, L.
Techniques for acidified dairy products. J. Dairy Sci., 50:434-440. 1967.

(44) Loney, B. E.
Some chemical and flavor changes of sterile concentrated milk during storage. Unpublished M. S. Thesis, Kansas State University, Manhattan, Kansas. 1967.

(45) Marth, E. H.
Certain aspects of starter culture metabolism. J. Dairy Sci., 45:1271-1281. 1962.

(46) McNurlin, T. F. and Ernstrom, C. A.
Formation of curd by direct addition of acid to skimmilk. (Abstract) J. Dairy Sci., 45:647. 1962.

(47) Morgan, M. E. and Day, E. A.
Simple on-column trapping procedure for gas chromatographic analysis of flavor volatiles. J. Dairy Sci., 48:1382-1385. 1965.

(48) Morgan, M. E., Lindsay, R. C., Libbey, L. M., and Pereira, R. L.
Identity of additional aroma constituents in milk cultures of Streptococcus lactis var. maltigenes. J. Dairy Sci., 49:15-18. 1966.

(49) Nageotte, G. J.
Correspondence. Va. Polytechnic, Blacksburg, Va., 1967.

(50) Nelson, J. A. and Trout, G. M.
Judging dairy products. 4th ed. Milwaukee, Wis.: The Olsen Publishing Co., 295pp. 1964.

(51) Olson, H. C.
Correspondence, Okla. St. Univ., Stillwater, Okla., 1967.

(52) Reddy, M. C., Bassette, R., Ward, G., and Dunham, J. R.
Relationship of methyl sulfide and flavor score of milk. J. Dairy Sci., 50:147-150. 1967.

(53) Sommer, H. H.
Market milk and related products. 3rd ed. Madison, Wis.: The Olsen Publishing Co., 561pp. 1952.

(54) Toan, T. T., Bassette, R., and Claydon, T. J.
Methyl sulfide production by Aerobacter aerogenes in milk. J. Dairy Sci., 48:1174-1178. 1965.

(55) Vincent, D. D.
Evaluation of a bacterial stimulant for lactic starter cultures in cottage cheese manufacture. Unpublished M. S. Thesis, Kansas State University, Manhattan, Kansas. 1961.

(56) Wilkowske, H. H.
Relationship between titratable acidity and pH during lactic acid fermentation in reconstituted nonfat milk. J. Dairy Sci., 37:22-29. 1954.

APPENDIX

DAIRY PRODUCTS AROMA SCORE CARD

PRODUCT: BUTTERMILK

NAME: _____ DATE: _____

Circle the number which most nearly expresses your attitude toward the product. Check any unusual characteristics in the proper space below.

	Code: A	Code: B	Code: C	Code: D	Code: E	Code: F	Code: G	Code: H
Like - Very much	1	1	1	1	1	1	1	1
Moderately	2	2	2	2	2	2	2	2
Slightly	3	3	3	3	3	3	3	3
Indifferent	4	4	4	4	4	4	4	4
Dislike-Slightly	5	5	5	5	5	5	5	5
Moderately	6	6	6	6	6	6	6	6
Very much	7	7	7	7	7	7	7	7
Defect:								
High acid	___	___	___	___	___	___	___	___
Cheesy	___	___	___	___	___	___	___	___
Yeasty	___	___	___	___	___	___	___	___
Lack	___	___	___	___	___	___	___	___
Misc.	___	___	___	___	___	___	___	___

Fig. 6. Aroma score card used in organoleptic evaluation of buttermilk.

DAIRY PRODUCTS FLAVOR SCORE CARD

PRODUCT: BUTTERMILK

NAME: _____ DATE: _____

Circle the number which most nearly expresses your attitude toward the product. Check any unusual characteristics in the proper space below.

	Code: I	Code: J	Code: K	Code: L	Code: M	Code: N	Code: O	Code: P
Like - Very much	1	1	1	1	1	1	1	1
Moderately	2	2	2	2	2	2	2	2
Slightly	3	3	3	3	3	3	3	3
Indifferent	4	4	4	4	4	4	4	4
Dislike-Slightly	5	5	5	5	5	5	5	5
Moderately	6	6	6	6	6	6	6	6
Very much	7	7	7	7	7	7	7	7
Defects:								
High acid	___	___	___	___	___	___	___	___
Green	___	___	___	___	___	___	___	___
Metallic	___	___	___	___	___	___	___	___
Stale	___	___	___	___	___	___	___	___
Bitter	___	___	___	___	___	___	___	___
Cheesy	___	___	___	___	___	___	___	___
Flat	___	___	___	___	___	___	___	___
Yeasty	___	___	___	___	___	___	___	___
Misc.	___	___	___	___	___	___	___	___

Fig. 7. Flavor score card used in organoleptic evaluation of buttermilk.

Table 20. Aroma evaluation of commercial buttermilk.

Sample	Aroma criticisms[a]										
	High acid	Cheesy	Yeasty	Lack	Harsh	Cooked	Cabbage	Foreign	Unclean	Putrid	Misc.
A	1			2	1	1					
B	1			1	1						1
C		3	1	2							
D				1	1						1
E								1			1
F				2		1	1		1		
G				1		1	1		1		
H				1			1	1			1
I	1			1							
J				1		1					1
K	1	1		1							1
L		1		1				1		1	dirty
M	1				1	1					dirty, fishy

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

Table 21. Flavor evaluation of commercial buttermilk.

Sample	Flavor criticisms[a]										
	High acid	Green	Metallic	Stale	Bitter	Cheesy	Flat	Yeasty	Foreign	Rancid	Misc.
A	2	1			1			1			old
B	3		1		1			1			unusual acid, 1
C	1	1	1	1		3	1				
D	3	1			1				1		sauerkraut
E	2	1					1		1	1	
F	3	1							1		1
G	2				1	1					cooked
H	2	1	1	1					1		unclean (2)
I	2									1	dirty, 1
J	2			1		1					astringent, dirty
K	3		2		2	2	1				
L	1			1	1	2		1	1		
M	2			1	1				1		unclean, powder

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

Table 22. Aroma evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 2)

Lactic culture no.	Trial[b]	Aroma score[c]	Aroma criticisms[a]						
			High acid	Unclean	Lacks	Cooked	Cabbage	Green	Misc.
2 Control	1-A	3.7		1	4		1		
	1-B	3.6		2	2				bland
	2-A	3.8	1		2			2	alcoholic
	2-B	3.2			3	1			cheesy
	3-A	5.3						2	
	3-B	3.8			3				
2 Acidified	1-A	3.6			2		1		
	1-B	3.7		1	1			2	stale
	2-A	3.0	1	1	2	1			
	2-B	2.4	1	1	2				
	3-A	3.8					1		
	3-B	3.8	1		2				

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 23. Aroma evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 3)

Lactic culture no.	Trial[b]	Aroma score[c]	Aroma criticisms[a]						
			High acid	Unclean	Lacks	Cooked	Cabbage	Green	Misc.
3 Control	1-A	2.7	1		1				yeasty
	1-B	3.5			2	2	1		stale, cheesy
	2-A	3.9		2	2				cheesy
	2-B	3.4	1	1	1				cheesy
	3-A	4.2		1	2				
	3-B	4.0	1		1				fishy
3 Acidified	1-A	3.3			2	1			cheesy, yeasty
	1-B	3.7			3				cheesy, musty (2)
	2-A	3.1		1					
	2-B	3.5		1					
	3-A	3.9		1	2				
	3-B	3.9							fishy

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 24. Aroma evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 7)

Lactic culture no.	Trial[b]	Aroma score[c]	Aroma criticisms[a]						
			High acid	Unclean	Lacks	Cooked	Cabbage	Green	Misc.
7 Control	1-A	2.1	1		1				
	1-B	3.2		2	1				metallic
	2-A	3.1	2		2				cheesy
	2-B	2.8			2				
	3-A	3.5		1	1	1			
	3-B	3.2		1	1				
7 Acidified	1-A	3.4		1	3				
	1-B	2.9		1	2				
	2-A	3.1	1	1	3				
	2-B	2.9			2			1	
	3-A	3.2			1				
	3-B	2.9			2	1			

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 25. Aroma evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 8)

Lactic culture no.	Trial[b]	Aroma score[c]	Aroma criticisms[a]						
			High acid	Unclean	Lacks	Cooked	Cabbage	Green	Misc.
8 Control	1-A	3.0		1					yeasty
	1-B	3.4			1				cheesy
	2-A	3.3			4			1	
	2-B	2.8			3				
	3-A	3.1			1				
	3-B	2.4		1	1				
8 Acidified	1-A	2.9		1				1	yeasty
	1-B	3.2			1				
	2-A	3.4	1		4				
	2-B	2.6			2				
	3-A	2.7			2				
	3-B	2.7			3				

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 26. Aroma evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 9)

Lactic culture no.	Trial[b]	Aroma score[c]	Aroma criticisms[a]						
			High acid	Unclean	Lacks	Cooked	Cabbage	Green	Misc.
9 Control	1-A	3.2	2					1	1
	1-B	2.2	2		2				cheesy
	2-A	3.2	1		1				
	2-B	2.6	1						cheesy
	3-A	3.3			3				
	3-B	3.0		1	3				
9 Acidified	1-A	3.0			1				cheesy, oily
	1-B	3.0			3				cheesy
	2-A	2.8	1		2				
	2-B	2.6			1				cheesy, sour
	3-A	3.1		1	1				
	3-B	2.8			2				

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 27. Aroma evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 10)

Lactic culture no.	Trial[b]	Aroma score[c]	Aroma criticisms[a]						
			High acid	Unclean	Lacks	Cooked	Cabbage	Green	Misc.
10 Control	1-A	3.4			4			1	
	1-B	2.6							
	2-A	2.9			2	2			
	2-B	2.7	1		4	1			
	3-A	3.4			2				
	3-B	2.9	1		2				
10 Acidified	1-A	4.0			5				fishy
	1-B	2.9		1					foreign, metallic
	2-A	2.9		1	3	2			
	2-B	2.9	1		3	1			metallic
	3-A	3.1			3	1			
	3-B	2.9			2				

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 28. Flavor evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 2)

Lactic culture no.	Trial[b]	Flavor score[c]	Flavor criticisms[a]						
			High acid	Green	Bitter	Cooked	Metallic	Flat	Misc.
2 Control	1-A	4.3	2	2	1		1	2	foreign
	1-B	4.2	3	2				1	cheesy, powder
	2-A	5.0	2	4	1		1	1	
	2-B	3.6	4	1	1		1		stale, unclean
	3-A	5.6	3	3			1	1	stale
	3-B	5.2	2				2	1	astringent
2 Acidified	1-A	4.5		1	2		1	3	cheesy, foreign
	1-B	5.0	1	3	1			2	cheesy, powder
	2-A	3.0	3	1	1	1	1		cabbage
	2-B	3.2	5	1	1	1	1		unclean
	3-A	4.5	1	2	1			2	cheesy, salty
	3-B	3.8	1		1			1	astringent

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 29. Flavor evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 3)

Lactic culture no.	Trial[b]	Flavor score[c]	Flavor criticisms[a]						
			High acid	Green	Bitter	Cooked	Metallic	Flat	Misc.
3 Control	1-A	4.8	1	1				3	stale, unnatural
	1-B	4.9			2			2	astringent, dirty
	2-A	3.8	2	1	1			1	stale, unclean, yeasty
	2-B	4.0	4	1	2		1		
	3-A	4.4	1	1					cheesy, unclean
	3-B	2.7	2						astringent
3 Acidified	1-A	5.1	2	1				2	stale, unnatural
	1-B	4.4	1		1			2	cheesy, stale
	2-A	3.9	1	1	3			3	cheesy, stale, yeasty
	2-B	2.9	2	1	2				cheesy
	3-A	3.9	1					3	cheesy, salty
	3-B	3.8	1		1	1		3	

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B): A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 30. Flavor evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 7)

Lactic culture no.	Trial[b]	Flavor score[c]	Flavor criticisms[a]						
			High acid	Green	Bitter	Cooked	Metallic	Flat	Misc.
7 Control	1-A	4.3	3	2			2		unclean
	1-B	3.7	4	1				1	
	2-A	4.4	3	4	1			2	unclean
	2-B	4.0	3	3					unclean
	3-A	4.4	4	2			1		unclean, cheesy
	3-B	3.4	4	1					unclean
7 Acidified	1-A	2.8	3	1				2	
	1-B	2.5	2		1				unclean
	2-A	4.4	1		1			4	
	2-B	3.8	2						salty
	3-A	3.0	1	1	1			2	
	3-B	3.5	3	1	1				

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 31. Flavor evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 8)

Lactic culture no.	Trial[b]	Flavor score[c]	Flavor criticisms[a]						
			High acid	Green	Bitter	Cooked	Metallic	Flat	Misc.
8 Control	1-A	5.4	5	2	2				
	1-B	4.3	5		2		1		unclean
	2-A	3.2	3					1	astringent
	2-B	4.2	5	1					stale
	3-A	4.3	1	2	2				
	3-B	2.8	3	1			1		
8 Acidified	1-A	3.0	2	1	2			2	
	1-B	3.9	3	1	4				
	2-A	4.5			2			2	cheesy, astringent
	2-B	3.9	3	1				2	unclean, salty
	3-A	3.3		1	1			2	
	3-B	3.3	3		1		1	1	stale

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 32. Flavor evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 9)

Lactic culture no.	Trial[b]	Flavor score[c]	Flavor criticisms[a]						
			High acid	Green	Bitter	Cooked	Metallic	Flat	Misc.
9 Control	1-A	3.8		4					
	1-B	4.2	2		2				
	2-A	2.5	2		1			1	
	2-B	3.7	4						
	3-A	3.8	1	1				1	
	3-B	3.7	4						
9 Acidified	1-A	4.8	1	1				2	stale
	1-B	3.8	2		3				
	2-A	2.0	2		1			1	
	2-B	3.5	4						
	3-A	3.3	1	2	1			1	
	3-B	3.7	3	1	1		1		stale

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 33. Flavor evaluation of cultured buttermilk with and without supplementary acidification.

(Culture no. 10)

Lactic culture no.	Trial[b]	Flavor score[c]	Flavor criticisms[a]						
			High acid	Green	Bitter	Cooked	Metallic	Flat	Misc.
10 Control	1-A	4.2	2	1			1	3	cheesy
	1-B	2.7	2						
	2-A	3.2	3	1		1		4	
	2-B	3.4	4	1		1		1	stale
	3-A	3.6	1	1	1			3	
	3-B	3.3	2		1			1	stale
10 Acidified	1-A	3.4		1	1			3	
	1-B	3.4	2	1	1		1	1	
	2-A	2.8	3		1	1		2	
	2-B	3.6	4	1	4	1	2	1	
	3-A	3.3	3		2	1		1	
	3-B	3.2	1	1	1			1	

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Three trials with two samples each (A and B); A - sample evaluated same day as removed from incubator. B - sample evaluated after four days storage.

[c] 1-7 hedonic scale; 1 - like very much, 7 - dislike very much.

Table 34. Aroma evaluation of cultured buttermilk with and without supplementary acidification.

Lactic culture no.	Sample	Aroma criticisms[a]							
		High acid	Cheesy	Yeasty	Lack	Harsh	Cooked	Unclean	Green	Misc.
7 Control	1				1					mild, 1
	2				1					
	3				1					pickle, 1
	4	1			3					1
7 Acidified	1				1					mild, 1
	2				1					
	3				2					1
	4	1			2					
9 Control	1				4	1	1			
	2				1					musty
	3				2					bland
	4				1					
9 Acidified	1				3	1				
	2				2					musty
	3				2					bland, 1
	4				1					

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

Table 35. Flavor evaluation of cultured buttermilk with and without supplementary acidification.

Lactic culture no.	Sample	Flavor criticisms[a]								
		High acid	Green	Metallic	Stale	Bitter	Cheesy	Flat	Yeasty	Misc.
7 Control	1	1	1		1					1
	2	2			1					astringent, 1
	3	3								
	4	1	1			1		1		chemical
7 Acidified	1	1	1				1			1
	2	2		1	1			1		astringent
	3	3				1				
	4	2	1			1		2		
9 Control	1	1	1	1				1		1
	2	4	1					1		
	3	1						1		
	4	3						1		
9 Acidified	1	4	1	1				1		salty, 1
	2	3				1		1		salty
	3	2								salty
	4	2			1					1

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

Table 36. Aroma evaluation of commercial buttermilk.

Sample[b]	Aroma criticisms[a]								
	High acid	Cheesy	Yeasty	Lack	Harsh	Cooked	Unclean	Green	Musty	Misc.
A-1		1		1		1		2		chemical, burned, 1
A-2	1							2		2
B-1			1	1		1				chemical, burned, putrid
B-2				1	1	1	1			1
C-1							1			1
C-2				1		1			1	burned, cabbage, 2
D-1										1
D-2				2						heated, 1
E-1		1								pickle
E-2					1					volatile, wine, 1
F-1				1						caramelized, 1
F-2		1		1		1	1			1
G-1			1	1		1	2			old, 1
G-2				1	1		1			medicinal, 2
H-1				1		1				sulfide
H-2				2						scorched

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Letters designate companies and corresponding numbers the samples for each company.

Table 37. Flavor evaluation of commercial buttermilk.

Sample[b]	Flavor criticisms[a]									
	High acid	Green	Metallic	Stale	Bitter	Cheesy	Flat	Yeasty	Foreign	Misc.
A-1		4					1	1		
A-2	1	3	1	1				1		
B-1	3				1	1	1	1		1
B-2	3									rancid, salty
C-1	1	1	1	2	1	1				unclean, 1
C-2	2		1	1	1		1			sharp, chemical, medicine
D-1			1		1	1	1			1
D-2	1									unclean, sharp, 1
E-1	2				1		1			varnish, 1
E-2					1			1	1	fruity, fruit acid
F-1	1			1						heated, 1
F-2	2		1	1		1	1			unclean, sharp, heated
G-1				1						rancid (2), 1
G-2	3									unnatural, 1
H-1	3	1					1			unclean, cooked
H-2	4	1								

[a] Number of times each defect was marked by the judges for each duplicate set of samples.

[b] Letters designate companies and corresponding numbers the samples for each company.

EVALUATION OF COMMERCIAL BUTTERMILK AND PRE-ACIDIFIED CULTURED BUTTERMILK BY ORGANOLEPTIC AND GAS CHROMATOGRAPHIC ANALYSES

by

HAROLD A. ROBERTS

B. S., Kansas State University, 1959

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy & Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Much investigation has been conducted with lactic starter cultures used in commercial buttermilk. Most of these cultures consist of a combination of two or more species of streptococci and leuconostocs or a mixture of a number of strains of each species which are combined to produce desirable flavor and aroma characteristics.

Some volatile chemical components present in buttermilk as determined by gas chromatographic analysis include acetaldehyde, acetone, ethanol, diacetyl, and acetoin. Acetaldehyde and diacetyl are considered to be the two most important volatile chemical components affecting flavor and aroma.

In recent years there has been considerable research into the direct acidification of milk products. However, very little has been done using a combination of lactic starter cultures and direct acidification to produce buttermilk. Most direct acidification processes have been used in the production of sour cream and cottage cheese, although buttermilk produced by this method recently has been placed on the market. With sour cream and buttermilk, an imitation or synthetic flavor must be added to produce the desired flavor required in a high quality product.

The objectives of the present investigation were to determine some standards for buttermilk and whether an improved quality, a more uniform product, and a savings of production time could be achieved by pre-acidification of cultured buttermilk. In this study, five commercial mixed lactic starter cultures and one single species of Streptococcus diacetilactis were used. Control buttermilk was prepared by a standard

procedure. The pre-acidified buttermilk was prepared by the combination of direct acidification and culture procedure. Samples were analyzed for differences in culture characteristics and were compared with commercial cultured buttermilk. Organoleptic evaluation by a selected panel of five members was used to determine flavor and aroma quality with a 1-7 hedonic scale (1 - like very much, 7 - dislike very much). Gas chromatographic analysis was performed on all samples to determine neutral volatile chemical components and their peak heights which were converted to concentration expressed in parts per million. Titratable acidity, pH, and total volatile acids also were determined on each sample and these results were compared with flavor and aroma score and/or component concentration.

The results of a Kansas State University buttermilk clinic study consisting of one sample from each of 18 companies and evaluation of 29 additional commercial samples from 13 companies indicated a marked variation in the quality of buttermilk found on the market today. In addition to culture differences, these variations appeared to be the result of the different techniques used in processing buttermilk and/or handling in distribution channels. It also was evident that the organoleptic evaluation of buttermilk lacks the standardization that exists in judging certain other dairy products and that accurate evaluation is extremely difficult.

The six different lactic starter cultures studied indicated that the amount of volatile chemical components produced varied between cultures, although there was less variation in different trials using

the same culture. A more uniform product with fewer flavor defects was noted in the pre-acidified cultured buttermilk than in control and commercial buttermilk samples. The flavor of pre-acidified cultured buttermilk generally was preferred over the control and commercial samples with the control samples preferred to the commercial cultured buttermilk. The time saved in the pre-acidified process varied from 1/2 to 4 hours depending upon the lactic starter culture used in the preparation of the buttermilk. Using pre-acidification of cultured buttermilk, a more consistent product resulted with a more uniform production of volatile chemical components than in control and commercial cultured buttermilk.