BACTERIAL FERMENTATION AS A POSSIBLE SOURCE OF THE TITRATABLE ACIDITY OF FRESH MILK.

H. S. Samson, B. Sc. (1934).

April, 1936.

A SUB - THESIS

SUPPLEMENTING A THESIS ENTITLED

"THE CAUSE OF THE ACIDITY OF FRESH MILK."

SUBMITTED TO

THE COMMITTEE ON POST GRADUATE STUDIES

OF THE

UNIVERSITY OF MANITOBA

IN PARTIAL FULFILMENT

OF THE DEGREE

OF

MASTER OF SCIENCE

APRIL 1936.

The writer wishes to express his appreciation and thanks for the assistance and many kind suggestions received from Professor N. James.

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BACTERIAL FERMENTATION AS A POSSIBLE SOURCE OF THE TITRATABLE ACIDITY OF FRESH MILK.

A discussion of the source of the titratable acidity of fresh milk would not be complete without a survey of the role played by organisms capable of producing acid fermentation in milk. Especially is this true since titratable acidity is used generally as a basis for judging the quality of milk on the assumption that an increase in acidity from the normal indicates undesirable bacterial fermentation at some stage of the production of the milk.

Several instances have been reported which would tend to show that this assumption is of doubtful value. Fresh mixed herd milk has been reported as varying in acidity over a wide range in certain specific cases and to give no visible evidence of bacterial fermentation as the underlying cause for the deviation from the normal acidity. In the particular case under investigation, acidities of fresh herd milk ranged from 0.16% to 0.21% as compared to the normal average acidity of 0.14% to 0.16% generally accepted for most herds.

Inspection at the farm on which the milk was produced failed to show any factor or factors which might lead to the belief that insufficient attention was being paid to the proper handling of the milk from a bacteriological standpoint. The herd consisted of normal purebred Jersey cows maintained in sanitary surroundings. Milking was done by milking machines and cows stripped by hand. With regards to utensils, these were found to be in good condition and sterilization accomplished with boiling water. Temperatures of cooling and holding subsequent to shipment were satisfactory. Were bacterial fermentation to be the cause of the high titratable acidity, such fermentation must occur at the time of milking.

It is proposed therefore, to show by means of references to available literature and also by direct experimental evidence, that the possibility of the cause of the high titratable acidity being due to bacterial fermentation is slight, if not entirely negligible.

HISTORICAL.

Acid-forming Bacteria found in Milk.

The acid-forming bacteria of milk are of a large group comprising such types as microccoci, staphyloccoci and streptoccoci from the udder, mouth and feces of the cow; true lactic acid bacteria and variants; gas-forming bacteria of the colon-aerogenes group; types producing volatile acids such as acetic, propionic and butyric acids; and those producing acids as decomposition products. Mixed fermentations may occur which in addition to lactic acid, produce small quantities of acetic, propionic, succinic, formic and butyric acids. Under suitable conditions with regard to temperature, thermophilic types may produce acid.

For the purpose of this discussion, since acid production of the fresh milk by bacterial action is of main interest, the types requiring special conditions for growth will not be considered. Similarly, consideration of the types producing volatile acids in minute quantities or traces will be omitted.

Hammer (1928), lists as the groups mainly responsible for the production of lactic acid, the following:

(1). The *Streptoccocus lactis* group.

(2). The *Lactobacillus* group.

(3). The *Escherichia-Aerobacter* group.

Streptococcus lactis and variants produce chiefly lactic acid with a small amount of acetic acid. Together with the lactobacillus group, they are considered the "true lactic acid bacteria" and usually predominate in numbers and in production of acid in the early stages of fermentation. The temperature favoring the most rapid acid development is from 30°C to 40°C. Associative action of other types of bacteria may cause a variation in the quantity of lactic acid produced by these types as has been pointed out by Hammer.

The Escherichia-Aerobacter types produce both acid and gases. If the true lactic acid bacteria are low in numbers, the Escherichia-Aerobacter types may predominate to give a gassy type of fermentation with a high percentage of volatile acids. The amount of acid produced varies with oxygen conditions, more acid being produced in the anaerobic than in the aerobic fermentation. Most favorable temperatures are around 37°C.

Frazier and Sanders (1935) state that many of the micrococci present in milk are acid producers but that in comparison with the lactic streptococci, they are inactive and play only a minor part in the early stages of fermentation.

The amount of the various acids formed by types of organisms present in milk varies, according to Sommer (1935), with the types present. The bulk of the acid formed is lactic acid, to the extent of from 80% to 90% with acetic acid as the next largest proportion.

Sources of Contamination.

Organisms gaining access to the milk may be many and varied in types depending upon conditions of production. It is agreed that organisms may be present in the ducts and teat canal of the udder and subsequently carried into the milk. Sherman (1914) states that the types most commonly found in the udder are of four groups; staphylococci, streptococci, diptheroids and minute Gram-negative rod forms. The streptococci of the udder were differentiated from Streptococcus lactis by Rogers and Dahlberg (1914) and do not form lactic acid to the same extent. Other investigators found that the udder would not support the growth of Streptococcus lactis, as the organism when injected into the udder through the teat canal dies off rapidly. Lactobacilli and organisms of the Escherichia-Aerobacter group are not found in the udder.

The commonest sources of the three acid-producing types are, the coat of the animal, manural pollution, feeds, especially grains and silage, and utensils. Unsterile utensils provide the main source of Streptococcus lactis contamination. The milking machine in particular is a potential seed bed of contamination if not properly sterilized.

<u>The Number of Organisms Contributed by Various Sources.</u>

The number of bacteria present in milk in the udder is generally low. Harding and Wilson (1913) give 500 per c.c. as an average value for the number of bacteria present in the milk of the normal udder. Other investigators report similarly low figures. The types present have been shown to contribute little to acid production of the freshly drawn milk. The numbers gaining access to the milk from such sources as air, dirt and manure falling into the milk, etc., may vary considerably but with average conditions of cleanliness, the number so introduced is as a rule, less than 5000 per c.c. of milk. (Sherman 1914).

The main source of contamination with acid-producing bacteria has been shown to be utensils, strainers and milking machines. Here again the number introduced depends upon the state of cleanliness and sterility observed. Sherman (1935), states, "While it is true that under ordinary farm conditions the utensils are responsible for the largest number of bacteria of any single source of contamination, it should be recognized, on the other hand, that under good conditions where proper methods of sterilization and drying of equipment are practiced, the utensils constitute only a minor source of contamination."

Taken collectively, the number of bacteria introduced by the various sources of contamination can be reduced to a small figure by proper attention to cleanliness and sterilization of equipment in the production of milk. Freshly drawn milk under ordinary conditions shows a bacteria content of less than 50,000 per c.c.

Rate of Growth.

The factors favoring rate of growth of bacteria in milk are temperature and time depending upon species present for rate of acid production. (Heineman et al.). Since freshly drawn milk is considered in this discussion, the time factor is limited to three hours at the most. Most of the data available is concerned with growth rates involving periods of time over 12 hours. Tapernoux (1928) states that the lactic acid fermentation in milk is a function of time and follows the law of geometric proportions. The holding time in this case however, is so short that the germicidal property of fresh milk would have more effect on the numbers of bacteria then any growth factors.

The germicidal property of fresh milk has not been explained in a satisfactory manner but the effect has been noted by many investigators. The effect is a reduction in the number of bacteria present in freshly drawn milk as if the milk possessed a property similar to blood and certain other physiological fluids of causing a reduction in numbers of bacteria through a germicidal action. This germicidal property is more active in the udder.

It has been shown by Heineman to last from four to six hours in milk kept at 37°C. and in milk kept at a lower temperature, may continue as long as twenty-four hours. The effect while feeble and variable, is capable of resulting in a reduction in the numbers of bacteria contained in milk during a three hour time factor. Cooling of the freshly drawn milk to 40°F. is an added factor in retarding acid production by bacteria in fresh milk.

The Number of Bacteria Needed to Produce Acid Fermentation.

Van Slyke and Bosworth (1916) using sterile milk inoculated with streptococci, show that the most rapid acid formation occurs during the interval from the tenth to the twenty-fifth hour. This would indicate that in the case of fresh milk, regardless of the number of bacteria present, the rate of acid formation would not be rapid in the first three hours. To increase the acidity but not show distinct sourness, Hammer and Hix (1916) report requires at least a bacteria content in milk of and average of 56,000,000 per c.c. Also, to show any change or abnormality whatever, requires a bacteria content of over 1,000,000 per c.c. Sommer (1935) states that Swanner, using the Breed direct microscopic count reports the number of bacteria in milk to increase to about 60,000,000 per c.c. before there is any measurable rise in titratable acidity.

EXPERIMENTAL.

Source of Samples.

Samples were obtained from the milk of 15 purebred cows owned by Mr. N. N. Smith of Birds Hill, Manitoba. Each sample was from the mixed complete milking of the individual cow, and taken from the evening milking. Samples were taken in sterile half-pint bottles with sterile pipettes and held at 40°F. until analyzed. Time interval between sampling and analysis was approximately three hours.

Methods.

Macroscopic colony counts were made on each sample in duplicate according to the method outlined in "Standard Methods of Milk Analysis", Fifth Edition, 1927. Dilutions were; 1/10, 1/100, and 1/1000 . Results recorded in Table 1. represent in each case the average of duplicate determinations.

On the same samples, the Methylene Blue reduction test was made according to method shown in "Standard Methods of Milk Analysis", Fifth Edition, 1927. Results of the Methylene Blue reduction tests are recorded in terms of hours required for reduction to the leuco-base.

Titratable acidity was determined by titrating a 50 c.c. sample of milk with 0.1 N. sodium hydroxide using ten drops of a 1% alcoholic solution of phenolphthalein as the indicator. Results are expressed as percent acidity calculated as lactic acid.

RESULTS.

Table 1. gives the results of the Macroscopic Colony counts obtained from the various samples compared to the Titratable acidity.

Table 11. gives the results of the Methylene Blue tests as compared to Titratable acidity. The system of classification shown in " Standard Methods", is adopted, viz;

"Class 1. - Good milk, not decolorized in five and a half hours, developing, as a rule, less than one-half million colonies per c.c. on agar plates."

- TABLE 1. -

Showing Relation of Titratable Acidity to Macroscopic Colony Count.

Sample No.	Titratable Acidity %	Bacteria per c.c. Official Method.
1	0.18	121,000
2	0.22	80,000
3	0.17	54,000
4	0.195	90,000
5	0.18	170,000
6	0.23	60,000
7	0.21	210,000
8	0.19	44,000
9	0.215	162,000
10	0.20	33,000
11	0.17	230,000
12	0.185	163,000
13	0.17	97,000
14	0.24	105,000
15	0.215	183,000

- TABLE 11. -

Showing Relation of Titratable Acidity to Methylene Blue Reduction.

Sample No.	Titratable Acidity %	Reduction Time Hours	Class
1	0.18	over $5\frac{1}{2}$ hours	1.
2	0.22	"	"
3	0.17	"	"
4	0.195	"	"
5	0.18	"	"
6	0.23	"	"
7	0.21	"	"
8	0.19	"	"
9	0.215	"	"
10	0.20	"	"
11	0.17	"	"
12	0.185	"	"
13	0.17	"	"
14	0.24	"	"
15	0.215	"	"

The results show little apparent evidence of cor-realation between acidity and bacterial counts. The sample with 0.17% titratable acidity, the lowest reported, gave bacterial counts ranging from among the lowest to the highest. Those with the highest acidity, 0.24%, 0.23%, and 0.22% , showed counts intermed-iate between these limits.

Similarly, the variation in titratable acidity shows no correlation to Class as determined by the Methylene Blue reduction, all samples grading Class 1.

DISCUSSION.

As has been pointed out by reference to the work of various investigators, the types of bacteria found in fresh milk which are capable of producing acid by fermentation, are of three main groups. Further, these are not present in the milk in the udder but enter the milk by subsequent contamination from other sources. The numbers of bacteria contributed by the various sources are low, even under only average conditions of cleanliness and sterilization. In the particular case under investigation, sanitary conditions and methods employed in the production of the milk were mush better than average conditions. This would suggest that the possibility of contamination of the milk with large numbers of acid-producing types of bacteria is slight. The time and temperature factors in fresh milk held at 40°F. for three hours are not suggestive of rapid acid production even if we assume the presence of large numbers of the proper species of bacteria.

It was shown by investigators that to produce any abnormality in milk, at least 1,000,000 bacteria per c.c. must be present in the milk. The results in Table 1., show the general low bacterial count of the milk examined, all samples being below

300,000 bacteria per c.c. If, as has been shown by various workers, 50,000,000 bacteria per c.c. at least are nesessary to produce any appreciable increase in titratable acidity, then there is little possibility of 300,000 bacteria per c.c. causing an increase.

Experimentally it is shown that the bacterial count of the milk under examination was low and also that there is no evidence apparent of correlation between titratable acidity and the number of bacteria present. Table 11. presents more convincing evidence in that the milk of highest acidity, i.e., 0.24% graded as Class 1.

This demonstrates the fallacy of assuming in all cases that the titratable acidity, when over 0.18%, is evidence of acid production by bacterial fermentation.

SUMMARY AND CONCLUSION.

Reference to the work of various investigators and direct experimental evidence is offered to show that bacterial fermentation is not the cause of the titratable acidity of fresh milk.

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