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A field test for titratable acidity and the determination of lactic acid by gas chromatography

Mahala Garrell Pearsall

To the Graduate Council:

I am submitting herewith a thesis written by Mahala Garrell Pearsall entitled "A field test for titratable acidity and the determination of lactic acid by gas chromatography." 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.

Hugh O. Jaynes, Major Professor

We have read this thesis and recommend its acceptance:

W. W. Overcast, Curtis C. Melton

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

To the Graduate Council:

I am submitting herewith a thesis written by Mahala Garrell Pearsall entitled "A Field Test for Titratable Acidity and the Determination of Lactic Acid by Gas Chromatography." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

Hugh O. Jaynes, Major Professor

We have read this thesis
and recommend its acceptance:

Herbert C Hall

Curtis C. Melton

Accepted for the Council:

L Evans Roth

Vice Chancellor
Graduate Studies and Research

A FIELD TEST FOR TITRATABLE ACIDITY AND THE DETERMINATION OF LACTIC ACID BY GAS CHROMATOGRAPHY

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Mahala Garrell Pearsall

June 1979

DEDICATION

This thesis is dedicated to my husband, Dixon, whose love and patience have helped me to have the endurance to finish this work.

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ABSTRACT

A simple, convenient kit was developed to measure titratable acidity (TA) of milk in farm bulk tanks. The kit was based on the conventional determination of TA with standard base and phenolphthalein. Glass tubes were calibrated to contain a calculated amount of milk and base to show the phenolphthalein endpoint up to a chosen TA level. Samples remaining white would be rejected as TA above the chosen level.

A survey using the kit and standard TA titration was carried out in winter and summer on manufacturing and Grade A milks. In 192 samples, TA averaged 0.164% and ranged from 0.11 to 0.31% TA with a standard deviation of 0.021% TA. There was no difference from grades or seasons. The kit test failed on only four samples.

Lactic acid content of milk was measured in selected samples by an ether extraction-gas chromatographic method. Good agreement was obtained between the method and added and/or developed acidity determined by titration. Fresh milks with varying TA were shown to contain no lactic acid.

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CHAPTER I

INTRODUCTION

Titratable acidity is one of the traditional tests applied to measure the quality of raw milk. The test measures the acidity in milk as lactic acid, the predominant acid produced by microbial degradation of lactose. Lactic acid produced in milk by microbial action serves as a useful indicator of spoilage. The amount of lactic acid in milk greatly affects how the milk can be used and how long the product will last.

Milk freshly drawn from the udder exhibits a measurable titratable acidity known as natural acidity. This is distinguished from developed acidity in the form of lactic acid.

Natural acidity is caused by buffering agents, such as proteins, phosphates, citrates, and carbon dioxide, with no lactic acid actually present. This value, which varies directly with the solids-not-fat content of milk, averages 0.16 to 0.18% as lactic acid in mixed herd milks.

Several firms in Tennessee which purchase manufacturing milk are using titratable acidity as a basis for accepting or rejecting milk. There is also an interest in this area among Grade A plants according to Herbert Holt (15). Thus, the purpose of this project was to develop a field test for acidity which the milk hauler could use at the point of milk pick up. A limited survey of titratable acidity was conducted among manufacturing and Grade A milks. These milks were also used to

determine the accuracy of the field test. Eighteen milk samples covering a range of acidities were used to correlate the titratable acidity with the actual lactic acid present using gas chromatography.

CHAPTER II

REVIEW OF LITERATURE

The pH or hydrogen ion concentration of freshly drawn normal milk averages about pH 6.5 with variations from 6.4 to 6.8 (24). The hydrogen ion concentration is not normally used to judge the condition of milk or the changes taking place in it. Instead, titratable acidity has been found to be more useful. "The nature of milk with its high content of buffer substances is such that the pH tends to remain constant regardless of additions or changes" within the milk according to Rice and Markley (21).

Titratable acidity determinations on raw milk have been used to grade milk with milk above a set acidity being rejected. This practice of rejecting milk with a high acidity is used most commonly by condenseries to guard themselves against losses as a result of coagulation upon sterilizing evaporated milk according to Sommer and Hart (25). The lactic acid found in these products indicates fermentation of the lactose by micro-organisms since freshly drawn milk contains no lactic acid. Gould and Jensen (11) stated that the lactic acid content of concentrated evaporated milk products reflects the quality of the raw milk used in their manufacture. No one objects to rejecting milk with a high titratable acidity due to lactic acid production; however, there is considerable objection when a sample with high natural acidity is rejected (10). Milk with a high natural acidity is not undesirable since its acidity is due to other factors and does not indicate a high

hydrogen ion concentration or a sour taste or odor. Sommer and Hart (25) further concluded that "there is no relationship between apparent acidity and heat coagulation under pressure at 136°C."

The cause of natural acidity has been studied extensively. It has been attributed to many different components found in milk. Van Slyke and Bosworth (26) proposed that the apparent acidity was due primarily to the acid phosphates present in milk. Richmond (22) agreed with Van Slyke and Bosworth but also attributed part of the acidity to dissolved carbon dioxide. Bordas and Touplain (7) investigated the titratable acidity of the milk serum, the coagulum containing the insoluble salts, the casein plus the insoluble salts, and the pure casein separated by the alcoholic method. They concluded that the original acidity is due to casein and that no free acids or acid salts exist in milk when freshly drawn. Bordas (6) later reported that the increase in acidity is due to casein liberated from combination with calcium by the formation of calcium lactate.

In 1924, Rice and Markley (21) did an extensive study to look at the components causing natural acidity. They concluded that the majority of investigators to that date believed acidity in milk to be due to one or all of the following components: monobasic phosphates, casein, acid citrates, and carbon dioxide. Therefore, they investigated these components and concluded that carbon dioxide contributed 0.01 to 0.02% acidity; citrates, 0.01%; basein, 0.05 to 0.08%; and the phosphate the remainder of the acidity. They also concluded that there "seemed to be no striking relation between acidity and albumen content, citric acid, ash, alkalinity of ash, or CaO." Van Slyke and Bosworth (26) found

substantial results to conclude that from .06 to .10% acidity may be caused by chemical reactions involving calcium salts.

A condition of equilibrium exists among certain components of milk according to Rice and Markley (21), particularly

> citric acid, phosphoric acid, casein, and the bases, and the acidity due to any one of these depends upon its relationship to the others. It is the equilibrium which fixes the hydrogen ion concentration; and a change in the proportion of a constituent or ion will result in a shift in the equilibrium and a change in the influence which each has upon the acidity.

Other factors which influence the acidity of milk include breed, individuality of the cow, stage of lactation, variations between morning and evening milk, health of the cow, and age. More specifically, Caulfield and Riddell (8) concluded that the acidity for the different breeds averaged as follows: Ayrshire, 0.160%; Holstein, 0.161%; Guernsey, 0.172%; and Jersey, 0.179%. The milk from all breeds averaged 0.166% acid. They observed that the acidity of individual milks varied from 0.08% to 0.295%. They also concluded there was a gradual decline in acidity throughout lactation with a marked decline during the last month.

Now that we have discussed what causes the natural acidity of fresh milk, we need to look at what happens to the titratable acidity upon the production of lactic acid by bacteria. Once the milk is drawn, it is stored in bulk tanks at the farm until it is picked up by a milk hauler to be transported to the dairy plant. It is here where the problems of lactic acid production occur. First of all, the tank used must be very sanitary to prevent the introduction of contaminates to the milk. The tank must also maintain a temperature of 33 to 40°F to

limit bacterial growth according to Barnard and Glass (4). In a study conducted by Randolph, Langlois, and Conner (20) in 1966 among 534 Grade A producers, the actual temperatures of the milk at the farm ranged from 32 to 55°F. Differences were observed in the temperature after agitation. Butterfat particles and ice were found in approximately 8% and 2% of the tanks, respectively.

They found that 6% of the bulk tank thermometers were either broken or out of order. About 20% of the tank thermometers' readings did not check within ±3°F with a test thermometer, and the majority of the bulk tank haulers did not carry or use a test thermometer to check the temperature of the milk.

Obviously, if the milk is not handled under proper conditions and checked periodically to ensure proper conditions, one risks the possibility of bacterial growth resulting in lactic acid production. The lactic acid content of milk greatly influences how the milk can be used and the quality of the final product. The majority of dairy plants will reject milk with high acid content on these grounds.

There are several methods for determining acidity of milk. The most common method is the titration test using a Nafis tester containing 0.1 Normal Sodium hydroxide. To 9 ml of milk and four drops of phenolphthalein, enough Sodium hydroxide is added to obtain a persistent pink color. The Nafis tester reads directly in percent acidity as lactic acid. It does not distinguish between natural acidity and developed acidity as lactic acid. This is its major disadvantage (9).

Several other methods have been used to measure the lactic acid present in milk. Troy and Sharp (27) developed a method in which lactic

acid is oxidized to acetaldehyde and carbon dioxide after the proteins have been precipitated. After a series of steps, the aldehyde is distilled and titrated. This method indirectly measures the lactic acid originally present in the sample.

In the Hillig method (13,14), the proteins are precipitated and the serum extracted with ethyl ether. The lactic acid is recovered as barium lactate which is then treated in several steps before the lactic acid content is estimated by the color developed on adding ferric chloride. The readings from a photoelectric colorimeter are used to determine the concentration of lactic acid from a standard curve. This method was found to be reliable and accurate according to Gould (10) and Gould and Shiver (11); however, it has a definite time disadvantage since it takes approximately 8 hours to do an analysis.

Another method for determining lactic acid is the method used by Salwin and Bond (23). This method was originally used to determine the lactic and succinic acids present in eggs. It has also been used according to Salwin and Bone (23) in beef, shrimp, and cottage cheese whey. The author modified their procedure and applied it to the determination of lactic acid in fresh milk.

To determine the quantity of lactic acid in milk, the lactic acid is liberated from the milk with sulfuric and phosphotungstic acids (18). It is extracted with anhydrous ether, and then the ether is allowed to evaporate. The remaining lactic acid is esterified with boron trifluoride-n-propyl-alcohol. Various solutions, including an internal standard, are added to the esterified lactic acid and shaken before the layers are allowed to partition. Then the top layer

containing the lactic acid is injected into a gas chromatograph. The percent lactic acid present can then be calculated.

CHAPTER III

MATERIALS AND METHODS

Samples for the study were collected through a Grade A plant in Knoxville, Tennessee, and two manufacturing milk plants in Greeneville, Tennessee. The survey consisted of 192 samples collected during two seasons, winter and summer. There were 77 winter samples collected during the months of December through February, which consisted of 40 manufacturing samples and 37 Grade A samples. The summer samples collected in July and August consisted of 96 manufacturing samples and 19 Grade A samples.

The titratable acidity was determined in duplicate for each sample using a Nafis titrator, four drops of 1% phenolphthalein, 9 ml of milk, and enough .10 N NaOH to obtain a persistent pink color (1). The field test for acidity was also conducted on each sample.

The field test for titratable acidity (hereafter referred to as TA) was developed for milk haulers to use at the farm. It works on an acceptance-rejection principle, whereby milks with TAs above a certain breakpoint are rejected, and those below the breakpoint are accepted.

The field test for acidity consisted of a capped 25 X 150 mm Pyrex test tube calibrated to contain 45 ml of refrigerated solution (37.5 ml milk and 7.5 ml .10 N NaOH). A line was scribed in each test tube at this desired volume with a carborundum pencil. This volume was determined by establishing the maximum desired acidity at 0.17% and the volume of .10 N NaOH to be 7.5 ml. To prevent the rejection of a

borderline sample, a 0.01% TA error was calculated into the volume; therefore, 0.18% TA was used to calculate the parameters for a rejection level of 0.17% TA:

$$\text{ml of milk} = \frac{\text{ml}_{\text{NaOH}} \times \text{N}_{\text{NaOH}} \times \text{meq. wt. of lactic acid} \times 100}{\% \text{ acidity}}$$

$$37.50 \text{ ml} = \frac{7.5 \text{ ml} \times .10 \text{ N} \times .09 \times 100}{.18\%}$$

volume in tube = 37.5 + 7.5 ml = 45 ml

The above formula can be adjusted to accommodate any desired acidity. In this study, 0.18% and 0.19% TA were used in the winter study. For the summer study, the breakpoint was lowered to 0.17% TA because almost all of the samples had a TA below 0.18%. Leaving the breakpoint at 0.18% TA would have rejected very few samples and would not have given a rigorous test to the method.

The test kit included a wooden color comparator. The base of the comparator consisted of a black block with a shallow hole in the center designed to hold the calibrated test tube (Figure 1). The back of the comparator is divided in half with one side painted cameo white and the other light pink. The cameo white is a Benjamin Moore high gloss impervo enamel (133-77) corresponding to Munsell 10 YR 9.0/1.5. The pink is a Glidden Spred latex gloss enamel (3325 decorator white plus 30XR per quart) corresponding to Munsell 8.7 YR 8.7/1.5. Other brands of enamel corresponding to these colors would work as well; however, glossy enamel should be used to correspond to the reflectance on the surface of the glass tubes.

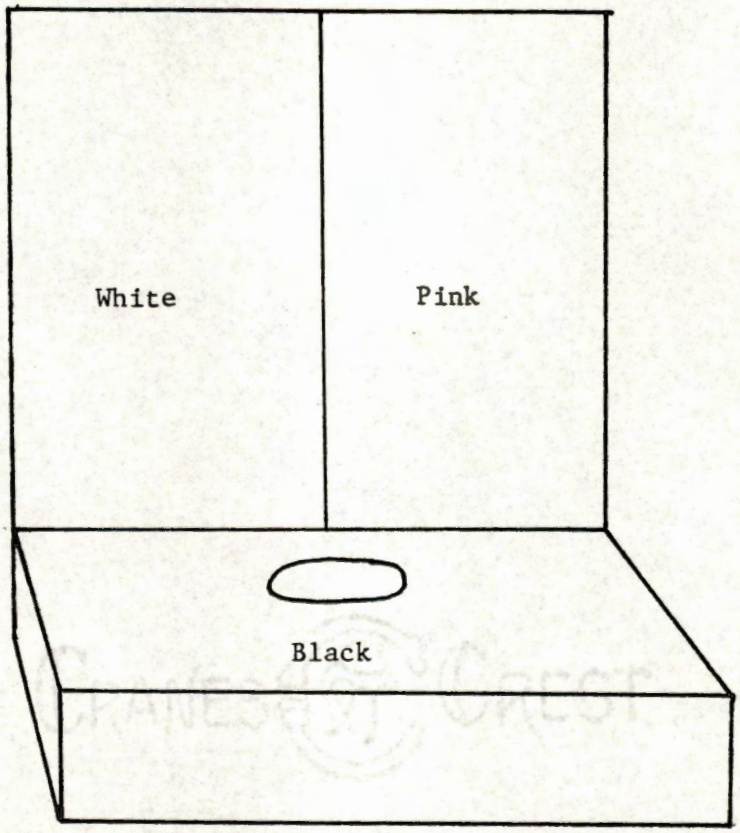

Figure 1. Illustration of color comparator used in field test.

The color of the two enamels was measured using a Hunterlab Digital Color-Difference Meter (D2502 D/M) with a white ceramic tile as the reference. Reference values of the ceramic tile used to standardize the instrument are: L, 93.4; a, -1.10; and b, +1.90. The CIE,[1] x, y, and Y values of the two enamel standards were determined and then converted to the Munsell color notation (3).

Procedure for Field Test

To conduct the field test for acidity with a breakpoint of 0.17% TA, one adds 7.5 ml of .10 N NaOH to a calibrated test tube using a Nafis titrator. One then fills the test tube to the calibrated mark with the milk sample and adds 12 drops of phenolphthalein. The tube is capped and gently inverted several times to mix the contents. The tube is then placed in the base of the color comparator. If the sample remains white, the sample is rejected because its acidity is above the desired acidity of 0.17%. If the sample is pink, it is accepted since its acidity is equal to or less than 0.17%.

Lactic Acid Determination

Fresh samples of milk were obtained and their TAs determined. The samples were stored in a 50°F incubator for several days and sampled periodically. The TA of each sample was measured before the samples were frozen for later use. Samples covering a range of acidities were selected from those frozen so that the developed lactic acid could be correlated with the increase in titratable acidity.

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[1] CIE = Commission Internationale de l'Eclairage.

The samples were prepared by liberating the lactic acid from a 50 g milk sample with sulfuric and phosphotungstic acid following AOAC method 15.008 (18). Fifty milliliters of filtrate were extracted with reagent grade anhydrous ether in a Bidwell continuous extractor according to AOAC method 15.012 (18). The extraction was allowed to continue at a rapid boil for 3 hours. The ether was driven off in a Buchler rotary evaporator at 45°C and 30" Hg. The lactic acid was then esterified with 2 ml of boron trifluoride-n-propyl alcohol on a steam bath for 10 minutes following the procedure of Salwin and Bond (23). The sample was allowed to cool before the addition of 4 ml of saturated $(NH_4)_2SO_4$ solution, 1 ml of acetophenone standard solution (1.6 mg acetophenone/ml n-propyl alcohol, 2.4 mg/ml, 3.2 mg/ml, 4.8 mg/ml, depending on initial acidity) and 2 ml of $CHCl_3$. The contents of the flask were then swirled and poured into a 30 ml separatory funnel. The separatory funnel was stoppered and shaken for 1 minute, then allowed to partition into two layers. The bottom one was discarded. The top layer was transferred to a screw-capped glass vial containing 3 g of anhydrous Na_2SO_4. The sample was stored in the refrigerator if it were not analyzed immediately.

To determine the quantity of lactic acid present, 3 µl of sample were injected in triplicate into a F & M Model 810 gas chromatograph to which was attached a Dohrman recorder equipped with a Disc integrator.

A 6-foot-by-1/8-inch stainless steel column was packed with 10% diethylene glycol succinate (DEGS) on 80-100 mesh Gas Chrom Q. The conditions used were as follows: column temperature, 200°C; detector temperature, 200°C; nitrogen flow rate, 80 ml/minute; hydrogen flow

rate, 37 ml/minute; air flow rate, 400 ml/minute. The electrometer sensitivity was adjusted to give peaks easily integrated.

Acetophenone (AP) was used as an internal standard. The concentrations of AP used ranged from 1.6 mg AP/ml of n-propyl alcohol to 4.8 mg AP/ml of n-propyl alcohol. The concentration used depended on the titratable acidity of the milk sample with the lower acidities corresponding to the lower concentrations of AP.

The following formula was used to determine the percent lactic acid present in the 50 g milk sample:

$$\% \text{ LA} = \frac{K\left(\frac{\text{mg of AP}}{\text{ml}}\right)\left(\frac{A_{\text{LA Peak}}}{A_{\text{AP Peak}}}\right)\left(\frac{\text{9 ml original solution}}{\text{3.53 ml final solution}}\right) \text{X } 100}{\text{50 g milk X 1000 mg/g}}$$

Relative Response Factor and Recovery of Lactic Acid

The relative response factor "K" between lactic acid and acetophenone was established using calcium lactate pentahydrate. Calcium lactate pentahydrate (1.712 g) was dissolved in distilled water and diluted in a 100 ml volumetric flask to give a lactic acid concentration of 10 mg/ml. Solutions containing 10 mg, 30 mg, 50 mg, and 70 mg/ml were made by adding 1, 3, 5, or 7 ml, respectively, of the 10 mg/ml lactic acid solution to a round-bottom flask. The solution was dried using a flash evaporator. This obtained 10 mg, 30 mg, 50 mg, or 70 mg of lactic acid in the final derivatized solution. The samples were then drivatized following the procedure previously described (23). Forty milligrams of acetophenone were used as an internal standard for all four samples. Aliquots were injected into the gas chromatograph in triplicate.

The area under the peaks was measured with the Disc integrator and the response factor calculated using the following formula:

$$K = \frac{\frac{Wt_{LA}}{Wt_{AP}}}{\frac{A_{LA}}{A_{AP}}}$$

An analysis of variance was performed on the K values to determine if they were significantly different over a range of relative concentrations. They were not significant at the 1% confidence level. Therefore, the mean of the K values was used to calculate the percent lactic acid in the milk samples.

Another set of samples was prepared in which .05 g, .10 g, and .15 g of lactic acid each were added to 100 ml of fresh milk with an acidity of .16% to give samples with a "high" TA. Fifty grams of the milk were extracted and derivatized in duplicate following the procedure previously described. These were then injected into the gas chromatograph in triplicate and used to establish the recovery of lactic acid.

CHAPTER IV

RESULTS AND DISCUSSION

The practice of rejecting milk with high titratable acidities due to the presence of lactic acid was most common among condenseries during the first half of this century. This was done to guard against losses as a result of coagulation on sterilizing evaporated milk (24). This practice worked well when milk was brought to the condensery in cans. Now that the bulk tank milk trucks are used, the milk with unacceptably high TA is mixed at the farm with the quality milk before either is tested for acidity. The manufacturer then has the choice of using milk of inferior quality or disposing of the entire truck load of milk (28). Either choice is costly. To aid the manufacturer, the field test for titratable acidity was developed for the milk hauler to use at the point of pick up. The test works on an acceptance-rejection principle. The test tubes in the kit were calibrated to accept acidities below a certain percent and reject those above that percentage.

The concept of the field test is based on the standard titration procedure used for acidity. The field test is easier to perform and more convenient to use than the standard titration procedure. The difference is that in the field test not only a set volume of sample and phenolphalein is used, but also a set volume of base is used. It does not determine the actual acidity; it determines whether the acidity is above or below a certain acidity based on the color of the final solution. If a pink color persists, then the milk is below the desired

acidity. If it remains white, then the milk is above the desired acidity and should be rejected.

For the survey of titratable acidity, 192 samples were collected. These samples were also used to test the field test. The samples were collected in two seasons, winter and summer. They consisted of both manufacturing and Grade A milk. For the winter samples, the breakpoint of the field test was established at 0.18% acidity. At this time, the safety factor of .01% TA was not allowed for in calibrating the test tubes. It was, however, accounted for in the summer samples. This idea is recommended because it ensures that a borderline sample will not be mistakenly rejected. Also, for the winter samples only four drops of phenolphthalein were added to each test tube. This caused problems when the acidity was close to the breakpoint because the pink color was very faint. For the summer samples, the phenolphthalein was adjusted to 12 drops per tube. This is the correct proportion needed to correspond to the standard titratable acidity test. It also alleviated the problem encountered in the winter samples of distinguishing the pink color.

In developing the kit for the field test, the pink color of phenolphthalein in the standard titration procedure was used as a color standard. A sample of fresh milk and a titrated sample of milk were taken to a paint store where the color of each was matched by professionals using color tint charts. These enamels were then used on the back of the test kit as a color standard.

The results of the survey of titratable acidities and the field test are shown in Tables 1 through 5. The tables are divided according to season, grade, and breakpoint. The (+) indicates an acidity below

Table 1. Field test results, Grade A winter samples.

Titratable Acidity[a]	Field Test[b]	Titratable Acidity	Field Test
-----%----		-----%----	
.16	+	.13	+
.17	+	.18	-
.17	+	.15	+
.17	+	.15	+
.16	+	.15	+
.17	+	.16	+
.15	+	.16	+
.18	-	.16	+
.25	-	.15	+
.15	+	.15	+
.17	+	.15	+
.17	-*	.17	-*
.20	-	.16	+
.17	+	.16	+
.17	+	.18	-
.15	+		
.16	+		
.18	-		
.17	-*		
.15	+		
.20	-		
.14	+		
.15	+		
.15	+		
.15	+		

*Samples rejected with TA at or below breakpoint.

[a]Means of duplicates.

[b]Tubes calibrated at 0.18% TA, 4 drops phenolphalein.

Table 2. Field test results, manufacturing grade, winter samples.

Titratable Acidity[a]	Field Test[b]	Titratable Acidity	Field Test
-----%----		-----%----	
.15	+		
.17	+	.13	+
.13	+	.17	-*
.15	+	.17	+
.17	+	.18	-*
.18	-*	.14	+
.16	+	.15	+
.16	+	.16	+
.16	+	.20	-
.18	-*	.16	+
.17	+	.16	+
.31	-	.13	+
.16	+	.17	-*
.18	-*	.16	+
.16	+		
.18	-		
.14	+		
.18	-		
.15	+		
.23	-		
.15	+		
.18	-*		
.16	+		
.16	+		

*Samples rejected with TA at or below breakpoint.

[a]Means of duplicates.

[b]Tubes calibrated at 0.18%, 4 drops of phenolphthalein.

Table 3. Field test results, manufacturing milk, summer samples.

Titratable Acidity[a]	Field Test[b]	Titratable Acidity	Field Test
-----%----		-----%----	
.18	+	.15	+
.15	+	.14	+
.15	+	.14	+
.15	+	.19	-*
.15	+	.12	+
.17	+	.17	+
.16	+	.13	+
.15	+	.22	-
.17	+		
.16	+		
.17	+		
.15	+		
.15	+		
.16	+		
.23	-		
.11	+		
.17	+		
.20	-		
.16	+		
.15	+		
.15	+		
.15	+		
.15	+		
.15	+		
.24	-		
.17	+		
.13	+		

*Samples rejected with TA at the established breakpoint.

[a]Means of sample duplicates.

[b]Tubes calibrated at 0.19% TA, 12 drops phenolphthalein, allowed for 0.01% TA error.

Table 4. Field test results, Grade A summer samples.

Titratable Acidity[a]	Field Test[b]
---------%--------	
.17	+
.14	+
.16	+
.17	+
.17	+
.18	+
.17	+
.17	+
.16	+
.16	+
.16	+
.17	+
.17	+
.15	+
.15	+
.16	+
.15	+
.15	+
.16	+

[a]Means of duplicate samples.

[b]Tubes calibrated at 0.17% TA, 12 drops phenolphalein, allowed for 0.01% TA error.

Table 5. Field test results, manufacturing milk, summer samples.

Titratable Acidity[a] -----%----	Field Test[b]	Titratable Acidity -----%----	Field Test	Titratable Acidity -----%----	Field Test
.11	+	.20	-	.15	+
.16	+	.21	-	.16	+
.20	-	.17	+	.15	+
.17	-*	.18	-	.15	+
.15	+	.15	+	.15	+
.16	-*	.16	+	.16	+
.14	+	.15	+	.15	+
.17	-*	.15	+	.15	+
.16	+	.15	+		
.24	-	.15	+		
.19	-	.15	+		
.19	-	.16	+		
.16	+	.16	+		
.14	+	.15	+		
.18	-	.17	+		
.18	-	.18	-		
.18	-	.14	+		
.19	-	.15	+		
.18	-	.17	+		
.15	+	.17	+		
.15	+	.16	+		
.15	+	.16	+		
.15	+	.14	+		
.16	+	.14	+		
.16	+	.15	+		
.18	-	.17	+		
.17	+	.17	+		

*Samples rejected with a TA at or below the established breakpoint.

[a]Means of sample duplicates.

[b]Tubes calibrated at 0.17% TA, 12 drops of phenolphthalein, allowed for 0.01% TA error.

or equal to the established breakpoint. The (-) indicates an acidity above the breakpoint.

Five samples from the winter survey which had a TA below or equal to the breakpoint failed to indicate this using the field test (Tables 1 and 2). These samples were all borderline samples that were probably affected by the small amount of phenolphthalein used. In the summer samples (Tables 3, 4, and 5), one sample was rejected with a TA below the established breakpoint and three with TAs at the breakpoint.

An analysis of variance (Table 6) was conducted to determine the difference in acidities among the four groups of samples. There was no significant difference among the groups at the 95% confidence level. The means, ranges, and standard deviations of the four groups of samples are found in Table 7. The acidities in the survey were lower than anticipated. There were only a few samples which were questionable as to their freshness, for example, a .31% found in the winter survey of manufacturing grade milk. This is indicative of the high quality of both Grade A and manufacturing milk in the market sampled. It also shows the desirability of a field test to maintain this quality and prevent the mixture of poor quality milk with quality milk in the haulers' bulk tank trucks.

The second half of this study included the determination of lactic acid actually present in milk over a range of acidities. A gas chromatographic procedure developed by Salwin and Bond (23) to determine the amount of lactic and succinic acids in eggs was adapted for milk. The lactic acid in a 50 g milk sample was ether extracted following AOAC method 15.012 (18). The ether was evaporated, and 9 ml of various

Table 6. Analysis of variance of the titratable acidity results.

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Cal.
Groups	3	.00081	.00027	.49091[ns]
Within	188	.10246	.00055	
Total	191	.10327		

[ns]Nonsignificant.

Table 7. Means, ranges, and standard deviations of the titratable acidity of the four groups of milk samples.

Season	Grade	Mean	Range	Standard Deviation
			% TA	
Winter	Grade A	0.164	0.13-.25	0.020
	Manufacturing	0.168	0.13-.31	0.031
Summer	Grade A	0.162	0.14-.18	0.010
	Manufacturing	0.162	0.11-.24	0.023

solutions were added to the dried lactic acid (2 ml of BF_3-n-propyl alcohol, 4 ml of saturated $(NH_4)_2SO_4$, 1 ml of acetophenone solution, 2 ml $CHCl_3$). Of the 9 ml, only 3.53 ml remained as the top layer when the solution was shaken and allowed to separate in a 30 ml separatory funnel. This top layer contained all of the lactic acid and acetophenone. The bottom layer containing the $(NH_4)_2SO_4$ and chloroform was discarded. Therefore, the concentration of the acetophenone and lactic acid must be considered as having partitioned into the propanol layer. The partition ratio, 9 ml/3.53 ml was introduced into the calculation to account for the actual concentration of acetophenone in the solution injected into the gas chromatograph.

The following equation was used to determine the concentration of lactic acid in the original sample:

$$\% \text{ LA} = \frac{K\left(\frac{\text{mg AP}}{\text{ml}}\right)\left(\frac{A_{LA}}{A_{AP}}\right)\left(\frac{\text{9 ml starting solution}}{\text{3.5 ml final solution}}\right) \text{ X } 100}{\text{50 g milk X 1000 mg/g}}$$

The K value was established by using known weights of lactic acid and acetophenone. The K value was determined by the following equation:

$$K = \frac{\frac{Wt_{LA}}{Wt_{AP}}}{\frac{A_{LA}}{A_{AP}}}$$

The K values were determined over a range of concentrations of lactic acid--10 mg, 30 mg, 50 mg, 70 mg/ml.

In the procedure, injections into the gas chromatograph for each trial were done in triplicate. An analysis of variance was conducted to determine if the K values obtained were significantly different. The data are shown in Table 8 and the analysis in Table 9. It was determined that they were not significantly different at the 1% confidence level. The K values obtained were then averaged to obtain 1.868 as the response factor to be used to determine the percent lactic acid present in the milk.

A simple linear regression of the weight of lactic acid compared to the peak area is found in Figure 2 and the analysis of variance in Table 10. Although there was a low significance for higher order regression, the function was fit to a linear equation since the linear coefficient of determination is $r^2 = .9915$, and the correlation coefficient is $r = .9957$. The regression equation was $Y = 79.433 + 15.76X$, where Y is peak area and X is lactic acid (mg).

Recovery data were obtained using fresh milk to which lactic acid was added in amounts thought to be representative of those encountered in milk held under adverse conditions. Enough reagent grade lactic acid was added to the milk to raise the TA from 0.05 to 0.15% TA. The determination was done in triplicate with triplicate injections into the gas chromatograph. Table 11 shows the recovery of the triplicate determinations. Acetophenone was used as an internal standard. An analysis of variance of the data is found in Table 12, and a linear regression of the percent lactic acid recovered compared to the weight of added lactic acid is found in Figure 3. The correlation coefficient between lactic acid recovered and lactic acid added was .9975.

Table 8. "K" values for lactic acid determination.

Lactic Acid mg/ml	"K" Values		
	1	2	3
10	1.1985	1.1579	1.3333
30	1.2205	1.2459	1.2688
50	1.3333	1.2459	1.2533
70	1.0148	.9607	1.0084

Table 9. Analysis of variance of "K" values.

Source	df	Sum of Squares	Mean Squares	F Ratio
Concentrations	3	0.151221	0.050407	1.73^{ns}
Injections	2	0.008176	0.004088	0.14^{ns}
Error	6	0.174931	0.029155	
Total	11	0.334328		

^{ns}Nonsignificant.

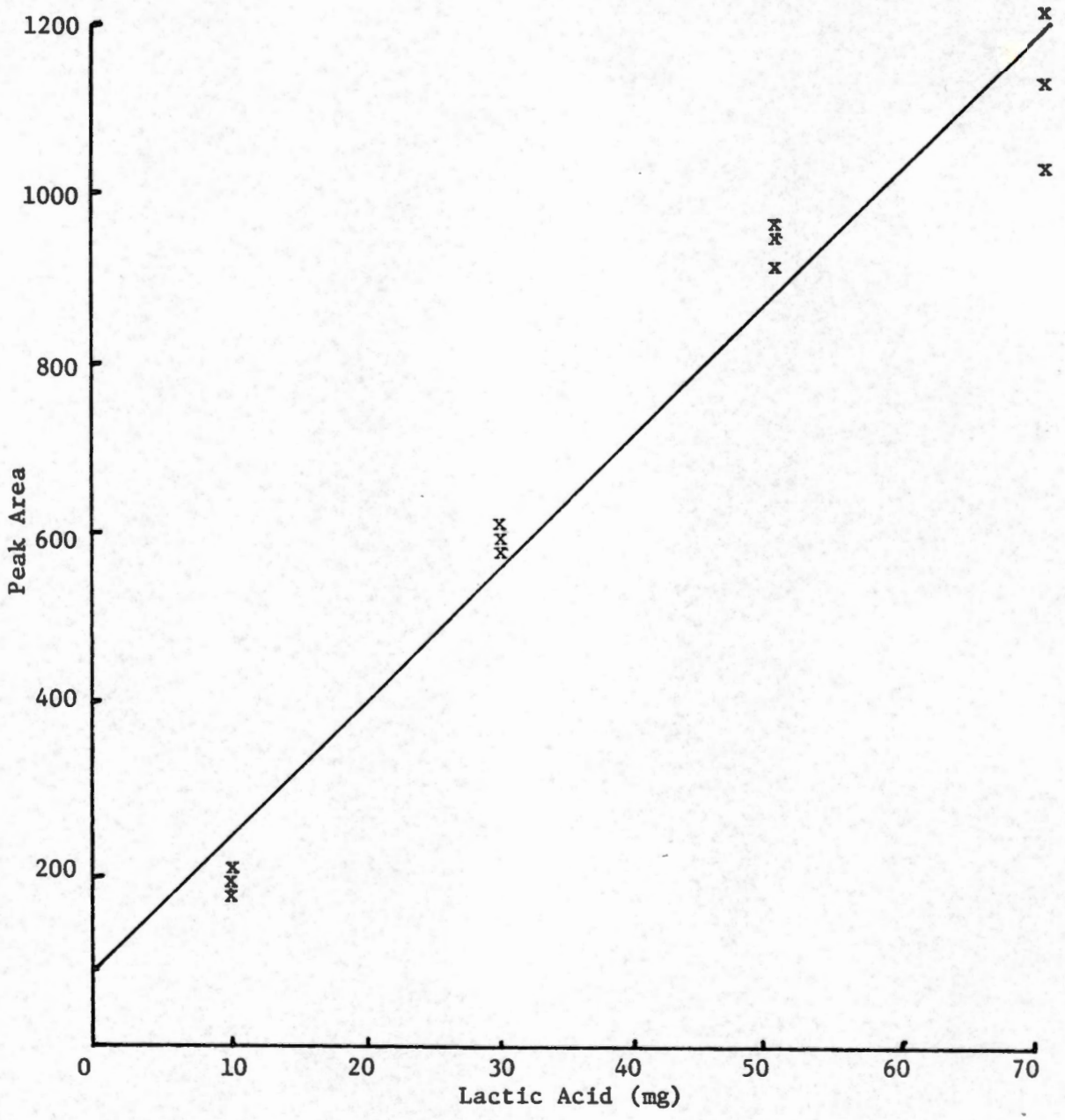

Figure 2. The relationship of peak area to weight of lactic acid by gas chromatography.

Table 10. Analysis of variance for regression of the peak area of lactic acid compared to weight.

Source	df	Sum of Squares	Mean Squares	F Ratio
Level of lactic acid	3	1,519,011	506,337	229.76***
Linear regression	1	1,490,265.6	1,490,266	676.23***
Higher order regression	3	28,745.4	14,343	6.52*
Error	8	17,630.2	220.4	
Total	11	1,536,641.2		

***$p < 0.0001$

*$p < 0.05$

Table 11. Recovery of lactic acid added to milk.

Lactic Acid mg/ml	Lactic Acid Recovered		
	1	2	3
	%		
.05	.0747	.0681	.0654
.10	.1270	.125	.138
.15	.1770	.181	.170

Table 12. Analysis of variance on recovery data of lactic acid when added to milk.

Source	df	Sum of Squares	Mean Squares	F Ratio
Level of lactic acid	2	.01715192	.0085755	243.95***
Linear regression	1	.0166037	.0166037	472.32***
Higher order regression	1	.0005482	.0002741	16.60**
Error	6	.00021092	.00004218	
Total	8	.01736284		

***$p < 0.0001$

**$p < 0.01$

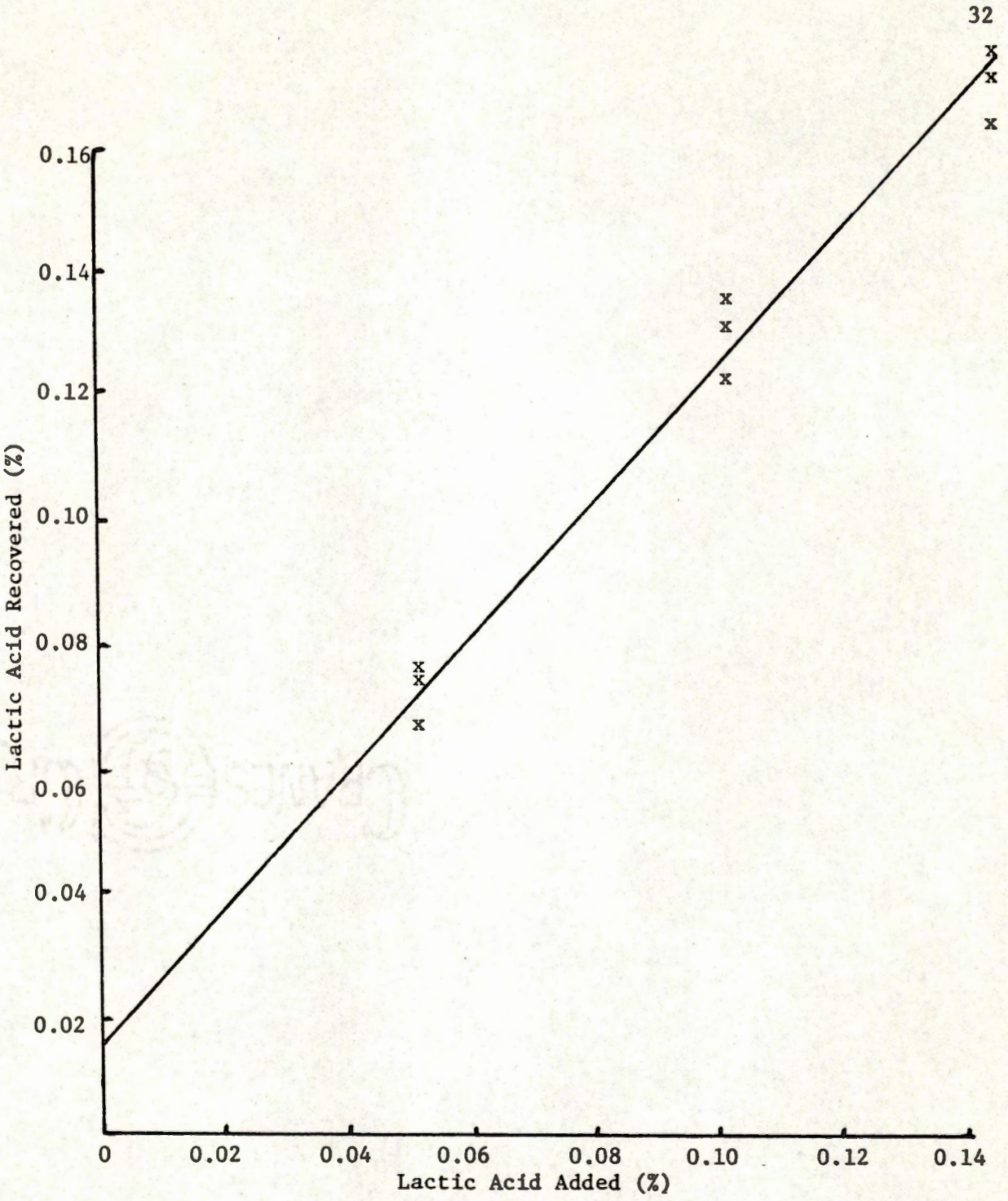

Figure 3. Recovery of lactic acid added to milk by gas chromatography.

Both sets of data, Tables 10 and 12, were presented as linear functions in Figures 2 and 3, even though there was significant higher power variation at a low order of significance. The high values of the correlation coefficient appeared to justify such an interpretation.

Two fresh milk samples with titratable acidities of .14% and .17% were stored in a 50°F incubator and sampled periodically over several days. The titratable acidity was measured in each sample and then was frozen for later use. The samples with developed lactic acid along with a fresh milk sample were extracted, derivatized, and injected into the gas chromatograph. The area of the peaks was determined and the percent lactic acid calculated. A gas chromatogram of a sample with 0.24% TA due to developed acid with an internal standard of 16 mg of acetophenone per milliliter is shown in Figure 4. The percent of lactic acid recovered was 0.0892%. In the chromatogram, there is an extra unidentified peak. It is presumed that this peak represents another short chain acid which developed over time as did the lactic acid. This acid contributes to the overall developed acidity of the milk. The percent developed TA in Table 13 is, therefore, not due totally to the development of the lactic acid.

Table 13 contains the data from the recovery of lactic acid developed over time in a 50°F incubator. It also lists the percent acidity due to the development of lactic acid and the unidentified acid. This value was obtained by subtracting the developed TA from the original TA.

The percent lactic acid recovered is within the range recovered when lactic acid was added to fresh milk (0.0654 to 0.181%). There was one sample that did not fall within the range; it fell below the range.

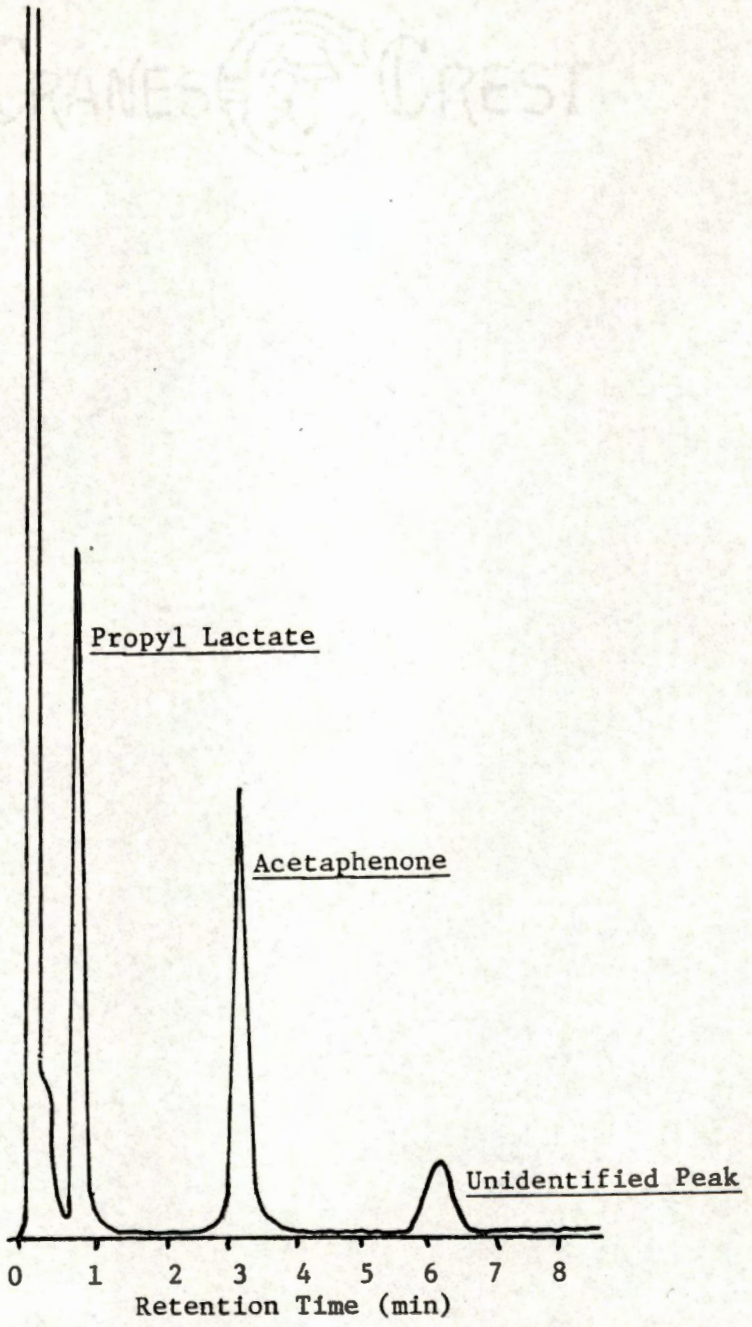

Figure 4. Gas chromatogram of a sample of milk with a developed acidity of 0.24%.

Table 13. Results of lactic acid recovery from milk samples allowed to develop acidity over time.

Original TA of Milk	TA after Incubation	TA Due to Developed Acidity	Lactic Acid Recovered
		%	
.14	.19	.05	.0327
.14	.19	.05	.0513
.14	.20	.06	.060
.14	.20	.06	.0655
.14	.22	.08	.075
.14	.22	.08	.0836
.14	.24	.10	.0892
.14	.24	.10	.0826
.14	.26	.12	.1041
.14	.26	.12	.1003
.17	.20	.03	.0124
.17	.20	.03	.0094
.17	.23	.06	.0634
.17	.23	.06	.063
.17	.26	.09	.0705
.17	.26	.09	.0648

The two fresh milk samples with TAs of 0.14% and 0.17% showed extremely small peaks at the lowest attenuation possible when injected into the gas chromatograph. Their peak area was considered to be non-significant. From this, it was concluded that there was no lactic acid present in fresh milk.

CHAPTER V

SUMMARY AND CONCLUSION

A field test for titratable acidity was developed for milk haulers to use at the point of pick up. The test is based on the standard test for titratable acidity. The field test is convenient to use at the farm, whereas the standard test for titratable acidity is not. The major difference between the two is that the field test uses not only a set volume of milk and phenolphthalein but also a set volume of base. It does not determine the specific acidity of a milk sample; rather, it determines whether the acidity of the sample is equal to or less than a chosen acidity. This is done by a color difference. Those samples which remain pink have acidities equal to or less than a chosen acidity; those which remain white have an acidity above the desired acidity. Tubes can be calibrated to accommodate any chosen level of titratable acidity by an acceptance-rejection breakpoint using a simple proportionality equation.

The field test is to be used to prevent the quality reduction of an entire tank by one farmer's unacceptable milk. It is not to be used to penalize farmers whose herds produce milk with a high natural acidity.

A procedure was adapted for milk to determine the actual amount of lactic acid present using gas chromatography. The procedure seems to obtain good results. Using this procedure, it was shown that there was no lactic acid present in samples of fresh milk with natural TAs of 0.14% and 0.17%. From this, it was concluded that milk with high natural acidity should not be rejected on the basis of a titratable

acidity test. However, since most milk is stored in bulk tanks before being transported to the dairy plant, the high natural acidity of one cow's milk is usually averaged due to a low natural TA of another cow's milk. This maintains a fairly uniform TA in the milk sampled by the milk hauler. Therefore, the field test for acidity can be used to detect milk with developed acidity due to lactic acid.

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