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The Hydrogen Peroxide Catalase Treatment of Milk for Swiss Cheese Manufacture

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THE HYDROGEN PEROXIDE CATALASE TREATMENT OF MILK FOR SWISS CHEESE MANUFACTURE

by

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Theodore R. Kowallis

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SUMMARY

Various temperatures and concentrations of the hydrogen peroxide catalase treatment of milk were studied in an attempt to discover the optimum temperature and concentration that would destroy unfavorable organisms and yet allow favorable ones to grow. The Federal Food and Drug Administration in their November, 1959, Definitions and Standards of Cheeses and Cheese Products states that "the amount of the hydrogen peroxide solution used shall be such that the weight of the hydrogen peroxide added thereby does not exceed 0.05 percent of the weight of the milk treated." Within the maximum level allowed, it was found that in day old manufacturing milk treated with 0.05 percent peroxide for 10 minutes at either 32 C or 49 C, more than 64 percent of all micro-organisms present were destroyed. Coliform organisms were very sensitive to peroxide, exhibiting a 92 percent kill at 32 C, and a 100 percent kill at 49 C. Lactic acid producing organisms were next in sensitivity to bacterial destruction, showing an 80 and 83 percent kill respectively for the same temperatures. Spore-forming organisms showed a 42 and 73 percent kill respectively at the above temperatures, but due to the refractory nature of spores to peroxide, the difference was not significant (p .05).

In addition to the foregoing, related studies were conducted on the hydrogen peroxide treatment of milk without the addition of catalase. Storage milk was treated over night at 4 C for 16 hours with two peroxide concentrations, 0.025 and 0.05 percent. At the lower concentration the kill was not satisfactory, while at the higher concentration 50 percent of the

spore-formers and 99 percent of the coliforms were killed. There was, however, a peroxide residue which would have to be eliminated to meet Federal Food and Drug Administration standards.

INTRODUCTION

Hydrogen peroxide has gained importance in the production of Swiss cheese because of its excellent germicidal properties. It would be beneficial to the cheese industry to know exactly what concentration of hydrogen peroxide to use in order to destroy unfavorable organisms and allow some of the favorable ones with the enzymes to survive. Also, it would be of importance to develop new procedures using hydrogen peroxide to reduce the cost or time involved in making Swiss cheese. This method of milk treatment may give greater uniformity in the quality of Swiss cheese.

Purpose

It is, therefore, the purpose of this study to reach the following objectives:

1. To determine the smallest effective concentration of hydrogen peroxide which will destroy spore-formers, coliforms, and other undesirable organisms and yet permit survival of a large percentage of favorable lactic acid producing organisms.

2. To adapt a technique and procedure in treating milk with hydrogen peroxide in order to reduce the cost of the process through the reduction of time, temperature, and the amount of materials used.

3. To ascertain the types and relative percentages of organisms which survive the various peroxide treatments.

REVIEW OF LITERATURE

Swiss cheese originated, as the name implies, in Switzerland. It is called Emmentaler after the Emmen Valley in the Canton of Bern. It is one of the oldest varieties of hard rennet cheese and was known to be exported from Switzerland as early as 1650 (Curren, Evans and Leviton, 1940). In the United States commercial production began in Ohio in the 1860's and has since expandedtto other states, notably Wisconsin, New York, Pennsylvania, Wyoming, Utah and Idaho (Fernou, 1923).

Hydrogen peroxide was first brought to the attention of the scientific world by the French chemist Thenard (1819). His discovery was brought about by the combined use of hydrochloric and sulphuric acid reacting upon barium peroxide to produce hydrogen peroxide and barium sulfate. He recognized the value of this discovery and later presented it in a paper to the French Academy of Science.

Jablin and Gonnet (1901) used hydrogen peroxide in the preservation of milk and found that consumption of the treated milk produced no noticeable ill effects.

Budde (1903) presented a new method of treating milk called "buddized" milk. This process consisted of heating the milk to 50 C and then adding enough hydrogen peroxide to give a final concentration of from 0.03 to 0.035 percent. After stirring the milk for 15 to 30 minutes it was poured into tightly stoppered bottles, held in a 50 C water bath for two or three hours, cooled and placed on the market.

Much and Romer (1906) experimented with the use of hydrogen peroxide and a catalase containing material as a means of sterilizing milk.

They soon discovered, however, that the impurities associated with the catalase materially limited the usefulness of the two chemicals. This particular method for treating milk could not be utilized to any extent in any part of the world for the next several years because the chemical industry was not yet able to supply peroxide and catalase of high stability, high concentration, and of such a degree of purity as public health authorities required for chemical products used in food (Rifaat, 1950).

Matheson, Boyer, and Warren (1927) studied the effects of different forms of oxygen in the treatment of milk to check gassy and other abnormal fermentations in Swiss cheese. They reported that ozone and oxygen have similar effects in checking gassy fermentation in Swiss cheese caused by spore-forming anaerobes. The oxygen treatment brought favorable results by checking "nissler" fermentation. The action of oxygen seemed germicidal as well as inhibitory.

Curran, Evans and Leviton (1949) experimenting with the action of peroxide and crystalline catalase, found that the germ killing activity of peroxide is greatly influenced by the temperature at which it reacts.

From their results, it is apparent that even high concentrations of hydrogen peroxide cannot be relied upon to kill all of the spores in a culture, because a time lag of variable length occurs between the addition of hydrogen peroxide and its measurable effects on most spores. They further state that the growth of S. lactis was retarded, even up to 48 hours, in milk which had received the hydrogen peroxide-catalase treatment. This was in all probability due to the unfavorable oxidation-reduction potential.

They further conclude that temperature, pH, concentration of reagent, and concentration of exposed organisms are important factors influencing the sporicidal activity of hydrogen peroxide. In neutral

solutions the sporicidal action of peroxide tended to increase with rising temperatures; however,an exception to this rule has been noted. The influence of pH seemed to be closely correlated with its effect upon the stability of the peroxide, hence in acid solutions the sporicidal activity was enhanced while alkaline reactions which promote its decomposition tended to reduce the germicidal activity of peroxide. Organic matter, apart from its possible catalase content, seemed to have comparatively little influence upon the germicidal activity of peroxide.

Robertson, Roper and Bauer (1941) did work on the degradation of mucins and polysaccharides by ascorbic acid and hydrogen peroxide. They found that ascorbic acid and hydrogen peroxide react to cause the degradation of fluid mucin. This degradation involved a breakdown of the micro-molecules without the liberation of detectable amounts of reducing substances or amino-sugars. They also reported that the ascorbic acid hydrogen peroxide system acted on gastric and salivary mucins as well as on polysaccharides such as starch, protein, flaxseed mucilage and the polysaccharide of synovial mucins and cartilage. It also destroyed the capsules of various types of pneumococci. This system caused no change in vomucin, agar-agar of gelatin. It did however, cause a dephosphorylation of B-glycerophosphate (Rifaat, 1950).

Payne and Foster (1945) carried out a quantitative investigation on the action of hydrogen peroxide on glyceric aldehyde, erythritol, d-arabinose, d-glucose, and sucrose. They found that hydrogen was a characteristic reaction product in every case. A satisfactory reaction mechanism placed the origin of the hydrogen in formaldehyde, produced in the oxidative degradation of the compounds.

Glyceric aldehyde undergoes a dismutation reaction in the presence

of low concentrations of hydrogen peroxide.

At Linate, Italy, near Milan, in 1945, an electrolytic plant produced 39 percent hydrogen peroxide of high purity. This product was utilized for the treatment of milk in the Milan area as a substitute for pasturization. According to the investigation of Drs. L. Morandi and Squatrite, extensive laboratory studies had indicated its suitability for this purpose. It was indicated by the investigation that this practice would be expanded when production could be increased. A "solid" hydrogen peroxide of 35 percent strength plus 65 percent urea also had been developed at the same time for milk treatment (Department of Commerce, 1945).

Brown (1947) working with a modified peroxide treatment (a glycerite of hydrogen peroxide) found that when tested by a modified cylinder plate method, peroxide-glycerol solutions, made from either urea peroxide or hydrogen peroxide, showed bacteriostatic action on both gram-positive and gram-negative organisms. A greater bacteriostatic effect was noted with gram positive rather than gram negative bacteria.

In comparison with 12 mercurial solutions, the glycerol-peroxide solutions showed, in general, greater bacteriostatic action on gram-positive organisms than did the mercurial solutions. The latter were, in general, the more effective on gram-negative bacteria. In specific cases, however, the peroxide-glycerol solutions proved more efficacious than some of the mercurial solutions, particularly when water was the principal solvent for the mercurial compound.

Brown and Slanitz (1947) showed that a glycerite of hydrogen peroxide healed "cold abscesses" in tuberculosis patients when applied in wet dressings.

Wyss et al. (1948) discovered in experiments on irradiated broth

that there is a marked similarity between certain biological effects produced by ultra-violet irradiation of nutrient broth and by the addition of hydrogen peroxide to the broth. It was also found that the effects of both can be negated by catalase. This work further confirmed work done by Fernau (1923) when he concluded that the results of a treatment with Roentgen rays, ultra-violet, and alpha particles on albumin solutions were identical with those produced by peroxide.

From 1947 to the present time considerable interest has been directed toward the use of hydrogen peroxide in the dairy industry by Morris (1948-1960), who has published articles (1950, 1951) and directed research on three theses on this subject (Johnson, 1952; Nagmoush, 1949; Rifaat, 1950). Roundy also has done work in this field (1948-1950, 1958, 1959).

Nagmoush (1949) completed a thesis on the use of hydrogen peroxide in treating milk for making Cheddar cheese. He stated that the hydrogen peroxide treatment successfully reduced the bacterial count including coliforms, aerobic and anaerobic spore-former organisms. He also stated that treatment with hydrogen peroxide caused greater retention of moisture in the curd and the finished cheese. It was also noted that cheese treated with the peroxide catalase treatment seemed to ripen more rapidly than untreated cheese.

Rifaat (1950) made a study of the use of hydrogen peroxide as a substitute for pasteurization in market milk. He concluded that the use of a 0.20 percent solution showed a higher reduction than pasteurization in total bacterial count and aerobic spore-forming organisms. He also stated that this treatment destroyed anaerobic spore-formers entirely. Treated milk also seemed to hold up for longer periods of storage than did pasteurized milk. The peroxide-catalase treatment

appeared to retard the development of oxidized flavor in milk.

Johnson (1952) made a comparative study of raw, pasteurized and hydrogen peroxide treated milk in the production of Swiss cheese. He concluded that eye development, flavor, body and texture were all superior in the hydrogen peroxide treated cheese as compared to the raw and pasteurized cheese.

Teply et al. (1958) made composition and nutritional studies on cheese produced from milk treated with peroxide and catalase. The results of these studies indicate no marked changes in the composition or nutritional value of milk treated with 0.1, 0.2, and 0.5 percent hydrogen peroxide or in the cheese or whey obtained from such milk under the conditions described.

Jasiwicz and Porges (1959) in their work on whey preservation by hydrogen peroxide showed that peroxide addition to grossly contaminated wheys of 2.8×10^7 micro-organisms per milliliter resulted in a 97 percent bacterial kill within one hour. They also showed that the 0.02 percent hydrogen peroxide concentration was relatively ineffective against greater numbers of bacteria.

With these developments in the use of the peroxide-catalase treatment it is being successfully used as a tool in the Swiss cheese industry. Research is presently being done at the University of Wisconsin and other universities throughout the country to determine the effect of time of exposure and the temperature and concentration of hydrogen peroxide on various pathogenic organisms which have importance in the industry.

PROCEDURE

Selection and handling of milk

The milk used in preliminary studies on this experiment was obtained from the Utah State University Dairy Farm and from the Cache Valley Dairy Association. Both good and poor milk were used in the preliminary studies. It was soon found, however, that high quality raw milk could not be used because of the low number of micro-organisms present. As a result of the preliminary studies, only manufacturing milk was used for the final tests. This milk was obtained from mixed herd samples taken from plant storage tanks at the Cache Valley Dairy Association. This milk in all cases was held at least one day before use after arriving at the plant and being cooled to 4 C. The milk was collected in clean, well-tinned five gallon cans and held under refrigeration until used.

Preparation and treatment of the milk

In the first of three series of preliminary experiments each lot of milk was divided into six portions of 3000 cc each. These portions were heated to 49 C and immediately treated with 0.003, 0.005, 0.01, 0.03 and 0.06 percent hydrogen peroxide respectively. The treatment with hydrogen peroxide was applied by first heating the milk to 49 C in order to reduce the catalase enzyme normally present and thus increase the effectiveness of the hydrogen peroxide.[1] A control portion of milk was heated in like manner, but no hydrogen peroxide was added. The treated milk was agitated and held for 20 minutes at 49 C after which

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[1]Roundy (1948-1959) used this method in an attempt to increase the effectiveness of the hydrogen peroxide. Rogers et al. (1912) found that catalase was effectively destroyed with a flash heat treatment at 70 C.

it was cooled to 43 C or below in order to permit survival of the added catalase. The purified liquid catalase, of 100 keil units per ml, was added at the rate of one cc per 31.8 ml of hydrogen peroxide.[1] The milk was then cooled and agitated until the complete decomposition of hydrogen peroxide had taken place, usually within 10 minutes. The presence of hydrogen peroxide was checked by the Potassium Iodide Test. This test consisted of adding 5 cc of a 30 percent solution of potassium iodide solution to 10 cc of the treated milk (Morris, Larsen, Johnson, 1951). A brown colored milk indicated a positive test for hydrogen peroxide; a natural colored milk indicated a negative test. In running the Potassium Iodide Test, two test tubes were used, one containing treated milk plus catalase and the other containing the same plus potassium iodide solution. With this procedure a better comparison was made. As soon as a negative test was obtained bacteriological plates were run on the samples.

The first series of preliminary tests were performed on eight different lots of milk. Figure 1 illustrates the procedure on each lot.

The second series of preliminary tests were performed on five different lots of milk. The procedure in the second series was the same as for the first except that the concentrations were increased to 0.08, 0.10 and 0.12 percent.

The third series of preliminary experiments were run the same as the first two except that the concentrations of hydrogen peroxide were increased to 0.14, 0.16 and 0.18 percent with a control in every lot of five tested.

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[1]The hydrogen peroxide used was approximately a 35 percent solution of a purified edible grade manufactured by the E. I. DuPont de Nemours and Company, Electrochemical Division, Elmonte, California. Its brand name is "perone."

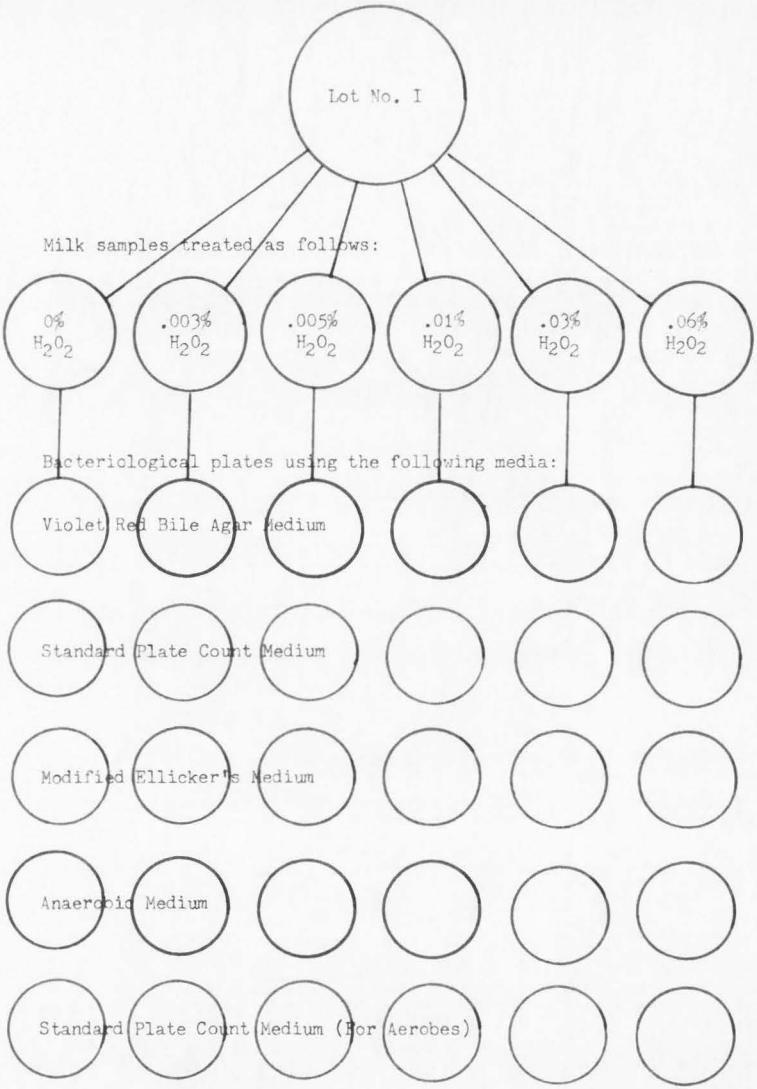

Figure 1. Plating procedure used on the preliminary lots in the first series

After the preliminary tests were completed and the results studied a practical application of the procedure was attempted at a nearby Swiss cheese plant. It was found that the application of hydrogen peroxide treatment accomplished work of organism destruction in approximately 10 minutes at the 0.18 percent level of concentration in a 25,000 pound Swiss cheese vat where the milk had been previously preheated to 49 C and cooled back to 32 C. It was also found that catalase added at the rate of 5 cc per pound of "perone" dissipated all hydrogen peroxide in the vat within 10 minutes after application was made, as shown by the Potassium Iodide Test. As a result of the preliminary studies and the practical application in a plant, certain changes were made for the final experiment.

In the final series of experiments four lots of manufacturing milk were used. Each lot of milk was divided into eight 100 ml samples and placed in 8 oz sterile screw-top bottles. In the first lot, four of these samples were heated in a water bath to 32 C. A theromometer was inserted in one sample to determine when it reached 32 C. Upon reaching this temperature the samples were removed, and two of them were treated with 0.02 percent hydrogen peroxide and another two with 0.05 percent hydrogen peroxide and then all four samples were thoroughly agitated and returned to a water bath which had been previously tempered to 32 C. Here they were held with agitation for 10 minutes after which the peroxide was dissipated with catalase. At this time, the samples were again agitated, and then held for 10 minutes and plated.

The other four samples of each lot were treated the same as the first except that they were heated to 49 C before adding hydrogen peroxide and were cooled to 43 C before adding catalase.

Eighty percent of the four lots of milk were treated in the above manner for three of the five groups of organisms tested. In the treatments for the other two groups of organisms (aerobic and anaerobic spore-forming) the remaining 20 percent of the four lots of milk used were heated in a steam injection water bath at 80 C for 10 minutes in order to destroy all of the vegatative and facultative organisms present. After this holding period, the samples were immediately cooled to 49 C. At this point half of these samples were treated with hydrogen peroxide, held with agitation for 10 minutes, and cooled to 43 C before adding catalase. The other half of these samples were cooled to 32 C and handled as the other lots in the final series.

Bacteriological tests on the milk

The milk was tested for five groups of organisms: coliforms, aerobic and anaerobic spore-formers, lactic acid producing organisms and standard plate count organisms. In the first series, these tests were plated according to Standard Methods for the Examination of Dairy Products (American Public Health Association, 1960) with the exception of the lactic acid producing organisms and the anaerobic spore-formers.

In the case of the lactic acid producing organisms, an agar culture medium recommended by Elliker (1955) was used, but with modification. The agar consisted of 20 grams tryptone, 2.5 grams gelatin, 5 grams sucrose, 5 grams yeast extract, 4 grams sodium chloride, 1.5 grams sodium acetate, 15 grams agar and water added to make 1000 ml. The modifications were as follows: The agar was buffered with $CaCO_3$ (calcium carbonate) at the rate of 0.4 percent or 4 grams/liter of agar. Two-tenths ml of a 1.6 percent solution of brom cresol purple was added. These modifications were to improve the readibility of the plates by buffering the acid zones produced by the lactic acid producing organisms

and thus retard them from spreading into one another. The dye indicator colored the agar purple, and the acid zones produced by the organisms were immediately changed to a clear yellow as the indicator was changed by the acid. These modifications facilitated a quick and accurate determination of the plate colony numbers after the incubation period.

The anaerobic organisms were plated in the following manner: Standard Baltimore Biological Laboratory anaerobic agar medium was used. Milk samples were first heat-treated to 80 C for 10 minutes to destroy the vegetative cells and then were plated with 10 cc of the medium. In the first series the procedure was modified by using regular petri dishes. After the initial 10 cc of medium was poured and innoculated the agar was allowed to solidify and an additional amount of agar was poured on its surface until each plate was nearly full. The agar was allowed to harden and the plates were inverted and incubated at 30 C from three to four days until the slow growing colonies were readily discernable. Plates were counted according to Standard Methods for the Examination of Dairy Products. After the first trial series was completed the results on the anaerobes proved inconclusive, so further tests were plated according to standard methods. After plating, the plates were evacuated and flooded with nitrogen gas before incubation.

RESULTS AND DISCUSSION

Results of this study are tabulated in table and graph form showing statistical analyses on each of the five groups of organisms tested as well as the percent kill at the various temperatures and peroxide concentrations. Milk samples in Tables 2 through 12 were held for 10 minutes after peroxide-catalase treatments before bacterial plate counts were made.

Table 1 shows the general trends or effects of increasing concentrations of peroxide on milk organisms. It is evident from the table that coliform organisms are most sensitive to the destructive effects of hydrogen peroxide. Lactic acid producing organisms and aerobic spore-forming organisms rank next in sensitivity to the treatment. The results are inconclusive regarding the spore-forming organisms. The organisms grown on the standard plate count medium showed a generally increased kill with an increase in percent of peroxide used.

Table 2 shows that the temperature had very little effect on bacterial destruction. Table 3 bears this out in the analysis of variance showing that the difference in temperatures is not significant. The interaction between temperatures and concentrations is also not significant ($p > .05$). It can be readily noted from Figure 2, that the concentrations of hydrogen peroxide have a highly significant ($p < .01$) effect on bacterial destruction.

Two possible reasons for this destruction are: First, it may be due to the coliform organisms present which are sensitive to the peroxide treatment; and secondly, the presence of lactic acid producing

Table 1. Preliminary series showing average percent kill at various concentrations of hydrogen peroxide[a]

	Coliform Count	Lactic Acid Producing Organisms	Standard Plate Count	Aerobic Plate Count	Anaeribic Plate Count
			Series I		
Total Raw Count[b]	556,000	2,271,000	2,367,000	3,175,000	1,000
.003% H_2O_2	100	57.16	31.82	58.52	
.005	99.7	53.28	40.90	53.64	
.01	100	76.49	73.43	83.66	99.92
.03	100	76.97	76.68	82.58	97.80
.06	100	99.38	99.32	98.02	99.98
			Series II		
Total Raw Count	714,000	4,400,000	15,860,000	23,200,000	28,300
.08	100	97.32	75.22	86.16	96.82
.10	100	97.27	95.65	97.28	99.29
.12	100	93.64	94.01	96.34	98.94
			Series III		
Total Raw Count		16,250,000	6,280,000	10,520,000	6,500
.14		98.03	99.57	97.43	46.16
.16		98.03	96.98	97.62	52.31
.18		99.14	96.18	99.98	53.85

a All organisms were treated with the stated amount of hydrogen peroxide and then held for 20 minutes before dissipation with catalase. The plates were incubated according to Standard Methods for the Examination of Dairy Products with the exception of the anaerobic spore-formers. (See preparation and treatment of the milk, page 8.)

b These counts are average figures taken from Appendix A.

organisms on the plate count medium would also influence the count because these organisms are sensitive to the peroxide treatment.

Table 2. Standard plate counts showing bacterial destruction with different concentrations of hydrogen peroxide at two temperatures

	Original Count 0% H_2O_2	.02% H_2O_2	Percent Killed	.05% H_2O_2	Percent Killed
32 C	2×10^7/ml[a]	2.23×10^6	89	1.1×10^6	94
49 C	1.85×10^7	2.56×10^6	86	4.6×10^5	98

a All figures are accumulated totals derived from four lots of milk with five replicate plates on each concentration and temperatures in each lot.
b Bacterial plate counts on nutrient agar incubated at 35 C for 48 hours.

Table 3. Analysis of variance on standard plate count organisms[a]

Source of Variation	d.f.	s.s.	m.s.	F
Replications	3	26,956		
Temperature	1	285	285	.13
Error (a)	3	6,750	2,250	13,419.53**
Concentrations	2	208,809	104,405.5	.85
Temp. x Conc.	2	723	361.5	
Error (b)	12	93,599	7,799.9	
Sampling	96	9,271	96.6	
Total	119	346,393		

** Highly significant at p .01.
a From data presented in Table 2.

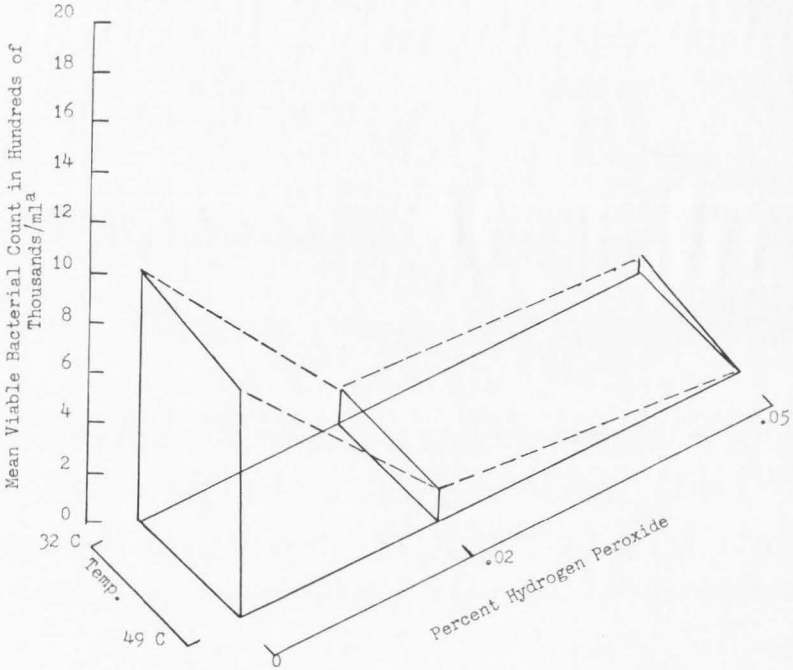

Figure 2. Comparison of mean standard plate counts showing a three dimensional representation of bacterial destruction in day old manufacturing milk using three concentrations of hydrogen peroxide and two temperature levels for 10 minutes

[a]Plate counts on nutrient agar medium incubated at 36 C for 48 hours.

Table 4 shows a complete destruction of coliforms organisms at the 0.05 percent peroxide level even at initial bacterial concentrations of 158,000 organisms per milliliter. Temperature alone has some effect on coliform destruction at the concentrations shown. From the analysis of variance in Table 5, it is indicated that temperatures alone have some effect on coliform destruction but this is not significant (p .05). Coliform organisms are very sensitive to the destructive effects of 0.02 and 0.05 percent hydrogen peroxide (p .01). The interaction between temperatures and concentrations on coliform organisms is also highly significant (p .01) as shown in Figure 3.

Table 4. Plate count on coliform organism showing bacterial destruction in day old manufacturing milk treated with different concentrations of hydrogen peroxide at two temperatures

	Original Count 0% H_2O_2	.02% H_2O_2	Percent Killed	.05% H_2O_2	Percent Killed
32 C	3.65×10^5/ml[ab]	7.2×10^4	80	2.75×10^4	92
49 C	1.58×10^5	1×10^2	99	0	100

a All figures are accumulated totals derived from four lots of milk with five replicate plates on each concentration and temperature in each lot.
b Bacterial plate counts on violet red bile agar incubated at 35 C for 48 hours.

Table 5. Analysis of variance on coliform organisms[a]

Source of Variation	d.f.	s.s.	m.s.	F
Replications	3	296,324		
Temperature	1	78,387	78,387	3.46
Error (a)	3	67,911	22,637	
Concentrations	2	374,895	187,448	69,220.00**
Temp. x Conc.	2	122,248	61,124	22,572.00**
Error (b)	12	34,291	2,708	
Sampling	96	296,991	3,094	
Total	119	1,268,247		

** Highly significant at p .01.
a From data presented in Table 4.

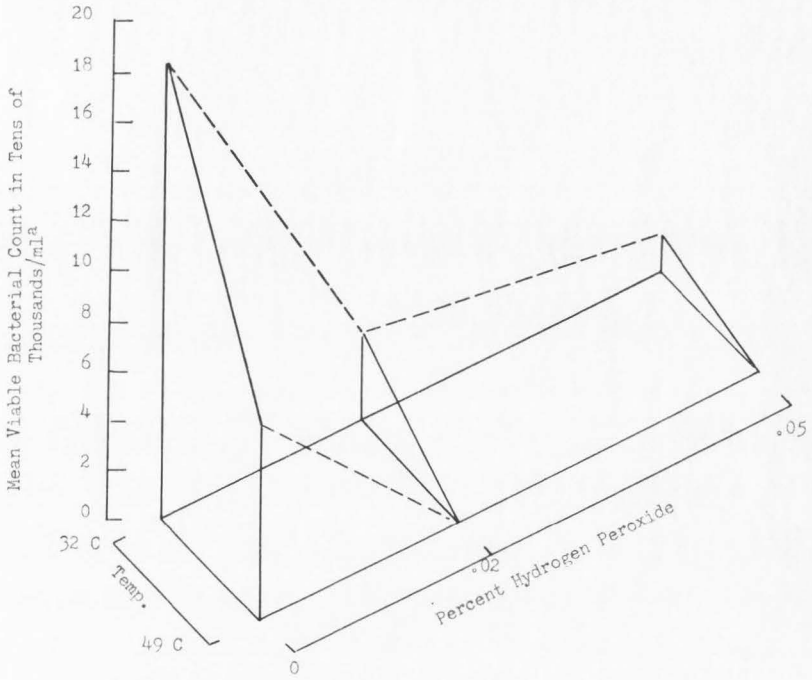

Figure 3. Comparison of mean coliform plate counts showing a three dimensional representation of bacterial destruction in day old manufacturing milk using three concentrations of hydrogen peroxide and two temperature levels for 10 minutes

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[a]Bacterial plate counts on violet red bile agar incubated at 35 C for 48 hours

It is possible that the destruction of coliform organisms in the presence of hydrogen peroxide may be due to the excessive amount of peroxide to which the organisms are exposed. It is understood that coliform organisms produce catalase as a protective mechanism but this mechanism does not seem to protect them at the concentrations to which they were exposed in this experiment.

In analyzing the data on the lactic acid producing organisms the information in Tables 6 and 7 as well as Figure 4 shows a very marked similarity to the data on coliforms in Figure 3. The only marked difference is in the extent of destruction which is less in the lactic acid producing organisms than in the coliforms.

Table 6. Plate counts on lactic acid producing organisms showing bacterial destruction in day old manufacturing milk treated with different concentrations of hydrogen peroxide at two temperatures

	Original Count 0% H_2O_2	.02% H_2O_2	Percent Killed	.05% H_2O_2	Percent Killed
32 C	2.97×10^7/ml[ab]	1.34×10^7	55	5.89×10^6	80
49 C	1.55×10^7	4.36×10^7	72	2.62×10^6	83

a All figures are accumulated totals derived from four lots of milk with five replicate plates on each concentration and temperature in each lot.
b Bacterial plate counts on modified Ellikers (1955) medium incubated at 30 C for 48 hours.

From Table 8 it can be noted that a greater bacterial destruction of aerobic spore-formers was obtained at 32 C than at 49 C at both levels of peroxide (see Figure 5). This may be explained by the procedure used in treating the milk. The samples were heated to 80 C for 10 minutes, then cooled to 49 C and treated. The remaining samples were cooled to 32 C before treatment. This heat treatment was meant to destroy all vegetative cells. After cooling the samples to 32 C the

spores may have begun to germinate again, thus making them more susceptable to the peroxide than at the higher temperature of 49 C. At the higher temperature the spores may not have begun to germinate, thus explaining the lower bacterial destruction at the higher temperature.

The analysis of variance on the aerobic spore-formers, Table 9, was not significant (p .05) at the temperatures and concentrations of peroxide applied. This was probably due to the resistance of the organisms to peroxide while in the spore state.

Table 7. Analysis of variance on lactic acid producing organisms[a]

Source of Variance	d.f.	s.s.	m.s.	F
Replications	3	294,714		
Temperature	1	58,565	58,565	4.23
Error (a)	3	41,668	13,889	
Concentration	2	181,508	90,754	
Temp. x Conc.	2	73,412	36,706	22.30**
Error (b)	12	48,874	4,073	9.01**
Sampling	96	110,563	1,152	
Total	119	809,304		

** Highly significant at the p .01.
a From data presented in Table 6.

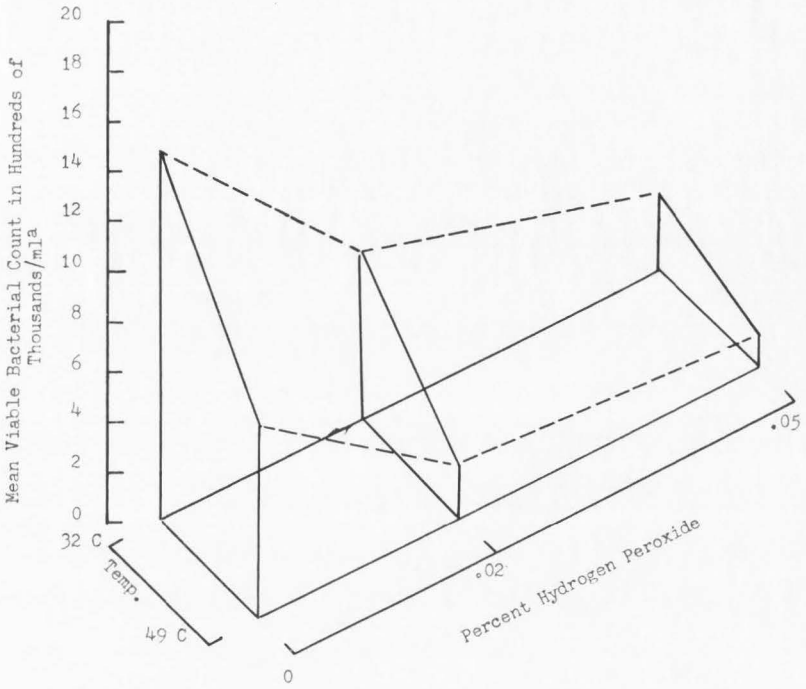

Figure 4. Comparison of mean plate count for lactic acid producing organisms showing a three dimensional representation of bacterial destruction in day old manufacturing milk using three concentrations of hydrogen peroxide and two temperature levels for 10 minutes

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[a]Bacterial plates on modified Ellikers medium incubated at 30 C for 48 hours

Table 8. Plate counts on aerobic spore-forming organisms showing bacterial destruction in day old manufacturing milk treated with different concentrations of hydrogen peroxide at two temperatures

	Original Count 0% H_2O_2	.02% H_2O_2	Percent Killed	.05% H_2O_2	Percent Killed
32 C	2.16×10^4/ml[ab]	1.54×10^4	51	1.15×10^4	64
49 C	3.61×10^4	3.1×10^3	14	2.07×10^4	42

a All figures are accumulated totals derived from four lots of milk with five replicate plates on each concentration and temperature in each lot.
b Bacterial plate counts on nutrient agar incubated at 30 C for 48 hours.

Table 9. Analysis of variance on aerobic spore-forming organisms[a]

Source of Variance	d.f.	s.s.	m.s.	F
Replications	3	2,647		
Temperature	1	720	720	.99
Error (a)	3	2,173	724	
Concentration	2	1,588	794	1.39
Temp. x Conc.	2	875	838	1.46
Error (b)	12	6,862	572	
Sampling	96	50,747	529	
Total	119	65,612		

a From data presented in Table 8.

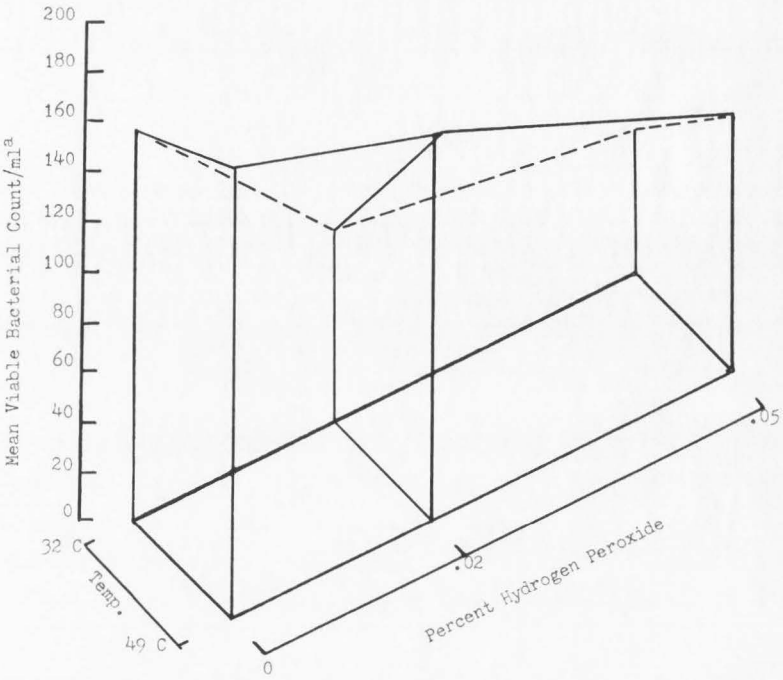

Figure 5. Comparison of mean aerobic plate counts showing a three dimensional representation of bacterial destruction in day old manufacturing milk using three concentrations of hydrogen peroxide and two temperature levels for 10 minutes

[a]Bacterial plate counts on nutrient agar incubated at 30 C for 48 hours

The anaerobic spore-forming organisms in Table 10 show some resistance to peroxide treatment. At 49 C and 0.05 percent peroxide, with an initial count of 13,700 organisms per milliliter, a 73 percent kill was obtained. From the analysis of variance in Table 11 it is evident that some of the destruction is due to concentration alone as may be observed in Figure 6; this however, is not significant (p .05). The remainder of the analysis on anaerobic spore-formers showed no significance at the 0.05 level. This lack of destruction of anaerobes is again probably due to the resistance of the organisms to the peroxide treatment while in the spore state.

Table 10. Plate counts on anaerobic spore-forming organisms showing bacterial destruction in day old manufacturing milk treated with different concentrations of hydrogen peroxide at two temperatures

	Original Count 0% H_2O_2	.02% H_2O_2	Percent Killed	.05% H_2O_2	Percent Killed
32 C	9.3×10^3/ml[ab]	4.6×10^3	50	2.8×10^3	70
49 C	1.37×10^4	5.2×10^3	62	3.7×10^3	73

a All figures are accumulated totals derived from four lots of milk with five replicate plates on each concentration and temperature in each lot.
b Bacterial plate counts on BBL anaerobic agar incubated at 37 C for 72 hours after being evacuated and flooded with nitrogen gas.

Table 11. Analysis of variance on anaerobic spore-forming organisms[a]

Source of Variance	d.f.	s.s.	m.s.	F
Replications	3	130		
Temperature	1	29	29	17.4
Error (a)	3	5	1.67	
Concentrations	2	381	190.00	1.6
Temp. x Conc.	2	52	26	.2
Error (b)	12	1,440	120	
Sampling	96	10,028	104	
Total	119	12,065		

a From data presented in Table 10.

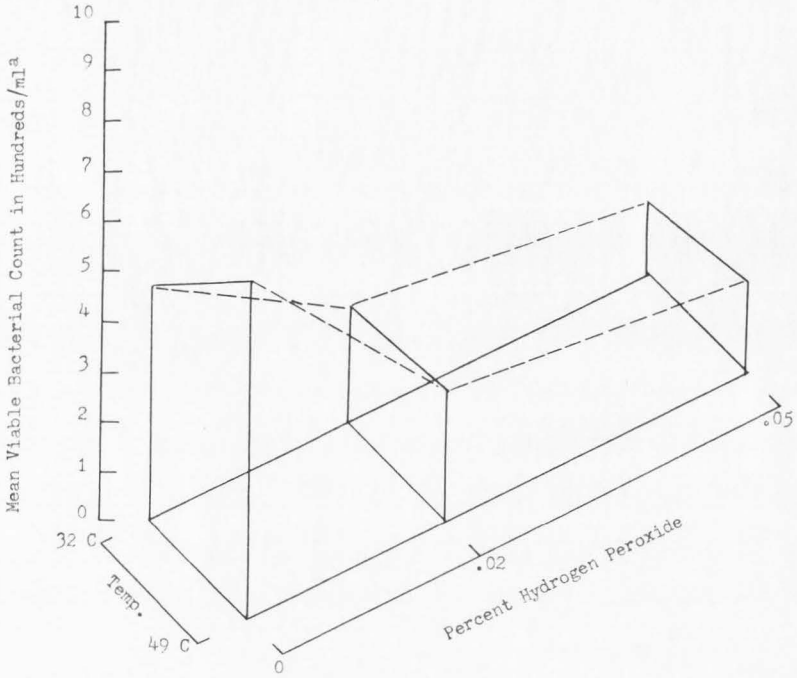

Figure 6. Comparison of mean anaerobic plate counts showing a three dimensional representation of bacterial destruction in day old manufacturing milk using three concentrations of hydrogen peroxide and two temperature levels for 10 minutes.

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[a]Bacterial plate counts on BBL anaerobic agar no. 139 incubated at 37 C for 72 hours after being evacuated and flooded with nitrogen gas.

Table 12 brings together for comparison the data in Tables 2 through 10. According to the data presented it can be concluded that the groups of organisms rank in sensitivity to hydrogen peroxide in the following order: Coliform organisms are most sensitive followed by the organisms grown on standard plate count medium. The next most sensitive group is the lactic acid producing organisms, closely followed by the two groups of spore-formers.

It is desirable to know just what would happen when manufacturing tank milk was treated with hydrogen peroxide and held for 16 hours at 4 C and then plated as in the author's previous work. This work was done and the results are tabulated in Table 13. Details of the procedure may be found in Appendix C.

When fresh milk was treated with two concentrations of hydrogen peroxide at 4 C and held for 16 hours before plating, the results were as follows: The coliforms, as expected, were nearly all destroyed ($>$ 99 percent). Sixty-six percent of the lactic acid producing organisms were destroyed at the 0.05 percent level, showing that they were next to coliforms in sensitivity at this temperature. These were closely followed by aerobic spore-formers, 55 percent, standard plate count organisms, 52 percent, and anaerobic spore-formers with 50 percent destruction at the 0.05 percent level of hydrogen peroxide. At the lower concentration of peroxide, the number of spore-forming organisms actually increased markedly. One theory to explain the increased spore counts at the lesser concentration is: The 0.025 percent concentration of peroxide seemed to stimulate an increased number of vegetative cells to form spores which protected them from being destroyed in the heat treatment which followed. When these spores were bacteriologically plated they germinated, thus accounting for the high number of organisms.

Table 12. Bacterial destruction in day old manufacturing milk treated with hydrogen peroxide as affected by temperature and concentration

	Standard Plate Counts				
	Original Count[a]	.02%	Percent Killed	.05%	Percent Killed
32 C	2×10^7/ml	2.23×10^6	89	1.1×10^5	94
49 C	1.85×10^7	2.56×10^6	86	4.6×10^5	98
Bacterial plate counts on nutrient agar incubated at 35 C for 48 hours.					
	Plate Counts on Coliform Organisms				
32 C	3.65×10^5	7.2×10^4	80	2.75×10^4	92
49 C	1.58×10^5	1×10^2	99.99	0	100
Bacterial plate counts on violet red bile agar incubated at 35 C for 48 hours.					
	Plate Counts on Lactic Acid Producing Organisms				
32 C	2.97×10^7	1.34×10^7	55	5.89×10^6	80
49 C	1.55×10^7	4.36×10^6	72	2.62×10^6	83
Bacterial plate counts on modified Elliker's (1955) medium incubated at 30 C for 48 hours.					
	Plate Counts on Aerobic Spore-forming Organisms				
32 C	3.16×10^4	1.54×10^4	51	1.15×10^4	64
49 C	3.61×10^4	3.1×10^3	14	2.08×10^4	42
Bacterial plate counts on nutrient agar incubated at 30 C for 48 hours.					
	Plate Counts on Anaerobic Spore-forming Organisms				
32 C	9.3×10^3	4.6×10^3	50	2.8×10^3	70
49 C	1.37×10^4	5.2×10^3	62	3.7×10^3	73
Bacterial plate counts on BBL anaerobic agar incubated at 37 C for 72 hours after being evacuated and flooded with nitrogen gas.					

[a] All figures are accumulated totals derived from four (4) lots of milk with five (5) replicate plates on each concentration and temperatures in each lot.

Table 13. Bacterial destruction in fresh manufacturing milk treated with hydrogen peroxide and held for 16 hours at 4 C as affected by concentration

Mean Organisms/ml	0% H_2O_2	.025% H_2O_2	Percent Killed	.05% H_2O_2	Percent Killed
Standard Plate Count	5.87×10^6	3.64×10^6	47	2.83×10^6	52
Coliform Count	4.66×10^4	1.5×10^3	68	2	99
Lactic Acid Organisms Count	3.2×10^6	2.2×10^6	31	1.1×10^6	66
Aerobic Spore Count	3×10^4	1.14×10^5	increase 381	1.35×10^4	55
Anaerobic Spore Count	1.1×10^3	3.17×10^3	increase 286	5.51×10^2	50.

See page 12 for plating procedure on all groups of organisms.

Conversely, at the 0.05 percent concentration of peroxide, 50 percent of the spore-forming organisms were destroyed in the vegetative state, thus accounting for the increased kill at this level of treatment.

Practically speaking, if we apply the peroxide catalase treatment to raw manufacturing milk in a Swiss cheese plant, just how should the treatment be applied?

If there are approximately two million organisms per milliliter in the raw milk (normally distributed as in Table 13) the application of 0.05 percent hydrogen peroxide for 16 hours at 4 C will assure the destruction of over 99 percent of the coliform organisms and at least 50 percent of other normally present types of organisms in bulk tank milk for manufacturing purposes. With this type of treatment it is necessary to check the milk before use with the Potassium Iodide Test to make sure that it is free from residual peroxide. If it is not, it must be treated with catalase so as not to destroy the starter organisms

added later to the cheese milk.

It would be desirable to perform further experiments to determine the effect of hydrogen peroxide on specific types of organisms including pathogens. The use of the peroxide-catalase treatment of milk appears to be both practical and economical for use in the cheese industry.

The results of these experiments agree favorably with the work of other researchers in this field (Jasewicz and Porges, 1959; Johnson, 1952; Morris, 1950; Negmoush, 1949; Roundy, 1958).

The Federal Food and Drug Administration has tentatively set a maximum limit of 0.05 percent hydrogen peroxide to be used in the treatment of milk for cheese products. This level of peroxide appears from all practical purposes to be sufficient to destroy enough organisms to permit satisfactory manufacture of cheese.

CONCLUSIONS

1. Hydrogen peroxide added to manufacturing milk at a concentration of 0.05 percent killed 92 to 100 percent of the coliform organisms at 4 C, 32 C and 49 C under the conditions of this experiment.

2. Lactic acid producing organisms normally present in manufacturing milk were found to be more sensitive to the peroxide treatments than were the spore-forming organisms.

3. Spore-forming organisms were more resistant to peroxide treatment than the other groups of micro-organisms studied. Further study is needed to determine resistence of specific varieties of spore-formers.

4. The bacterial destruction by 0.05 percent hydrogen peroxide at 32 C and 49 C was practically the same. Therefore, the use of the lower temperature may prove to be an economical procedure.

5. From 50 to 99 percent of the bacteria in fresh milk were destroyed when exposed to 0.05 percent peroxide for 16 hours at 4 C; however, most of the samples showed the presence of residual peroxide at the end of the treatment. If by some procedure peroxide residues can be eliminated, this process may provide a more economical and practical method for treating milk during storage.

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APPENDIXES

APPENDIX A

Appendix A contains the data from the preliminary series of experiments. Detailed information concerning the selction, preparation and treatment of the milk may be found on pages 8 through 12 in the body of this thesis. The procedure used in plating the various groups of organisms may be found on pages 12 and 13.

Lot I

49 C for 20 minutes using raw Grade A milk

Coliforms 1/100

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - negative

E - negative

F

Lactic Organisms 1/1000

Blank - negative

R - 4

A - 3

B - negative

C - negative

D - 2

E - 3

Standard Plate 1/1000

Blank - negative

R - 16

A - 2

B - 8

C - 3

D - 1

E - 2

Aerobic Plate 1/1000

Blank - negative

R - 16

A - 6

B - 4

C - 5

D - 1

E - negative

Anaerobic Plate 1/1000

Blank - negative

R - 1

A - negative

B - negative

C - negative

D - 1

E - negative

Lot II--first series

49 C for 20 minutes using raw Grade A milk

Coliforms 1/100

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - negative

E - negative

Lactic Organisms 1/1000

Blank - negative

R - 1

A - 2

B - negative

C - negative

D - negative

E - negative

Standard Plate 1/1000

Blank - negative

R - 2

A - 2

B - 4

C - 2

D - 1

E - negative

Aerobic Plate 1/1000

Blank - negative

R - 1

A - 2

B - 5

C - negative

D - 1

E - 1

Anaerobic Plate 1/1000

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - negative

E - negative

Lot III--first series

49 C for 20 minutes using raw Grade A milk

Coliforms 1/1000

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - negative

E - negative

Lactic Organisms 1/1000

Blank - negative

R - 2

A - negative

B - negative

C - negative

D - negative

E - negative

Standard Plate 1/1000

Blank - negative

R - 5

A - 3

B - 4

C - negative

D - 15 1 spreading lg. colony

E - 2 surface colonies

Aerobic Plate 1/1000

Blank - 1

R - 3

A - negative

B - negative

C - negative

D - 1

E - 1

Anaerobic Plate 1/1000

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - 1

E - negative

Lot IV--first series

49 C for 20 minutes using raw Grade C milk

Coliforms 1/100

Blank - negative

R - 60

A - negative

B - negative

C - negative

D - negative

E - negative

Lactic Organisms 1/1000

Blank - negative

R - TNC

A - TNC

B - TNC

C - TNC

D - TNC

E - 440

Standard Plate 1/1000

Blank - negative

R - TNC

A - TNC

B - TNC

C - TNC

D - TNC

E - TNC

Aerobic Count 1/1000

Blank - negative

R - TNC

A - TNC

B - TNC

C - TNC

D - TNC

E - TNC

Anaerobic Plate 1/1000

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - negative

E - 1

Lot V--first series

49 C for 20 minutes using raw Grade C milk

Coliforms 1/100

Blank - negative

R-F - TNC - completely covered

G - 7

H - negative

I - negative

J - negative

K - negative

Lactic Organisms 1/1000

Blank - negative

R-F - TNC

G - TNC

H - TNC

I - TNC

J - TNC

K - TNC

Standard Plate 1/1000

Blank - negative

R - TNC

A - TNC

B - TNC

C - TNC

D - TNC

E - TNC

Aerobic Count 1/1000

Blank - negative

R-F - TNC

G - TNC

H - TNC

I - TNC

J - TNC

K - TNC

Anaerobic Count

Blank - negative

R-F - negative

G - negative

H - negative

I - 4

J - 1

K - 1

Lot VI--first series

49 C for 20 minutes using fresh raw Grade C milk

Coliforms 1/1000

Blank - negative

R - 243

A - negative

B - negative

C - negative

D - negative

E - negative

Lactic Organisms 1/10,000

Blank - negative

R - 200

A - 93

B - 80

C - 33

D - 49

E - 1

Standard Plate 1/10,000

Blank - 1

R - 107

A - 66

B - 73

C - 46

D - 47

E - negative

Aerobic Count 1/10,000

Blank - negative

R - 127

A - 70

B - 82

C - 20

D - 28

E - negative

Anaerobic Plate 1/100

Blank - negative

R - 2

A - 44

B - 2

C - 4

D - negative

E - negative

Lot VII--first series

49 C for 20 minutes using day old raw Grade C milk

Coliforms 1/1000

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - negative 1/1000

E - negative 1/1000

Lactic Organisms 1/10,000

Blank - negative

R - 9

A - negative

B - 13

C - 16

D - 3

E - negative

Standard Plate 1/10,000

Blank - 1

A - negative

B - 29

C - 35

D - 3

E - 2

R - 46

Aerobic Plate 1/10,000

Blank - negative

R - 36

A - 1

B - 31

C - 29

D - 23

E - 2

Anaerobic Plate 1/100

Blank - negative

R - negative

A - 38

B - 28

C - 3

D - negative

E - negative

Lot VIII--first series

49 C for 20 minutes using day old raw Grade C milk

Coliforms 1/1000

Blank - negative

R - negative

A - negative

B - negative

C - negative

D - negative

E - negative

Lactic Organisms 1/10,000

Blank - negative

R - 1

A - negative

B - negative

C - negative

D - negative

E - negative

Standard Plate 1/10,000

Blank - negative

R - 3

A - 1

B - 4

C - 1

D - negative

E - negative

Aerobic Plate 1/10,000

Blank - negative

R - 6

A - 5

B - 1

C - 2

D - negative

E - 2

Anaerobic Plate 1/100

Blank - negative

R - negative

A - 1

B - 69

C - negative

D - negative

E - negative

Lot IX--first series

49 C for 20 minutes using day old mixed raw Grade A milk

Coliforms 1/1000
Blank - negative
R - 307
A - negative
B - 17 1/100
C - negative
D - negative
E - negative

Lactic Organisms 1/1000	1/10,000
Blank - negative	negative
R - TNC	16
A - 263	2
B - 251	10
C - 230	4
D - 72	negative
E - 35	negative

Standard Plate 1/1000	1/10,000
Blank - negative	
R - TNC	76
A - TNC	17
B - TNC	12
C - 842	10
C - 189	3
E - 135	1

Aerobic Plate 1/1000	1/10,000
Blank - negative	negative
R - TNC	144
A - TNC	14
B - 782	16
C - 914	15
D - 238	4
E - 144	2

Anaerobic Plate 1/100
Blank - negative
R - negative
A - 2
B - 38
C - negative
D - 2
E - 1

Lot X--first series

49 C for 20 minutes using day old mixed raw Grade A milk

Coliforms 1/1000

Blank - negative

R - negative

A - 2 1/100

B - 5 1/100

C - negative

D - negative

E - negative

Lactic Organisms 1/1000

Blank - negative

R - 4

A - 18

B - 31

C - 4

D - 1

E - 1

Standard Plate 1/1000

Blank - negative

R - 24

A - 467

B - 133

C - 14

D - 5

E - 2

Aerobic Plate 1/1000

Blank - negative

R - 25

A - 409

B - 163

C - 17

D - negative

E - 1

Anaerobic 1/100

Blank - negative

R - negative

A - 30

B - 41

C - 1

D - negative

E - 1

Lot I--second series

49 C for 20 minutes using day old Grade C milk

Coliforms 1/1000

Blank - negative

R - 663

A - negative

B - negative

C - negative

Lactic Organisms 1/10,000

Blank - negative

R - 134

A - 23

B - negative

C - 10

Standard Plate 1/10,000

Blank - negative

R - 346

A - 24

B - negative

C - 16

Aerobic Plate 1/10,000

Blank - negative

R - 327

A - 25

B - 2

C - 10

Anaerobic Plate 1/100

Blank - negative

R - negative

A - negative

B - negative

C - negative

Lot II--second series

49 C for 20 minutes using day old Grade C milk

Coliforms 1/1000

Blank - negative

R - negative

A - negative

B - negative

C - negative

Lactic Organisms 1/10,000

Blank - negative

R - negative

A - negative

B - negative

C - negative

Standard Plate 1/10,000

Blank - negative

R - 2

A - negative

B - negative

C - negative

Aerobic Plate 1/10,000

Blank - negative

R - negative

A - negative

B - negative

C - negative

Anaerobic Plate 1/100

Blank - negative

R - negative

A - 5

B - negative

C - negative

Lot III--second series

49 C for 20 minutes using day old Grade C milk

Coliforms 1/1000

Blank - negative

R - 40

A - negative

B - negative

C - negative

Lactic Organisms 1/10,000

Blank - negative

R - 86

A - 25

B - 5

C - 6

Standard Plate 1/10,000

Blank - negative

R - 230

A - 99

B - 23

C - 36

Aerobic Plate 1/10,000

Blank - negative

R - 190

A - 74

B - 13

C - 34

Anaerobic Plate 1/100

Blank - negative

R - 53

A - 1

B - negative

C - negative

Lot IV--second series

49 C for 20 minutes using day old Grade C milk

Coliforms 1/1000

Blank - negative

R - 9

A - negative

B - negative

C - negative

Lactic Organisms 1/10,000

Blank - negative

R - 75

A - 6

B - negative

C - 2

Standard Plate 1/10,000

Blank - negative

R - 144

A - 38

B - 7

C - 10

Aerobic Plate 1/10,000

Blank - negative

R - 201

A - 40

B - 2

C - 10

Anaerobic Plate 1/100

Blank - negative

R - 3

A - negative

B - negative

C - 3

Lot V--second series

49 C for 20 minutes using day old Grade C milk

Coliforms 1/1000

Blank - negative

R - 2

A - negative

B - negative

C - negative

Lactic Organisms 1/10,000

Blank - negative

R - 1250

A - 37

B - 7

C - 10

Standard Plate 1/10,000

Blank - negative

R - 864

A - 232

B - 39

C - 33

Aerobic Plate 1/10,000

Blank - negative

R - 1602

A - 182

B - 46

C - 31

Anaerobic Plate 1/100

Blank - negative

R - 227

A - 3

B - 2

C - negative

Lot I--third series

49 C for 20 minutes using day old Grade C milk

Lactic Organisms 1/10,000

Blank - negative

R - 41

A - 11

B - 9

C - 6

Aerobic Plate 1/10,000

Blank - negative

R - 82

A - 14

B - 10

C - 6

Standard Plate 1/10,000

Blank - negative

R - 62

A - 11

B - 8

C - 7

Anaerobic Plate 1/100

Blank - negative

R - 39

A - 24

B - 26

C - 21

Lot II--third series

49 C for 20 minutes using day old Grade milk

Lactic Organisms 1/10,000

Blank - negative

R - 30

A - negative

B - negative

C - negative

Aerobic Plate 1/10,000

Blank - negative

R - 24

A - negative

B - negative

C - negative

Standard Plate 1/10,000

Blank - negative

R - 25

A - 2

B - negative

C - 1

Anaerobic Plate 1/100

Blank - negative

R - 1

A - 3

B - 2

C - negative

Lot III--third series

49 C for 20 minutes using day old Grade C milk

Lactic Organisms 1/10,000

Blank - negative

R - 182

A - negative

B - negative

C - negative

Aerobic Plate 1/10,000

Blank - negative

R - 146

A - negative

B - negative

C - 1

Standard Plate 1/10,000

Blank - negative

R - 149

A - 1

B - negative

C - 1

Anaerobic Plate 1/100

Blank - negative

R - 1

A - negative

B - negative

C - negative

Lot IV--third series

49 C for 20 minutes using day old Grade C milk

Lactic Organisms 1/10,000

Blank - negative

R - 949

A - 21

B - 10

C - 8

Aerobic Plate 1/10,000

Blank - negative

R - 800

A - 13

B - 18

C - 14

Standard Plate 1/10,000

Blank - negative

R - 392

A - 13

B - 11

C - 15

Anaerobic Plate 1/100

Blank - negative

R - 23

A - 8

B - 3

C - 9

APPENDIX B

The following tables show the experimental design used in this work as well as the data used to make up the analysis of variance on each of the five groups of organisms involved in the experiment.

CO, CO_2 and CO_5 designate the percent concentrations of peroxide used. T_{90} and T_{120} designate the temperatures in degrees fahrenheit used in the experiment. The four lot numbers conform to the four different lots of milk used on each group of organisms. Where there are double sets of figures the second set represents a second dilution concentration from the same sample of milk at the given temperature and hydrogen peroxide concentration shown.

Agar and water blank controls were run on all lots for every group of organisms and were negative in every instance.

The data given under the sum totals portion of the tables contains the sums of the plate counts for each individual lot as well as the totals of those sums.

Lactic acid producing organisms

Add four (4) zeros to all figures to convert them to raw numbers.

Lot I

	CO	CO_2	CO_5	Totals
T90	10	6	3	19
	10	8	3	21
	7	3	2	12
	12	5	3	20
	9	3	5	17
	48	25	16	89
T120	8	0	0	8
	4	1	0	5
	8	0	0	8
	6	0	0	6
	6	0	0	6
	32	1	0	33

Lot II

	CO	CO_2	CO_5	Totals
T90	35	43	18	96
	32	19	18	69
	42	23	18	83
	36	33	19	88
	31	22	20	73
	176	140	93	409
T120	25	1	1	27
	17	1	1	19
	26	0	1	27
	22	0	0	22
	20	0	0	20
	110	2	3	115

Lot III

	CO	CO_2	CO_5	Totals
T90	200	75	30	305
	366	130	29	525
	168	160	40	368
	240	220	29	489
	314	130	40	484
	1288	715	168	2171
T120	214	53	38	305
	140	62	43	145
	180	63	35	278
	200	52	28	280
	190	57	33	280
	924	287	177	1388

Lot IV

	CO	CO_2	CO_5	Totals
T90	334	86	65	485
	270	99	65	434
	262	71	57	390
	258	99	70	427
	330	109	55	494
	1454	464	312	2230
T120	89	25	11	125
	98	26	16	140
	125	32	22	179
	92	33	21	146
	80	30	12	122
	484	146	82	712

Sum Totals

	CO	CO_2	CO_5	Totals
T90	48	25	16	89
	176	140	93	409
	1288	715	168	2171
	1454	464	312	2230
	2966	1344	589	4899

Sum Totals

	CO	CO_2	CO_5	Totals
T120	32	1	0	33
	110	2	3	115
	924	287	177	1388
	484	146	82	712
	1550	436	262	2248

Anaerobic plate counts

Add two (2) zeros to all figures to convert them to raw numbers.

Lot I

	CO	CO_2	CO_5	Total
T90	15	8	0	23
	8	3	2	13
	13	0	1	14
	13	0	1	14
	1	0	1	2
	50	11	5	66
T120	17	0	0	17
	1	1	0	2
	28	1	3	32
	9	1	0	10
	19	1	1	21
	74	4	4	82

Lot II

	CO	CO_2	CO_5	Total
T90	1	5	3	9
	11	8	4	23
	3	1	1	5
	1	1	0	2
	1	3	1	5
	17	18	9	44
T120	1	1	3	5
	2	5	3	10
	22	1	0	23
	4	4	2	10
	5	2	2	9
	34	13	10	57

Lot III

	CO	CO_2	CO_5	Total
T90	1	0	3	4
	6	1	4	11
	1	6	1	8
	4	0	1	5
	6	1	0	7
	18	8	9	35
T120	3	2	1	6
	1	4	1	6
	5	8	3	16
	1	3	3	7
	3	1	3	7
	13	18	11	42

Lot IV

	CO	CO_2	CO_5	Total
T90	2	1	1	4
	1	1	1	3
	3	3	0	6
	1	2	1	4
	1	2	2	5
	8	9	5	22
T120	6	4	1	11
	1	0	3	4
	0	7	6	13
	4	4	1	9
	5	2	1	8
	16	17	12	45

Sum Totals

	CO	CO_2	CO_5	Totals
T90	50	11	5	66
	17	18	9	44
	18	8	9	35
	8	9	5	22
	93	46	28	167

Sum Totals

	CO	CO_2	CO_5	Totals
T120	74	4	4	82
	34	13	10	57
	13	18	11	42
	16	17	12	45
	137	52	37	226

Standard plate counts

Add four (4) zeros to all figures to convert them to raw numbers.

Lot I

	CO	CO_2	CO_5	Totals
T90	32	4	9	45
	31	8	3	41
	42	12	3	57
	62	23	4	89
	31	10	3	44
	198	57	22	277
T120	30	6	3	39
	33	4	2	39
	11	21	3	35
	204	7	0	211
	29	9	5	43
	307	47	13	367

Lot II

	CO	CO_2	CO_5	Totals
T90	102	11	14	127
	76	9	7	92
	130	20	5	145
	76	3	9	87
	74	10	9	93
	458	43	43	544
T120	25	5	4	34
	15	2	7	24
	22	13	2	37
	37	6	4	47
	18	4	2	24
	117	30	19	166

Lot III

	CO	CO_2	CO_5	Totals
T90	117	11	11	139
	181	11	7	199
	149	15	7	171
	110	9	7	126
	132	12	9	153
	689	58	41	788
T120	147	12	0	159
	82	17	1	100
	106	41	1	148
	121	16	1	138
	107	16	6	674
	563	102	9	674

Lot IV

	CO	CO_2	CO_5	Totals
T90	100	12	0	112
	92	12	0	104
	166	7	1	174
	164	26	1	191
	133	8	2	143
	655	65	4	724
T120	166	13	2	181
	207	14	0	221
	182	16	2	200
	144	9	0	153
	160	25	1	186
	859	77	5	941

Sum Totals

	CO	CO_2	CO_5	Totals
T90	198	57	22	277
	458	43	43	544
	689	58	41	788
	655	65	4	724
	2000	233	110	2333

Sum Totals

	CO	CO_2	CO_5	Totals
T120	307	47	13	367
	117	30	19	166
	563	102	9	674
	859	77	5	941
	1846	256	46	2148

Coliforms

Add three (3) zeros to all figures to convert them to raw numbers.

Lot I

	CO		CO_2		CO_5		Total	
T90	300	330	71	70	37	0	408	400
	238	340	94	40	19	10	351	390
	256	390	80	40	32	10	368	440
	342	350	51	110	10	0	403	460
	362	370	85	20	16	0	463	390
	1498	1780	381	280	114	20	1993	2080
T120	186	150	0	0	0	0	186	150
	150	120	0	0	0	0	150	120
	202	110	0	0	0	0	202	110
	130	130	0	0	0	0	130	130
	118	150	0	0	0	0	118	150
	786	660	0	0	0	0	786	660

Lot II

T90	398	420	68	60	41	30	507	510
	402	380	71	90	33	20	506	490
	378	410	55	50	27	50	460	510
	438	430	74	90	16	50	528	570
	384	470	71	50	44	10	499	530
	2000	2110	339	340	161	160	2500	2610
T120	230	290	1	0	0	0	231	290
	118	100	0	0	0	0	118	100
	138	160	0	0	0	0	138	160
	162	130	0	0	0	0	162	130
	142	270	0	0	0	0	142	270
	790	950	1	0	0	0	791	950

Lot III

T90	17	30	0	0	0	0	17	30
	8	30	0	0	0	0	8	30
	10	20	0	0	0	0	10	20
	18	20	0	0	0	0	18	20
	25	30	0	0	0	0	25	30
	78	130	0	0	0	0	78	130
T120	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Lot IV

	CO		CO_2		CO_5		Total	
T90	14	30	0	0	0	0	14	30
	12	10	0	0	0	0	12	10
	18	10	0	0	0	0	18	10
	16	20	0	0	0	0	16	20
	13	20	0	0	0	0	13	20
	73	90	0	0	0	0	73	90
T120	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Sum Total

T90	1498	1780	381	280	114	20	1993	2080
	2000	2110	339	340	161	160	2500	2610
	78	130	0	0	0	0	78	130
	73	90	0	0	0	0	73	90
	3649	4110	720	620	275	180	4644	4910
T120	786	660	0	0	0	0	786	660
	790	950	1	0	0	0	791	950
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	1576	1610	1	0	0	0	1577	1610

Aerobic plates

Add one (1) zero to all figures to convert them to raw numbers.

Lot I

	CO		CO_2		CO_5		Total	
T90	10	100	10	100	30	100	50	300
	20	0	10	0	0	0	30	0
	20	0	10	0	0	0	30	0
	10	0	0	0	0	0	10	0
	0	0	0	0	0	0	0	0
	60	100	30	100	30	100	120	300
T120	10	100	80	300	10	200	100	600
	20	300	10	0	10	0	40	300
	50	100	20	0	10	0	80	100
	0	0	0	0	10	0	10	0
	0	0	0	0	30	0	30	0
	80	500	110	300	70	200	260	1000

Lot II

T90	10	100	10	100	10	0	30	200
	10	100	10	100	10	0	30	200
	30	0	0	0	0	0	30	0
	40	0	0	0	0	0	40	0
	40	0	0	0	0	0	40	0
	130	200	20	200	20	0	170	400
T120	30	100	10	200	30	100	70	400
	20	100	20	0	50	100	90	200
	50	0	30	0	10	100	90	100
	70	0	20	0	0	0	90	0
	40	0	40	0	0	0	80	0
	210	200	120	200	90	300	420	700

Lot III

T90	21	10	7	20	8	10	36	40
	10	10	9	10	14	0	33	20
	21	40	12	10	6	10	39	60
	10	20	12	40	7	30	29	90
	14	20	8	10	1	10	23	40
	76	100	48	90	36	60	160	250
T120	8	40	11	0	6	30	25	70
	5	10	9	0	7	10	21	20
	2	10	7	20	6	30	15	60
	6	10	9	20	6	40	21	70
	12	20	7	0	5	0	24	20
	33	90	43	40	32	110	106	240

	CO		CO_2		CO_5		Total	
T90	6	10	13	20	5	20	24	50
	15	10	16	0	5	20	36	30
	10	20	10	20	8	0	28	40
	9	10	4	20	9	0	22	30
	10	30	13	30	2	10	25	70
	50	80	56	90	29	50	135	220
T120	8	20	9	20	2	10	19	50
	4	10	8	0	4	10	16	20
	10	10	10	20	5	0	25	30
	9	30	3	10	2	20	14	60
	7	10	7	10	5	20	19	40
	38	80	37	60	18	60	93	200

Sum Total

T90	60	100	30	100	30	100	120	300
	130	200	20	200	20	00	170	400
	76	100	48	90	36	60	160	250
	50	80	56	90	29	50	135	220
	316	480	154	480	115	210	585	1170
T120	80	500	110	300	70	200	260	1000
	210	200	120	200	90	300	420	700
	33	90	43	40	30	110	106	240
	38	80	37	60	18	60	93	200
	361	870	310	600	208	670	879	2140

APPENDIX C

Appendix C contains the data obtained from the treatment of various lots of milk with 0, 0.025 and 0.05 percent hydrogen peroxide at 4 C and held for 16 hours before plating. Prior to bacteriological plating, the treated milk was tested with potassium iodide to determine the presence of residual peroxide. The result of these tests may be found in Table 14, page 65. The plating procedure was the same as shown on pages 12 through13 .

Table 14. A comparison of potassium iodide tests on milk samples[a] treated with hydrogen peroxide and held at 4 C for 16 hours with variations caused by unknown initial amounts of catalase or catalase producing organisms

Sample	Standard Plate Initial Count	Untreated	.025%	.05%
Bulk (Nov. 7)	4×10^5	-	+	+
D 24	4.05×10^5	-	-	-
R 22	2.17×10^4	-	-	+
D 33	5.77×10^5	-	-	+
	Coliforms			
Bulk (Nov. 11)	2.5×10^5	-	-	+
A 21	8×10^5	-	-	+
D 35	8.4×10^3	-	+	+
A 38	2.38×10^5	-	-	-
	Lactic Acid Organisms			
Bulk (Dec. 16)	2.08×10^6	-	-	+
S 12	1.85×10^5	-	+	+
S 39	7.12×10^6	-	-	-
S 57	8.9×10^4	-	-	-
	Aerobic			
Bulk (Nov. 19)	1×10^4	-	-	+
R 30	6×10^3	-	-	+
R 29	7.2×10^4	-	-	-
R 22	2×10^3	-	-	+
	Aerobic Organisms			
Bulk (Nov. 28)	6.11×10^4	-	-	+
E 12	8.9×10^3	-	-	+
E 18	7.48×10^4	-	-	-
E 37	5.14×10^4	-	-	-

a Milk samples obtained from bulk tanks and individual patrons of the Cache Valley Dairy Association.

Coliform plate counts

Add three (3) zeros to all figures to convert them to raw numbers.

Lot I

	CO		CO_2		CO_5		Total	
T40	6	60	6	0	1	0	13	60
	14	0	5	0	1	0	20	0
	22	60	6	0	3	0	31	60
	27	10	4	10	0	0	31	20
	57	0	4	10	0	0	61	10
	126	130	25	20	5	0	156	150

Lot II

T40	1	10	3	0	0	0	4	10
	1	0	0	0	0	0	1	0
	1	0	1	10	0	0	2	10
	1	0	0	0	2	0	3	0
	0	0	2	0	1	0	3	0
	4	10	6	10	3	0	13	20

Lot III

T40	16	0	0	0	0	0	16	0
	4	0	0	0	0	0	4	0
	18	0	0	0	0	0	18	0
	2	0	0	0	0	0	2	0
	2	0	0	0	0	0	2	0
	42	0	0	0	0	0	42	0

Lot IV

T40	332	80	0	0	0	0	332	80
	248	50	0	0	0	0	248	50
	212	90	0	0	0	0	212	90
	200	110	0	0	0	0	200	110
	200	30	0	0	0	0	200	30
	1192	360	0	0	0	0	1192	360

Aerobic plate counts

Add one (1) zero to all figures to convert them to raw numbers.

	CO		CO_{25}		CO_5		Totals	
T40	325	80	715	180	188	110	1228	370
	975	80	690	90	142	70	1807	240
	650	90	388	150	150	0	1188	240
	455	80	500	90	142	40	1097	210
	650	90	390	100	80	0	1120	190
	3055	420	2683	610	702	220	6440	1250
				Lot II				
T40	100	40	1820	320	120	20	2040	380
	88	20	1700	240	115	30	1903	290
	92	10	1820	260	85	20	1997	290
	105	10	1365	300	60	40	1530	350
	60	30	1225	410	35	20	1320	460
	445	110	7930	1530	415	130	8790	1770
				Lot III				
T40	975	150	2600	300	390	20	3965	470
	715	150	2470	370	325	30	3510	550
	780	260	2600	420	390	30	3770	710
	520	250	2760	270	411	40	3691	560
	750	170	2500	300	300	40	3550	510
	3740	980	12930	1660	1816	160	18486	2800
				Lot IV				
T40	650	170	930	190	240	20	1820	380
	455	70	1040	400	380	100	1875	570
	585	110	1105	180	368	80	2058	370
	455	140	720	320	300	80	1475	540
	425	100	910	350	365	30	1700	480
	2570	590	4705	1440	1653	310	8928	2340

Anaerobic plate count

Add one (1) zero to all figures to convert them to raw numbers.

Lot I

	CO		CO_{25}		CO_5		Total	
T40	134	10	112	50	24	0	270	60
	72	10	72	0	28	0	172	10
	80	0	140	40	25	10	245	50
	35	0	120	40	30	0	185	40
	120	10	120	110	21	10	261	130
	441	30	564	240	128	20	1133	290

Lot II

T40	56	10	480	10	18	0	554	20
	48	0	410	30	27	0	485	30
	70	10	455	30	14	0	539	40
	45	0	390	40	4	0	439	40
	60	0	390	30	19	10	469	40
	279	20	2125	140	82	10	2486	170

Lot III

T40	455	110	1040	250	142	40	1637	400
	390	200	1235	250	236	0	1861	450
	325	110	1300	270	236	20	1861	400
	333	70	910	180	140	10	1383	260
	248	120	975	160	100	20	1323	300
	1751	610	5460	1110	854	90	8065	1810

Lot IV

T40	400	10	570	130	195	0	1165	140
	120	10	390	110	260	20	770	140
	240	10	455	130	150	10	845	150
	342	0	650	120	172	20	1164	130
	120	40	400	70	194	10	714	120
	1222	70	2465	560	971	50	4658	680

Lactic acid producing organisms

Add three (3) zeros to all figures to convert them to raw numbers.

Lot I

	CO		CO_{25}		CO_5		Totals	
T40	2600	2600	585	2480	184	1520	3369	6600
	3250	3250	650	1120	128	720	4028	5090
	1200	3900	650	1400	172	1000	2122	6300
	650	3900	600	1480	300	880	1550	6260
	2600	4550	520	2100	320	960	3440	7610
	10400	18200	3005	8580	1104	5080	14509	31860

Lot II

T40	188	180	14	10	10	10	212	200
	160	120	14	10	4	20	178	150
	214	160	14	20	11	20	239	200
	220	130	21	0	5	0	246	130
	145	100	15	0	5	0	165	100
	927	690	78	40	35	50	1040	780

Lot III

T40	10530	12350	2275	8450	1925	6500	14730	27300
	5850	10500	2275	4550	1500	3900	9625	18950
	8210	13000	1950	7150	1375	6500	11535	26650
	4720	11700	1190	9100	1625	4550	7535	25350
	6280	13000	1990	6500	1780	7150	10050	26650
	35590	60550	9680	35750	8205	28600	53475	124900

Lot IV

T40	60	130	83	90	65	30	208	250
	83	150	26	80	58	100	167	330
	100	260	0	90	78	120	178	470
	92	250	36	80	70	90	198	420
	110	300	67	50	60	80	237	430
	445	1090	212	390	331	420	988	1900

Standard plate count

Add two (2) zeros to all figures to convert them to raw numbers.

Lot I

	CO		CO_{25}		CO_5		Totals	
T40	4550	91200	2990	43400	1495	40200	9035	174800
	3250	97500	3900	65000	1950	65000	9100	227500
	3055	100000	1950	52000	2600	45500	7605	197500
	3960	78000	3250	45500	3250	39000	10460	162500
	5200	60000	2600	71500	2210	40000	10010	171500
	20015	426700	14690	277400	11505	229700	46210	933800

Lot II

T40	3990	117000	2925	71500	2925	65000	9840	253500
	3100	130000	2470	65000	3120	52000	8690	247000
	3700	104000	2600	45500	5000	58500	10700	208000
	5200	94500	3055	39000	3770	39000	12025	172500
	4875	116000	4000	52000	3445	52000	12320	220000
	20265	561500	15050	27300	18260	266500	53575	855300

Lot III

T40	256	2100	68	700	32	300	356	3100
	260	2000	68	600	80	200	408	2800
	256	3100	56	900	60	200	372	4200
	160	2000	132	600	60	200	352	2800
	152	2300	108	800	36	300	296	2400
	1084	11500	432	3600	268	1200	1784	16300

Lot IV

T40	7020	110000	4499	58500	1560	52000	13079	220500
	5850	104000	5200	65000	2350	0	13400	169000
	5850	130000	4550	65000	2795	65000	13195	260000
	5850	91000	5850	78000	3250	45500	14950	214500
	4290	78000	6000	71500	2600	45500	12890	195000
	28860	513000	26099	338000	12555	208000	67514	1059000