3-1976

Changes in buttermilk on storage with special reference to flavor

Sampath Kumar N. Bhoopalam

To the Graduate Council:

I am submitting herewith a thesis written by Sampath Kumar N. Bhoopalam entitled "Changes in buttermilk on storage with special reference to flavor." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

W. W. Overcast, Major Professor

We have read this thesis and recommend its acceptance:

B. J. Demott, M. B. Badenhop

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Vice Chancellor
Graduate Studies and Research

CHANGES IN BUTTERMILK ON STORAGE WITH SPECIAL REFERENCE TO FLAVOR

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Sampath Kumar N. Bhoopalam

March 1976

ACKNOWLEDGMENT

The author wishes to express his deepest gratitude to Dr. W. W. Overcast for his valuable and patient guidance and encouragement during the period of the graduate study. Appreciation is expressed to Dr. B. J. Demott and Dr. M. B. Badenhop for serving on the graduate committee and for their helpful suggestions. Special thanks are due to members of sensory panel for their participation and suggestions.

Appreciation is also expressed to Mr. Herbert Holt for his help in securing the buttermilk samples from various manufacturers; to Mrs. Anne Helms and Mr. Sung-Hsung Tsai for their assistance with the laboratory work.

Deepest appreciation is expressed to the author's brother Dr. Krishnadev for his understanding and encouragement in spite of his busy schedule as a medical doctor.

ABSTRACT

Four brands of buttermilk were secured from four buttermilk manufacturers and evaluated. Diacetyl level and pH of the buttermilk were determined. The buttermilk was evaluated for flavor by a taste panel.

During storage there was no significant change in pH level but there was significant change in diacetyl level for all brands of buttermilk.

The judgment of the sensory panel indicated that there was no significant difference among the different brands of buttermilk on the first day of storage but on the eleventh day of storage brand number 3 and 4 were inferior to brand number 1 and 2. The judgment also showed that the brand number 3 and 4 were significantly different from each other but there was no significant difference between brand number 1 and 2.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	4
III.	MATERIALS AND METHODS	10
	Sources of Buttermilk	10
	Sensory Panel	10
	Multiple Comparison Test	11
	Method for the Determination of Diacetyl	11
	Procedure for the Tests Conducted on Buttermilk	13
	Bacterial Count	14
	Statistical Analysis	14
IV.	RESULTS AND DISCUSSION	15
	Changes in pH Values	15
	Changes in Diacetyl Levels	15
	Multiple Comparison Test	19
	Percent Fat	26
	Percent Solids-Not-Fat	28
	Bacterial Count	28
V.	SUMMARY AND CONCLUSIONS	31
LIST OF REFERENCES		33
APPENDIX		37
VITA		39

LIST OF TABLES

TABLE		PAGE
1.	The Panelists in the Order of Number of Correct Answers and Tests	12
2.	Multiple Comparison Test of Four Brands of Buttermilk for the Eleventh Day of Storage	22
3.	The Analysis of Variance of Multiple Comparison Test	24
4.	Comparison of Flavor Scores by Duncan's Multiple Range Test	25
5.	Percent Fat for the Four Brands of Buttermilk	27
6.	Percent Solids not Fat for the Four Brands of Buttermilk	29
7.	Bacterial Count on Storage for the Four Brands of Buttermilk	30

CHAPTER I

INTRODUCTION

One of the most remarkable recent developments in the dairy industry which has taken place in all major dairying countries is the rapidly increasing production of cultured milk products; buttermilk being the most important among them (13, 20). Buttermilk has been in use since prehistoric times. "Ayurveda Shastra," the ancient Indian science of medicine, believes that buttermilk is the ambrosia of this world. Buttermilk can be made at home or manufactured commercially. Lassi, the buttermilk of India, is a by-product in the preparation of butter from Dahi (curd) by the indigenous process. With frequent addition of water, Dahi is churned until the butter granules are formed. The diluted beaten curd, remaining after the butter is removed, is the buttermilk (29).

The buttermilk that is manufactured commercially in the United States is called "cultured buttermilk." Cultured buttermilk is a skimmed or partly skimmed milk that has been cultured with lactic acid bacteria and closely resembles that derived from churning sour cream. In the manufacture of buttermilk a common practice is to inoculate the skim milk with special culture. In the Lactic starters Streptococcus and Leuconostoc species are the usual microorganisms found. Such a culture should be selected on the basis of flavor, viscosity and acid production. Cultures forming appreciable amounts of diacetyl, carbon dioxide and volatile acids are required for a mild pleasant flavor. A

harsh acid flavor is usually noted when Leuconostoc strains are absent or are present in small numbers (10, 16, 19, 33).

Usually fresh skim milk is used as a starting material for making cultured buttermilk. Skim milk with 9 to 10 percent solids content is necessary. A weak-bodies buttermilk is obtained from low solids skim milk. Highly viscous buttermilk is obtained from high solids milk. When considerable amount of fat is present homogenization of the skim milk is necessary to prevent fat rising during fermentation. The inoculated skim milk is incubated at a temperature of 21°C for 12 to 16 hours. After this incubation an acidity of 0.85 to 0.90 percent calculated as lactic acid is present; the developed acid being due to the action of lactic acid producing bacteria. During the same time the aroma producing bacteria develop the diacetyl and acetylmethylcarbinol which give a desirable flavor (10, 11, 19, 31). As the buttermilk is stored, changes occur resulting in a deterioration of the flavor.

Some alleged advantages of buttermilk over regular milk are:

1. Less fat concentration, more easily digetible.
2. More cooling effect to the body.
3. Can be stored longer than milk.
4. According to "Ayurveda Shastra" buttermilk corrects certain types of digestive disorders which milk cannot.

A buttermilk drink made in India is used during all seasons but more frequently during summer. This drink is made as follows: To a half cup buttermilk one half cup of water is added. A pinch of salt and lemon juice from a quarter of lemon are added to the above mixture and

stirred. In summer this drink is made on a large scale in the Indian homes, especially in southern India and is kept in pots made from clay. After keeping the buttermilk drink for some hours in a pot, the evaporation of water through the walls of the vessel cools the contents somewhat.

This study was conducted to determine the losses of diacetyl during storage and to correlate pH changes and diacetyl losses with flavor.

CHAPTER II

REVIEW OF LITERATURE

According to Lindsay and co-workers (22), one of the most important constituents of buttermilk is diacetyl. Diacetyl is a diketone with the formula $CH_3.CO.CO.CH_3$ (8). Pure diacetyl is a liquid of a slightly yellowish color with boiling point of 88°C. It is readily volatized with steam and has a penetrating odor which can be detected in solution in dilution of 1 in 100,000,000 (23).

Kandler (15) studied the metabolism of starter (culture) organisms. Some of the starter organisms convert sugars into carbon dioxide, lactic acid and alcohol or acetic acid. The lactic acid resulted from the reduction of pyruvic acid. Pyruvic acid may, however, to a small extent, be decarboxylized to carbon dioxide and acetaldehyde. Condensing activated acetaldehyde with pyruvic acid results in acetolactate which by decarboxylizing was transformed into acetoin. oxidation of which produces diacetyl. Anderson (5) studied the production of diacetyl and acetoin in cultures of aroma-producing bacteria. Streptococcus diacetilactis and Leuconostoc citrovorum were grown in a medium containing labelled sodium citrate and lactose. Both substances were used in diacetyl production. Lacrampe and Weber (18) found that production of diacetyl by experimental and industrial starter cultures were optimum at a temperature of 21°C and pH of 4.35.

In recent years cultured buttermilk with added flavoring materials has become available. Hedrick (12) gave guidelines for commercial

production of flavored buttermilk, covering the selection of culture, preparation of cultured buttermilk, factors to be considered in the choice of the type of flavoring and formula adopted and ingredient cost of flavoring.

Many methods for the determination of diacetyl and acetoin have been devised which are applicable to various types of investigation. Piet and co-workers (26) developed a colorimetric method for the determination of diacetyl with diamino benzene derivatives which, in the presence of strong acids, give compounds having a yellow color. They first used 3,4-diaminotoluene but later (27) found that diamino-benzidine gave a stronger color. Prill and Hammer (28) devised a colorimetric procedure for the microdetermination of diacetyl in butter and milk based on the formation of colored ammonoferrous dimethyglyoximate. Farren and co-workers (9) did the plarographic determination of diacetyl in buttermilk. Changes in diffusion current were related to the diacetyl content of buttermilk samples in parts per million range. The method eliminated the involved pretreatment of buttermilk and the results were unaffected by the presence of acetoin. Scanlan and Lindsay (30) did the quantitative determination of diacetyl by electron capture. The method involved a gas entrainment, on-column trapping technique and was applicable to concentrations as low as 0.002-0.003 parts per million.

Because diacetyl is one of the most important constituent of the buttermilk flavor, the next step is to develop methods to enhance and stabilize this in lactic starter cultures. Hydrogen peroxide-catalase treatment of milk, prior to inoculation with starter cultures containing

diacetyl-producing aroma bacteria, was found to increase synthesis and stabilization of diacetyl in the cultured milk (25).

Infrared spectroscopy and paper and gas liquid chromatography have been employed to attempt to characterize chemically the flavor of commercial buttermilk (6). Infrared spectroscopy of vacuum distillates of 35 commercial buttermilks revealed a relationship between the infrared spectra of the volatile compounds and the flavor quality. The complexity of the infrared spectra varied directly with the flavor quality of the buttermilk and a specific ratio between various adsorption peaks occurred with good-flavored buttermilk. The spectra indicated the presence of aldehydes, ketones, dicarbonyls, esters and small amounts of alcohols and acids in good-flavored buttermilk. Paper and gas-liquid chromatography were used to separate individual volatile flavor components. Acetaldehyde, propanol, acetone, acetoin, diacetyl, butanol, butanone-2, pentanal, ethyl acetate, ethanol, acetic acid, propionic acid, valeric acid and methyl sulfide were identified tentatively in the buttermilk. Methyl sulfide, propanol, acetone, ethyl acetate, acetoin, diacetyl, butanone-2, pentanal and three unidentified compounds were found in all good-flavored buttermilk. Results indicated that there was a balance between certain important flavor related compounds and flavor quality. This balance in volatile compounds was absent in fair- and poor-flavored compounds.

Objective methods for evaluating the physical and chemical properties of foods necessitate the use of sophisticated instruments. However, flavor factors in foods become a problem to evaluate completely

through instrumentation and are best evaluated by subjective methods, since flavor elements in foods exist in such small quantities. Even when sophisticated instruments are used, the flavor elements might contain large numbers of chemical substances which were not perceived as separate substances (2, 14, 17). Even like gas chromatography coupled with mass spectrometry cannot measure the flavor of a food as such because they respond to a certain single factor and not to total flavor. The mouth-feel or the flavor acceptability that can be evaluated by subjective methods cannot be evaluated by objective methods (1, 2, 17, 21). In the subjective evaluation of flavor of foods personal preference judgments are avoided by developing techniques which are most objective within subjective evaluation (17, 21).

Eight conditions influencing sensory interaction to be given careful consideration are (a) strength of accessory stimulus, (b) excitatory state of primary sense organs, (c) duration of accessory stimulation, (d) termination of accessory stimulation, (e) activity of stimulus, (f) physiological state, (g) diurnal variation, (h) summation, repetition and cumulation of accessory effects (1).

In the subjective evaluation of foods the senses of taste, smell and touch are most prominent. With the interaction of taste receptors and olfactory nerves, food quality in programs was evaluated for quality control and new product development. Several persons are required to carry out this evaluation. Statistical techniques are necessary to avoid wrong notions caused by human imperfections and for estimating the reliability of the panel's observation. Thus, a sensory panel

conducted on a statistical basis could be said to be a psychophysical test based on psychom trics (1, 4, 17, 21, 24).

Selection of panelists is an important factor in the sensory evaluation. For economic reasons for the sensory evaluation of foods panel members should be selected from office, plant, research staff and students whoever are closest to the evaluation room. Persons who prepare the samples and those concerned with the test product should not be included on the panel (1).

All panel members should be in good health. Smokers and nonsmokers both are found to be useful as panel members, but it is not advisable for smokers to smoke within one to two hours before a test. Those who smoke one or more packs of cigarettes per day are branded as heavy smokers and these people are generally less sensitive than nonsmokers. However, there are exceptions. Experience plays a bigger role than sensitivity as long as there is basic sensitivity. A person with high personal integrity, intellectual curiosity, ability to concentrate and willingness to spend time in evaluation coupled with average sensitivity may do a better job than a careless person with high acuity of taste and smell. Ability to detect differences and consistency in evaluation are the two most important factors to be considered in the selection of panelists. Some tasters do well with some foods while others do well in other foods; the exception is to find a person to be equally proficient in tasting all foods (1, 21).

Because of variations between individuals on the taste panel statisticians recommend more panelists than the researcher sometimes

thinks necessary. Four panelists is probably the minimum number although eight to ten is better. Panels consisting of individuals with high sensitivity and ability may be preferable to large panels whose members have less sensitivity. As far as possible the tasting schedule should not be less than one hour before a meal or two hours after a meal since hunger will have an effect on the test (1, 17, 24, 30).

Coding should be such that the judges would not be influenced by code bias. When A, B, C, D or 1, 2, 3, 4 are used it is likely that judges would consider A or 1 to be the best. When the coding is for example 778, 806, 798, 811 the coding bias is avoided (1, 21).

The two types of taste panel tests are the difference tests and preference tests. The first is an objective evaluation since panelists are merely asked if a difference exists between two or more samples. Preference tests conducted with 100 to 160 untrained persons are used to determine representative population preferences (21, 24). As little information about samples as possible should be given to panelists as more information than basically necessary may influence their judgment. Temperature of the samples should be uniform, but high or low temperatures make the taste buds less sensitive. Therefore as far as possible, room temperature should be used (1, 24).

The screening procedure for the selection of panelists are the multicomponent odor identification test, the intensity rating test, and triangle test. Among these procedures, the triangle test is economically able to rapidly eliminate candidates who have difficulty tasting. The ability to differentiate consistently is paramount. The screening test should be repeated at least three times (1, 2, 17).

CHAPTER III

MATERIALS AND METHODS

Sources of Buttermilk

Four different brands of fresh buttermilk were secured directly from the manufacturers.

Sensory Panel

Twenty-five panelists, selected on the basis of their "likeness" for buttermilk, were invited to each of the taste panel sessions. A combination of triangle test and actual testing was done at the same time. Brand number 1 was taken as the reference sample. There were four samples, brand number 1 being one of them. On day one (zero day is the day of packaging), panelists were asked to indicate whether each sample was better than, comparable to, or inferior to the reference sample. Then they were asked to mark the amount of difference that existed on a hedonic scale. On the eleventh day a similar procedure was used. A fresh reference sample was used on that day. Tasting on the first day and the eleventh day constituted one replication. Three replications were conducted. For the purpose of statistical analysis only fifteen members were selected. This selection was made as follows: One point was given to each panel member every time he identified the brand number 1 with the reference sample on the first day of each replication and again one point was given to each panel member every time he recognized brand number 1 inferior to the reference sample

on the eleventh day of each replication. Those panel members who scored three or more points out of the possible six points were selected for statistical analysis (Table 1).

Multiple Comparison Test

The panelists were asked to score samples on day one and day eleven in comparison to the reference sample using a nine point hedonic rating system (Appendix). The ratings were given numerical values 1 to 9 by the person analysing the results with "no difference" equaling 5, "extremely better than R" equaling 1, and "extremely inferior to R" equaling 9 (21). There were three replications of this nature.

Method for the Determination of Diacetyl

Diacetyl level was determined by using the colorimetric method of White and co-workers (34).

Principle. The method depends on the production of a colored compound by the reaction of diacetyloxime and urea in the presence of strong acid. The colored compound has not been identified.

Reagents: Urea—3 percent solution in water.

Hydroxylamine—a solution containing 10 milligrams per milliliter in water.

Sulfuric-Phosphoric Acid Mixture—1 volume of concentrated sulfuric acid and 3 volumes of syrupy phosphoric acid

TABLE 1

THE PANELISTS IN THE ORDER OF NUMBER OF CORRECT ANSWERS AND TESTS

Panelists	Number of Tests	Number of Correct Answers
P_3	6	6
P_1, P_2, P_4, P_8, P_{11}, P_{12}	6	5
P_5, P_9, P_{14}	6	4
P_6, P_7, P_{10}, P_{13}, P_{15}	6	3
Total 15	90	63

Diacetyl standard. A solution of 100 milligrams of diacetyl in 100 milliliters of water—this solution was kept in the ice box when not in use and, due to volatility of diacetyl, was freshly prepared about every two weeks. For assay this solution was diluted 1:10 to give a concentration of 100 gamma per milliliter.

Procedure. Various dilutions of a trichloracetic acid filtrate of the buttermilk were made in such a manner that 1 milliliter contained approximately 100 gamma or less of diacetyl. One milliliter of the dilution was then transferred to a test tube, and 1 milliliter of the hydroxylamine solution, 1 milliliter of urea solution and 2 milliliters of the sulfuric acid-phosphoric acid mixture were added. The volume was then adjusted to 6 milliliters with distilled water and the tube was rotated rapidly to mix the contents.

After mixing, the samples were placed in a boiling-water bath for 45 minutes, cooled and the optical density measured by a spectrophotometer at 470 wave length.

Procedure for the Tests Conducted on Buttermilk

On the first day fat percent was determined by the Babcock test; percent of solids-not-fat was determined by using the Cenco moisture balance; pH was determined by using pH meter; flavor was determined by the taste panel using Multiple Comparison Difference Analysis and diacetyl level was determined by using the colorimetric method of White and co-workers(34). Six replications of the same sample were analyzed by this procedure to ascertain the precision of the method. The values

of these six replications were 0.338, 0.333, 0.335, 0.336 and 0.333. As the differences among these values are within a reasonable limit, the procedure was taken to be precise enough.

On the fourth day of storage the diacetyl and pH levels were determined again. On the eleventh day of storage diacetyl and pH levels and the flavor were determined again.

Bacterial Count

Total bacterial count was made on the first and eleventh day of storage using standard method (3) except for the fact that volume instead of weight was used.

Statistical Analysis

The data obtained by taste panel was subjected to analysis of variance and if the samples were found significantly different at 5 percent level Duncan's Multiple Range Test (21) was applied to compare sample means.

Analysis of variance was applied to the data on diacetyl content to detect any significant difference among the different brands of buttermilk and also to detect any significant change in diacetyl level in each brand of buttermilk due to storage.

CHAPTER IV

RESULTS AND DISCUSSION

The changes in four brands of buttermilk on storage were determined. The factors that were considered were pH values, diacetyl levels, flavor panel scores and bacterial counts. Percent fat and percent solids-not-fat were determined once on each sample.

Changes in pH Values

The average of pH values for three replications for each brand of buttermilk on the first, fourth and eleventh day of storage are shown in Figure 1. Brand number 3 had the highest average value for the first and fourth day of storage with pH of 4.54 and 4.50 respectively. For the eleventh day brand number 2 and 4 had the same pH of 4.36 which was the highest. For the first day brand number 2 had the lowest pH of 4.46, for the fourth day brand number 1 had the lowest pH of 4.42 and for the eleventh day both brand number 1 and 3 had the same pH of 4.34 which was the lowest.

The analysis of variance for pH levels showed there was no significant change at 5 percent level in pH values due to storage.

Changes in Diacetyl Levels

Figure 2 shows standard curve for diacetyl which has optical density on X axis and diacetyl concentration on Y axis. Figure 3 shows the average diacetyl levels for three replications for each brand of buttermilk for the first, fourth and eleventh day of storage. The

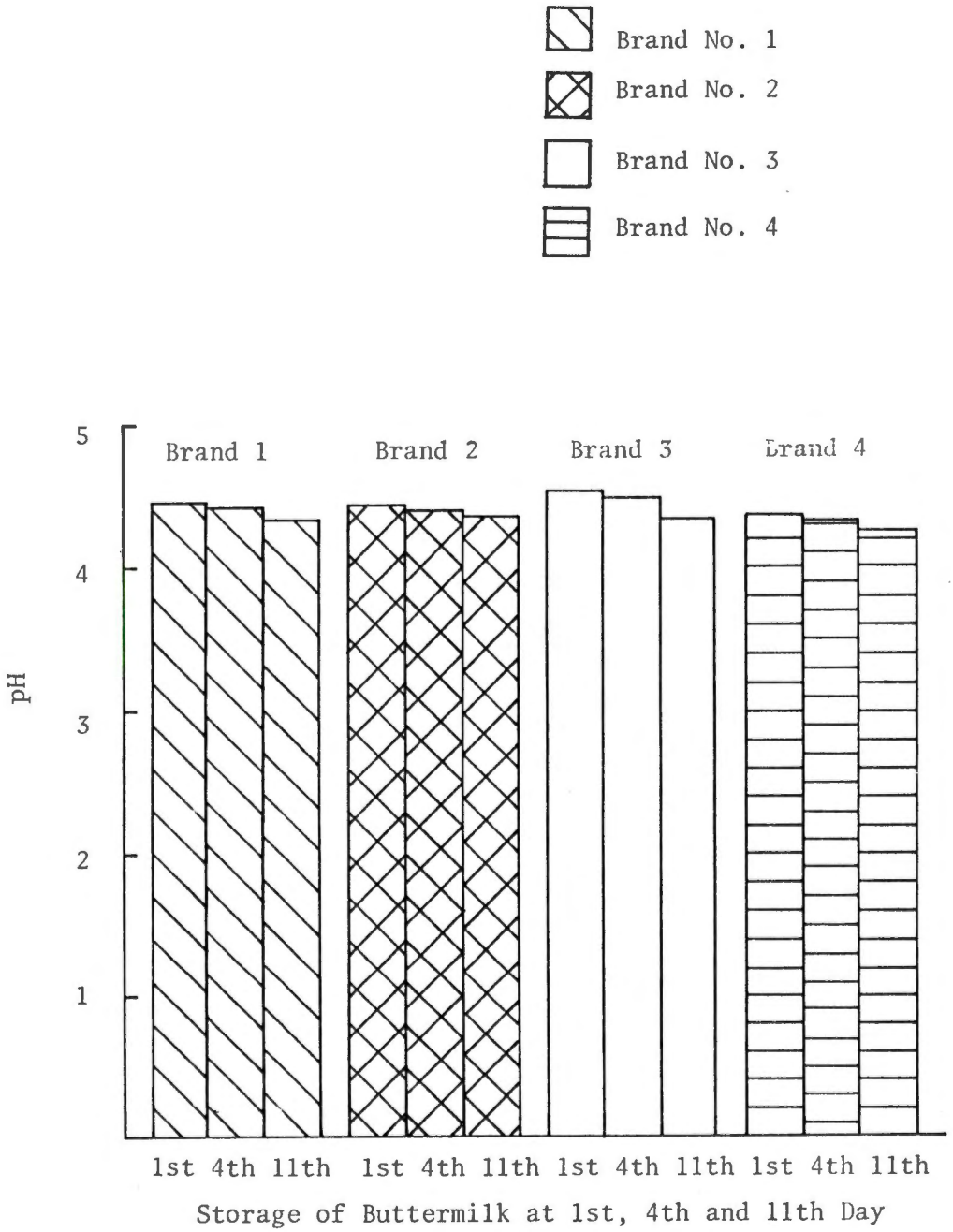

Figure 1. Average pH of four brands of buttermilk on the first, fourth and eleventh day of storage at 4°C.

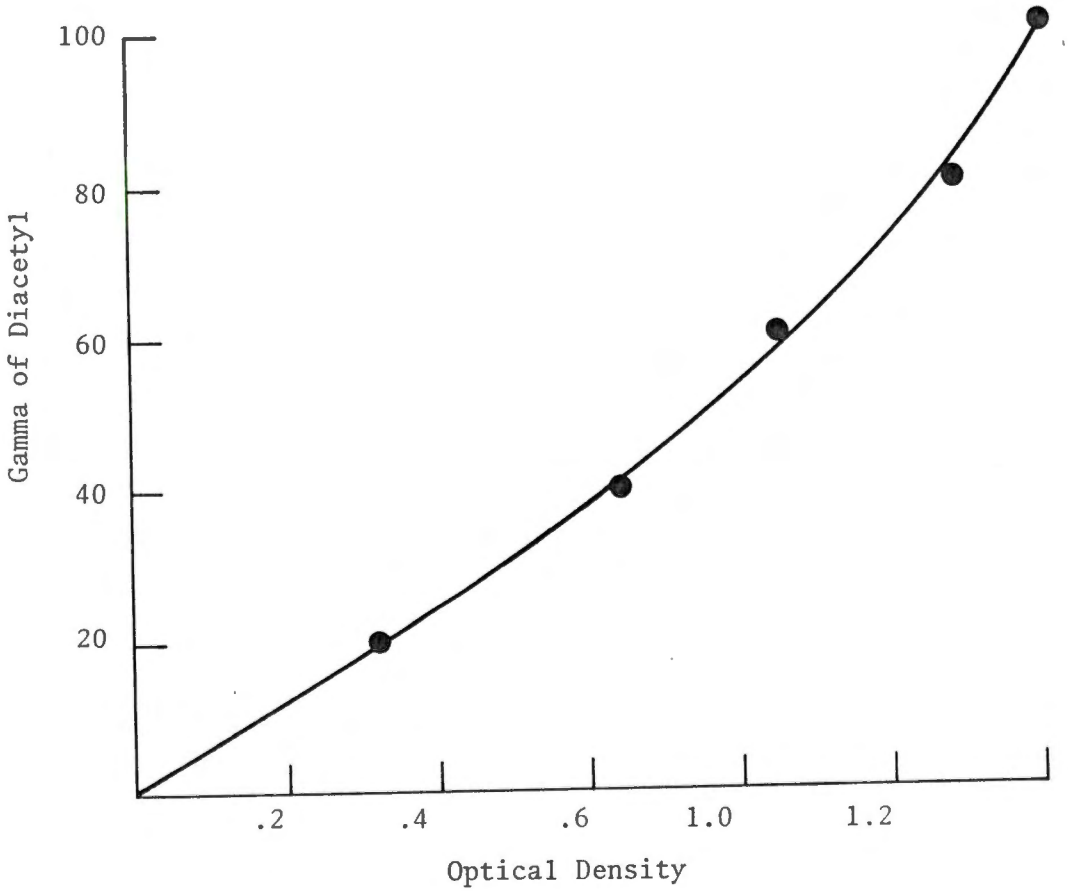

Figure 2. Standard curve for diacetyl.

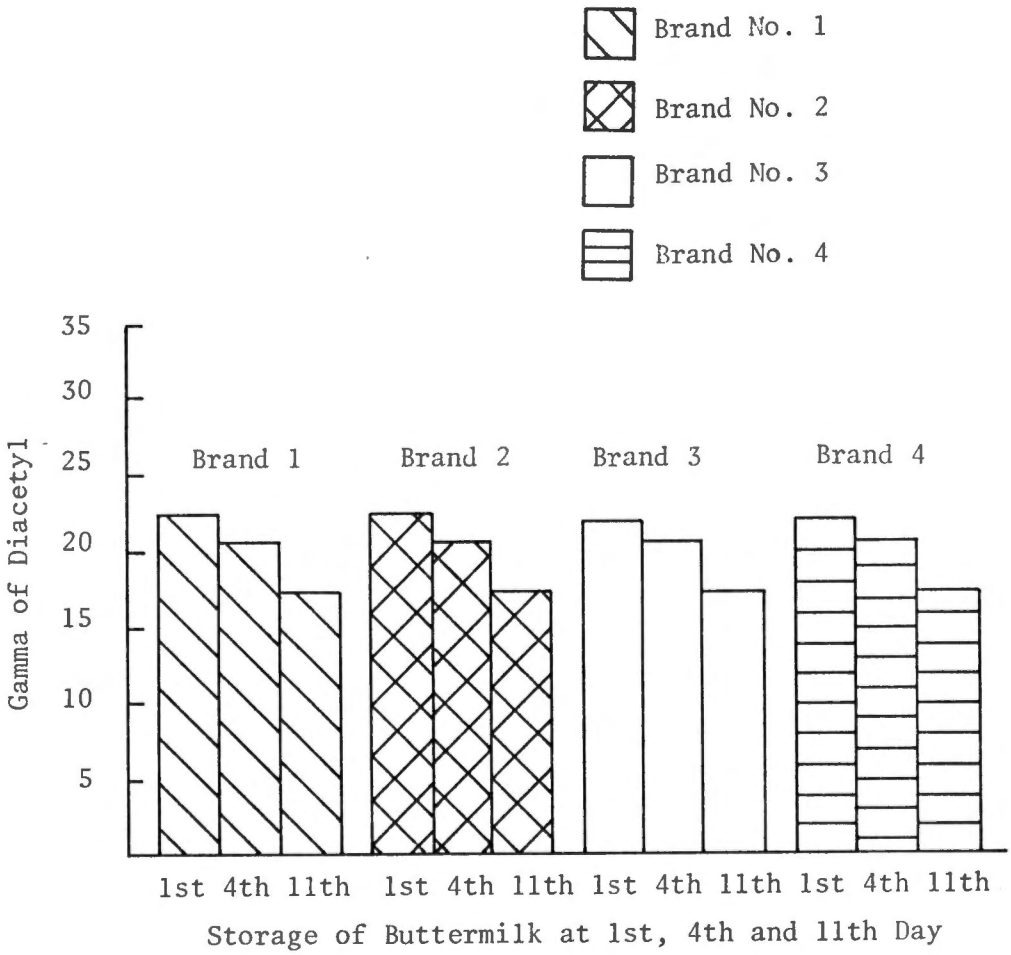

Figure 3. Average diacetyl levels of four brands of buttermilk on the first, fourth and eleventh day of storage.

difference in the average level of diacetyl was negligible among the four brands of buttermilk. For the first day the average diacetyl content was highest for brand number 1 with 22.55 gamma and lowest for brand number 3 with 22.06 gamma. For the fourth day the average diacetyl content was highest for brand numbers 2 and 3 with 20.81 gamma and lowest for brand number 1 with 20.63 gamma. For the eleventh day the average diacetyl content was highest for brand number 4 with 17.88 gamma and lowest for brand number 2 with 17.44 gamma. The analysis of variance showed no significant difference in the diacetyl levels of the four brands of buttermilk for the first, fourth or eleventh day of storage, at 5 percent confidence level. The analysis of variance showed that all four brands of buttermilk had significant changes at 1 percent confidence level in diacetyl concentration due to storage.

Multiple Comparison Test

Figure 4 shows the average panel score for the first day for each brand of buttermilk for each replication. The ratings were given numerical values 1 to 9 by the person analyzing the results as was mentioned earlier. There was not much difference in the scores for different brands of buttermilk for the first day of storage. The average lowest score was 4.93 and the average highest score was 5.73. When the analysis of variance was determined no significant difference at 5 percent significance level was found among the panelists or samples. The reason why there was not much difference among the brands on the first day of storage could be attributed to the following facts: there was

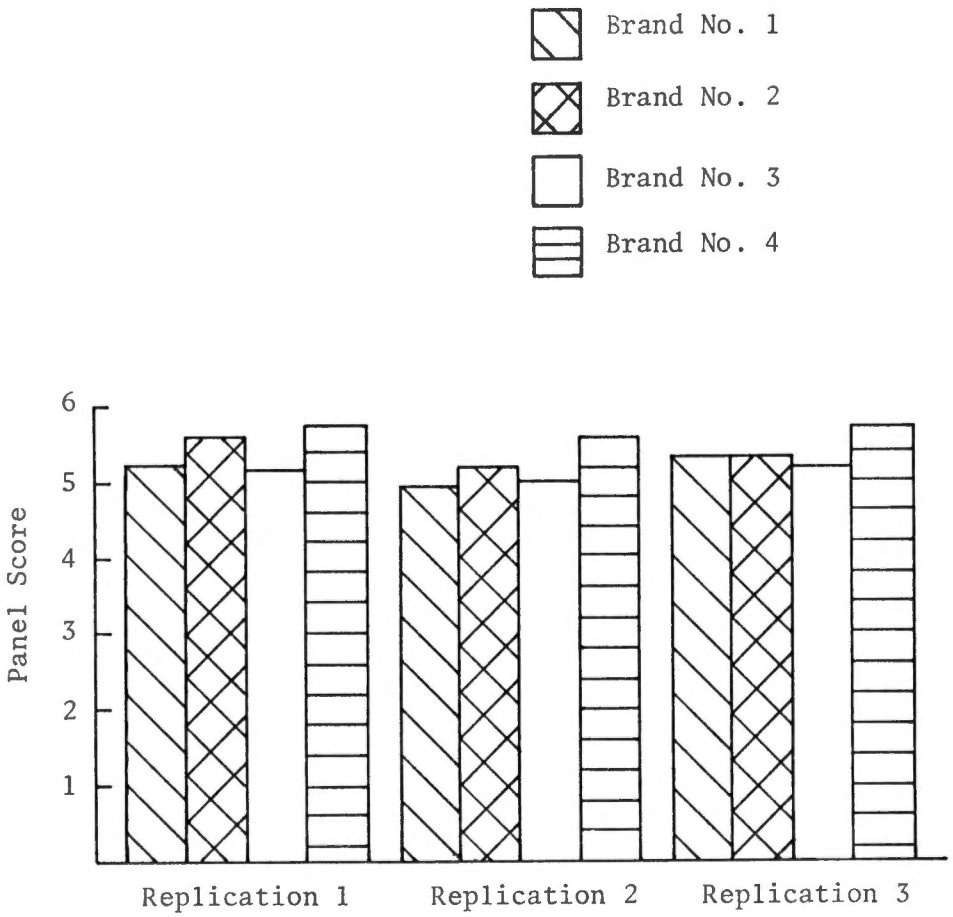

Figure 4. Average flavor scores on the first day of storage for four brands of buttermilk for each replication.

no significant difference either in pH levels or in the diacetyl levels and since all the samples were fresh, the difference that could arise due to the difference in the keeping quality had not arisen.

Table 2 gives the data for the Multiple Comparison Test of four brands of buttermilk for the eleventh day of storage. It shows the panel score of each panelist for each replication. Data in Table 2 demonstrates quite a difference in flavor among the panelists of the samples on the eleventh day of storage. Some panelists were more critical of the samples as shown by higher hedonic scores and some panelists made the judgments within a narrow range. Human psychological factors and human preference to the samples might be the reason for this. The data in Table 2 also indicates the widely different score given to the same brand of buttermilk by the same panelist during different replications. Panelist number 7 is a good example for this. This panelist gave as low a number as two in one replication while giving number seven in another replication. A factor to be considered for wider gap in the score of different brands of buttermilk for the eleventh day as compared to the first day was the keeping quality of each brand of buttermilk.

The analysis of variance in Table 3 from the data in Table 2 demonstrated there was significant difference at 1 percent confidence level both among the panelists and the samples. Duncan's Multiple Range Test (Table 4) showed: (1) brand number 3 was significantly different from brand number 1, 2 and 4, (2) brand number 4 was significantly different from brand number 1 and 2, (3) brand number 1 and 2 were not

TABLE 2

MULTIPLE COMPARISON TEST OF FOUR BRANDS OF BUTTERMILK FOR THE ELEVENTH DAY OF STORAGE

Panelisits	Replications	Score of Four Brands of Buttermilk				
		No. 1	No. 2	No. 3	No. 4	Total
P_1	3	6	6	8	6	26
		4	6	6	6	22
		6	6	6	6	24
P_2	3	6	7	6	7	26
		4	6	6	4	20
		6	5	9	9	26
P_3	3	6	5	6	6	23
		6	4	7	5	22
		6	7	6	6	26
P_4	3	6	7	7	8	28
		6	6	8	6	26
		6	6	7	8	27
P_5	3	6	7	9	6	28
		4	6	5	6	21
		6	6	9	6	27
P_6	3	6	5	6	7	24
		5	4	6	4	19
		6	5	9	7	27
P_7	3	5	2	2	4	13
		6	4	7	7	24
		5	6	7	6	24
P_8	3	6	6	6	6	24
		5	6	9	6	26
		7	8	8	8	31
P_9	3	7	6	9	8	30
		6	6	5	6	23
		6	6	9	8	29
P_{10}	3	6	6	8	6	26
		6	6	5	6	23
		6	7	6	7	26

TABLE 2 (continued)

Panelists	Replications	Score of Four Brands of Buttermilk				
		No. 1	No. 2	No. 3	No. 4	Total
P_{11}	3	5	6	9	8	28
		6	7	8	6	27
		7	6	6	6	25
P_{12}	3	6	7	6	8	27
		5	5	8	7	25
		6	6	9	8	29
P_{13}	3	6	7	9	7	27
		6	6	7	6	25
		5	7	6	6	24
P_{14}	3	5	6	9	7	27
		6	8	4	7	25
		7	6	8	6	27
P_{15}	3	5	6	7	6	24
		5	4	7	6	22
		6	6	8	8	28
Total	45	258	266	318	291	1133

TABLE 3

THE ANALYSIS OF VARIANCE OF MULTIPLE COMPARISON TEST

Source of Variance	df	SS	MS	F ratio
Samples	3	48.95	16.32	18.76*
Panelists	44	111.64	2.54	2.92*
Error	132	114.80	0.87	
Total	179	275.39		

*One percent level of significance

TABLE 4

COMPARISON OF FLAVOR SCORES BY DUNCAN''S
MULTIPLE RANGE TEST

Source	Brands of Buttermilk			
	No. 1	No. 2	No. 3	No. 4
Sample Score	258	266	318	291
Sample Means	5.73[a]	5.91[a]	7.07[b]	6.47[c]

Numbers denoted by the same letters are not significantly different at 5 percent confidence level.

significantly different from each other. The fact that there was no significant difference among the samples on the first day of storage but there was significant difference among the same samples in their flavor on the eleventh day of storage, lead one to suspect that the keeping quality of the buttermilk of different brands may not be the same. A good example for this is a particular brand may be more susceptible for oxidation on storage than another brand. As we have seen earlier there was no significant difference in the diacetyl or pH level of the samples either on the first day or the eleventh day. pH change for each brand of buttermilk was insignificant over the storage period. The change in diacetyl level was significant for each brand of the buttermilk over the storage period. Hence the difference in the flavor among the samples on the eleventh day cannot be due to either pH or diacetyl level.

The common comments for the flavor of number and number 4 brands of buttermilk were foul smell and flat tasting respectively, on the eleventh day of storage for each replication. Probably foul smell was due to oxidation. The reason for the flat taste is hard to find since brand number 4 had similar pH values as the other brands.

Percent Fat

The fat concentration of the four brands of buttermilk were similar (Table 5). The highest percent fat was 0.48 and the lowest was 0.38 and these values are in the range of the percent fat values found by different studies (7, 32).

TABLE 5

PERCENT FAT FOR THE FOUR BRANDS OF BUTTERMILK

Replication	Brand			
	1	2	3	4
1	0.46	0.47	0.44	0.42
2	0.48	0.43	0.48	0.38
3	0.43	0.44	0.43	0.37

Percent Solids-Not-Fat

The highest percent of solids-not-fat found was 8.82 and the lowest was 8.22 (Table 6), a fairly narrow range and are comparable to those found in other studies (7, 32).

Bacterial Count

Table 7 lists the bacterial count of the four brands of buttermilk. On each replication the bacterial count was lower on the eleventh day than on the first day. The highest count for the first day was 9.5×10^8 and the lowest count for the first day was 4.7×10^7 among the three replications. For the eleventh day the highest count was 13×10^7 and the lowest count was 8.2×10^5.

TABLE 6

PERCENT SOLIDS NOT FAT FOR THE FOUR BRANDS OF BUTTERMILK

Replication	Brand			
	1	2	3	4
1	8.54	8.88	8.24	8.54
2	8.60	8.72	8.41	8.22
3	8.61	8.79	8.37	8.26

TABLE 7

BACTERIAL COUNT ON STORAGE FOR THE FOUR BRANDS OF BUTTERMILK

	Replication					
	1		2		3	
	Day of Storage					
Brand No.	1st	11th	1st	11th	1st	11th
	Times 10^7					
1	7.1	0.082	44	1.1	95	13
2	9.5	1.5	36	2.3	42	1.7
3	44	6.1	49	6.5	4.7	0.035
4	23	5.4	17.0	5.2	11.0	4.2

CHAPTER V

SUMMARY AND CONCLUSIONS

Four different brands of buttermilk each made by a different manufacturer were used in the present study. The buttermilks were analyzed for pH and diacetyl levels on first, fourth, and eleventh day of storage. A taste panel was used to evaluate the flavor on first and eleventh day of storage using a Multiple Comparison Test with a nine point hedonic scale.

The general conclusions may be summarized in the following statements:

1. There was no significant difference at 5 percent confidence level in the flavor of four different brands of buttermilk on the first day of storage.
2. There was no significant change at 5 percent confidence level in the level of pH from first day to the eleventh day of storage.
3. Diacetyl level of each brand of buttermilk was reduced significantly at 1 percent confidence level after 10 days of storage.
4. The results of the taste panel on the eleventh day of storage showed that (a) brand number 3 was significantly different from brand number 1, 2 and 4; (b) brand number 4 was significantly different from brand number 1 and 2; (c) brand

number 1 and 2 were not significantly different from each other.

5. Buttermilks of brand number 1 and 2 were superior in flavor to brand number 3 and 4, since brands 1 and 2 received a higher score from the panelists and also because there was no panelist who found off odor or taste in them after 10 days of storage while common comments on the brand number 3 and 4 on eleventh day of storage were foul smelling and flat tasting respectively.
6. Neither the changes in the diacetyl levels nor the changes in the pH levels were responsible for significant differences in the flavor of different brands of buttermilk on the eleventh day of storage.

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APPENDIX

MULTIPLE COMPARISON SHEET

Name_____ Date_____

You are receiving four different kinds of samples to compare to a reference sample marked R. Please taste each sample; show whether it is better than, comparable to, or inferior to the reference. Then mark the amount of difference that exists.

Sample number	____	____	____	____	____	____	____
Better than R	____	____	____	____	____	____	____
Equal to R	____	____	____	____	____	____	____
Inferior to R	____	____	____	____	____	____	____

Amount of Difference

None	____	____	____	____	____	____	____
Slight	____	____	____	____	____	____	____
Moderate	____	____	____	____	____	____	____
Much	____	____	____	____	____	____	____
Extreme	____	____	____	____	____	____	____

VITA

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