The Influence of Ammonia on the Development of Rancidity in Milk

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AMONG the large and growing list of things that have been shown to influence the activity of lipase in milk, no reference can be found to ammonia. The results of the experiments described in this paper show that ammonia, either added to the milk in the form of an aqueous solution or absorbed by the milk in the form of a gas, has a marked effect on the development of rancidity.

EXPERIMENTAL

The source of the ammonia used in these experiments was a 28 percent solution prepared by Canadian Industries Limited. The quantities used are always designated as the amount of ammonia (NH_3) per volume of milk.

The experiments were all done with winter milk (between January 1 and May 1) from a herd of Jersey, Holstein, and Ayrshire cattle. There were also included two dual purpose Shorthorns. The general procedure was to obtain a composite sample of milk from each cow and place them immediately in an ice box held at 5° C. The activity of the lipase was followed by three different methods. The first was a modification of the method used by Rice and Markley (1): 10 ml. of milk were added to 100 ml. of cream (approximately 25 percent fat), saturated with cane sugar, and this mixture was incubated at 37° C. Increases in acidity were observed by periodically titrating 10 ml. of the mixture in 50 ml. of distilled water against N/10 NaOH with phenophthalein as an indicator. The second method followed the decreases of surface tension of milk when stored at 5° C., using a Cenco-du Nouy tentiometer. Thirdly, the development of rancidity was observed by noting changes in the flavor of the milk. In no instance was milk classified as rancid unless it had a definite and unmistakable butyric-acid-like flavor. Suspicious or slightly rancid flavors were ignored.

These are all preliminary experiments planned to determine whether addition of small amounts of ammonia to milk accelerates the production of rancidity. In this paper no attempt is made to work out a detailed relationship between the amounts of ammonia added and the effects they produce. Nor is any attempt made to determine whether the ammonia activates the lipase or simply changes the physical or chemical condition of certain constituents of the milk in such a way as to favor conditions for lipase activity. For this reason, most of the experimental work deals with the production of a rancid flavor and decreases in surface tension of the milk itself, rather than with the action of lipase on fatty substrates other than milk.

PRESENTATION OF DATA

The effect of ammonia on the milk from individual cows. The first tests were made with milk from 9 Holstein cows. One of these, No. 7, had a previous history of mastitis; the others were all normal cows. Ten ml. of milk from each cow were added to 100 ml.

of sugar-cream syrup and incubated at 37° C. Two sets of 100 ml. samples of milk from each cow were held at 5° C. To one set, sufficient ammonia was added to give a concentration of 1 : 5,000. After 4 days, the decreases in surface tension of the milk samples were compared with the increases in titratable acidity in the sugar-cream mixtures, measured in ml. of N/10 NaOH required to neutralize the increased acidity in sugar-cream syrups incubated for the same period at 37° C. The results are shown in Table 1.

TABLE 1

CHANGES IN SURFACE TENSION AND ACIDITY BY ADDITION OF AMMONIA

Cow No.	*Acidity*[1] Ml. N/10 NaOH	*Surface Tension* No NH_3 Added	*Surface Tension* NH_3 Added
11	1.25 (1)	50.0⊠	42.7
33	.85	48.5	45.3
26	.85	49.0	45.7
29	.75	46.0	46.6
14	.75	50.0	46.7
25	.70	48.6	46.9
27	.65	48.7	47.5
17	.65	50.0	48.0
7	.60	50.0	50.0

(1) Increase in acidity over control samples. ⊠ Dynes per cm. at 23° C.

Two things are noticeable in these results:

(1) Some of the milk samples to which ammonia had been added had a much lower surface tension than the corresponding untreated samples; others remained similar to the untreated milk; and in no instance was the addition of ammonia accompanied by an increase in surface tension over that of the normal milk. (2) There is a closer co-relation between the increases in titratable acidity in the sugar-cream mixture and the decrease of surface tension of the milk samples when ammonia had been added. The discrepancy between the increased acidity (in the cream-sugar mixture) and decreased surface tension with the milk from certain individual cows had been noted in periodic tests over a period of three months.

The second series of tests was made with milk from 32 cows. The ammonia was added to give a concentration of 1 : 3,000. Surface tension readings were taken at 24, 48, and 120 hours. They were also tasted for flavor changes. It should be stated that as well as No. 7, the mastitic cow, there was one other animal producing abnormal milk. Cow No. 79 was a ten year old Jersey at the very end of her lactation period. She had been previously giving milk that very rapidly developed a strongly rancid flavor when held at a low temperature.

The results of the surface tension readings were similar to those of the group previously given. The action of ammonia in lowering the surface tension below that of the untreated milk varied greatly with the samples from different cows. Figure 1 shows 5 examples where this effect was the greatest and 7 examples where the effect was the least for the first period of 24 hours. It is very significant that in the case of Cow No. 79, which had been producing milk with "naturally activated lipase," the results are practically the same with and without added ammonia. At 24 hours the average of surface tension readings of the samples

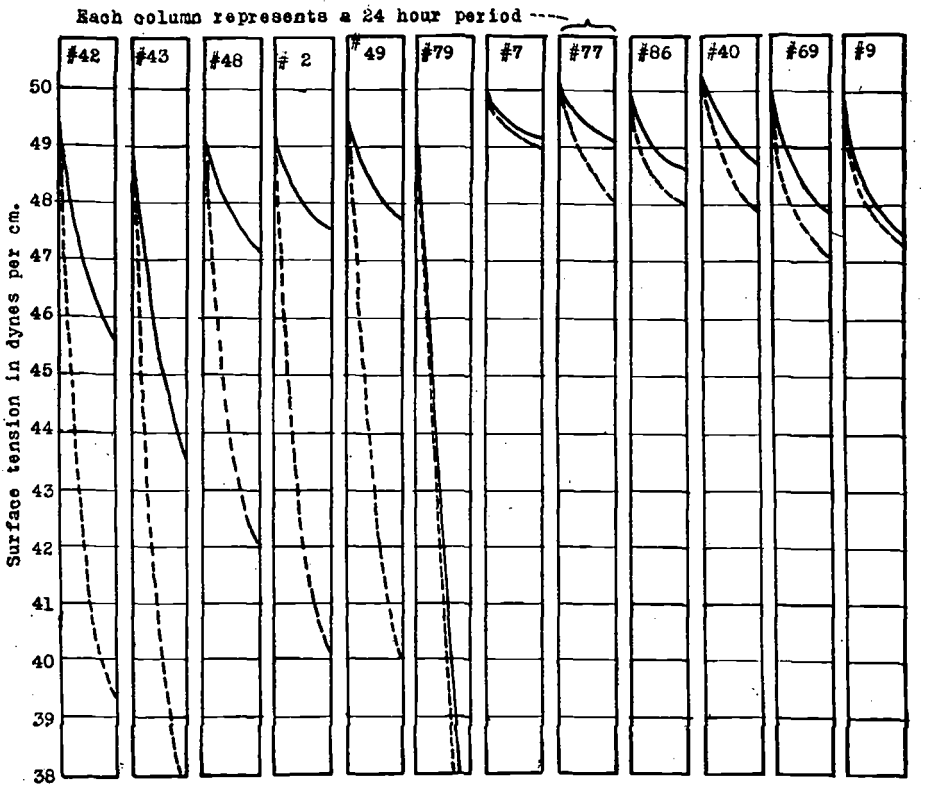

FIGURE 1

Decreases in surface tension during 24 hours storage at 5° C. for milk samples from 12 cows. The unbroken lines indicate untreated milk and the broken lines milk to which 1 part of ammonia had been added to 3,000 parts of milk.

to which ammonia had been added was 2.8 dynes lower than the average of the untreated milks; at 48 hours there was a difference of 3.0 dynes and at 120 hours it had increased to 4.7 dynes. Excluding the two abnormal cows (No. 7 and No. 79), and two Shorthorns, the following gives some idea of the effect of ammonia in lowering the surface tension below that reached by the untreated milk, for the three breeds tested:

That the reduction of surface tension was caused by formation of fatty acids is shown by the fact that such decreases were accompanied by the development of rancidity, as shown by the following:

	Average Decrease in S.T. below Untreated Milk Samples*		
Breed	At 24 hours	At 48 hours	At 120 hours
Jerseys (6 cows).....	2.43 dynes	1.76 dynes	2.83 dynes
Holsteins (12 cows).....	2.40 "	3.83 "	5.48 "
Ayrshires (10 cows).....	3.90 "	3.99 "	5.42 "

* S.T. = Surface Tension.

Treatment	Number of Rancid Samples		
	At 24 hours	At 48 hours	At 120 hours
Plain milk	1 (# 79)	1 (# 79)	4
Ammonia added	6	8	19

It is interesting to note that the milk from the 5 cows which developed a rancid flavor when ammonia was added, at 24 hours (not counting No. 79) were from 4 Ayrshires and 1 Holstein. At 120 hours, where ammonia was added, the rancid samples came from 2 Jerseys, 7 Holsteins and 8 Ayrshires.

Variations in Amounts of Ammonia Used. Milk samples from 8 cows were selected, of which 4 had been found previously to be markedly affected by the addition of ammonia and 4 which were only slightly affected. These samples were divided into 5 sets of 150 ml. each, and ammonia was added so as to give the following concentrations: 1:2,800, 1:14,000, 1:140,000, 1:1,400,000, and a control set without added ammonia. They were held at 5° C., and their flavor and surface tension tested at 36 hours, 4 days, and 7 days. Table 2 gives the average surface tensions for each of the groups at the various times when they were tested. These results indicate that ammonia to the strength of 1 part in 14,000 parts of milk is effective in increasing lipolytic activity in milk, but not so effective as 1 part in 2,800; and further, that 1 part in 140,000 is insufficient to bring about any noticeable change.

Absorption of Gaseous Ammonia. In the first of these tests, two glass desiccator jars were used, having a capacity of 2.5 liters. Into each jar were placed 7 duplicate milk samples in small, screw-topped, wide-mouthed bottles, holding 25 ml. each. One out of each pair of duplicate samples had the lid of the bottle tightly screwed down; the other was left uncapped. Into the bottom of the first jar was placed a dish containing 1 ml. of concentrated (28 percent) ammonia solution. Into the other, a similar dish was placed containing 1 ml. of concentrated ammonia that had been diluted 50 times. These were then held at 5° C., and samples were tested at 36, 48, and 110 hours for changes in flavor and surface tension.

In the desiccator jar containing 1 ml. of concentrated solution, so much ammonia was absorbed by the uncapped samples that marked changes occurred in the appearance of the milk. The portion of the milk below the cream layer lost its opaqueness and became almost watery. The samples had a very strong flavor of ammonia and the pH became strongly alkaline. These were discarded. Table 3 gives the results from the milk samples in the jar containing one fiftieth as much ammonia. There was no observable difference in the appearance of the capped and uncapped bottles in this jar. The milk from the first 4 of these cows (Nos. 42, 43, 48, and 53) had been shown in previous tests to undergo a larger additional reduction in surface tension when ammonia has been added than the remaining two (Nos. 76 and 71). These results indicate that ammonia absorbed by milk from the atmosphere is also effective in increasing the lipase activity in milk. In two of these cases the difference between the treated and untreated samples was sufficient to produce a rancid flavor in the former.

TABLE 2

THE EFFECT OF ADDING VARIOUS CONCENTRATIONS OF AMMONIA ON THE AVERAGE SURFACE TENSION READINGS OF TWO GROUPS OF MILK SAMPLES HELD AT 5° C.

Length of Holding Period		*Control* (Nothing added)	*Concentration of Ammonia* 1: 2,800	1: 14,000	1: 140,000	1: 1,400,000
36 hours	Group I	44.5☒ (1)	39.1 (3)	40.3 (2)	45.6 (1)	44.6 (1)
	Group II	47.6 (0)	44.7 (0)	46.2 (0)	46.9 (0)	47.9 (0)
4 days	Group I	44.7 (1)	36.5 (3)	42.0 (2)	44.4 (1)	44.7 (1)
	Group II	45.9 (0)	42.7 (1)	44.6 (1)	45.0 (0)	45.1 (0)
7 days	Group I	37.7 (4)	34.5 (4)	37.4 (4)	38.5 (4)	39.3 (4)
	Group II	43.9 (1)	41.8 (1)	44.3 (1)	44.5 (1)	44.6 (1)

☒ Dynes per cm. at 23° C.
The figures in brackets indicate the number of samples that had a rancid flavor in each group which had a total of 4 samples.

In a second test, where milk was exposed to an atmosphere containing ammonia, the period of exposure was greatly reduced. A glass box (26″ x 23″ x 19″), similar to those used for protecting balances, was used in place of the desiccator jar. Although closely fitted, it was not completely air tight. Two petri dishes, each containing 1 ml. of concentrated ammonia (28 percent) were placed in opposite corners of the glass box. Five hundred ml. of milk were placed in a wide-mouthed glass jar fitted with a syphon delivering into a similar jar below it. It took five minutes for the milk to run from one container to the other, and the milk was left exposed to the ammonia for another 25 minutes. With this treatment it was hoped that an approximation of the most extreme conditions of milking in an atmosphere containing ammonia could be obtained. The milk used was a composite sample from 4 cows.

TABLE 3

CHANGES IN SURFACE TENSION IN CAPPED AND UNCAPPED MILK IN A DESICCATOR JAR CONTAINING 1 ML. OF A 0.56 PERCENT AMMONIA SOLUTION

Cow No.	*Containers*	*36 Hours*	*48 Hours*	*110 Hours*
42	Uncapped	45.5☒	44.2	39.9 (rancid)
	Capped	45.3	44.5	41.9
43	Uncapped	43.3	41.5	39.2 (rancid)
	Capped	44.2	42.3	43.0
48	Uncapped	48.7	47.1	45.3
	Capped	48.9	48.7	47.2
53	Uncapped	44.3	43.9	41.9
	Capped	46.4	46.7	45.0
76	Uncapped	48.4	45.9	45.6
	Capped	45.4	47.5	45.5
71	Uncapped	46.2	46.2	43.2
	Capped	46.6	46.8	43.8

☒ Dynes per cm. at 23°C.

A control of the same milk in a tightly stoppered bottle was kept beside the treated sample so as to ensure approximately similar temperature treatment. The milk was held at 5° C. In this, as in all other tests, the glassware had been previously sterilized and every effort was made to keep conditions as aseptic as possible. As indicated by Table 4, even this half-hour exposure was sufficient to bring about

TABLE 4

EFFECT OF AMMONIACAL ATMOSPHERE ON SURFACE TENSION OF MILK

	14 Hours	*24 Hours*	*48 Hours*	*72 Hours*	*120 Hours*
Treated milk	47.4☒	46.0	45.7	45.0	45.3
Untreated milk	48.9	47.8	47.8	47.6	48.0

☒ Dynes per cm. at 23° C.

an increased reduction in the surface tension of the milk over that of the untreated sample.

RELATION OF THE EFFECT OF AMMONIA TO OTHER CHARACTERISTICS OF THE MILK

From the college herd 8 cows were selected whose milk was most affected by the addition of ammonia, and these were compared with the 8 which were least affected by the addition of ammonia. After 24 hours at 5° C. the average additional drop in surface tension of the first group when ammonia (1:3,000) was added, was 5.7 dynes per cm.; the corresponding decrease for the second group was 0.6 dyne. The average of the results of these comparisons is as follows:

	Group I	*Group II*
a. Surface tension of untreated milk after 24 hours at 5° C. (dynes per cm.)	46.7	47.7
b. Increased titratable acidity in sugar-cream syrup after 12 days at 37° C. (ml. of $N/_{10}$ NaOH)	1.75	1.77
c. Amount of milk at the milking when these samples were taken (pounds)	23.1	18.9
d. Age of cows (years)	4.1	4.7
e. Length of lactation period (days)	128	85

Although there is insufficient data to make any positive statements regarding these relationships, these results strongly suggest that the "ammonia factor" is independent of the amount of lipase, the volume of milk secreted and the age of the cows, and that it may be related to the changes that occur in milk as the lactation period progresses.

SUMMARY AND DISCUSSION

It has been shown that the addition of ammonia accelerates the production of rancidity in milk, as indicated by the development of a typical butyric-acid-like, rancid flavor, and the reduction of its surface tension when held at 5° C. This effect differs with the milk from different cows, and to a lesser degree with different breeds of animals. It increases as the lactation period progresses.

From the results obtained in these preliminary experiments it seems doubtful, except in very extreme cases, if the concentration of ammonia in the atmosphere of the barn where cows are being milked would ever be a major factor causing rancidity to develop in the milk or cream. However, during the late winter and early spring a strong odor of this gas is not uncommon in many barns, and might very well be a contributing factor to those undesirable flavors in milk and cream which precedes the actual development of butyric-acid-like rancidity. The question also arises as to whether the occasional breakdown in refrigeration units, accompanied by a release of ammonia in the atmosphere, may also accelerate the development of rancidity in unpasteurized milk or cream.

Apart from these practical considerations it seems probable that further study of these findings may shed some more light on the total mechanism of

lipase action in milk, in contrast to the action of the enzyme in a less complex substrate.

CONCLUSIONS

It has been shown that small amounts of ammonia added to milk held at low temperatures, accelerates the reduction of surface tension and hastens the development of rancid flavors.

REFERENCE

1. Rice, F. E., and Markley, A. L. *J. Dairy Sci.*, **5**, 64–84 (1922).

STUDY CURD STRENGTH OF EVAPORATED MILK

So-called re-made evaporated milk, which is simply evaporated milk to which water has been added, has an exceptionally soft curd according to findings in the dairy laboratory at the State Experiment Station at Geneva. The results of the studies are reported by Prof. J. C. Marquardt in a recent issue of the *Journal of Dairy Science*. The study was conducted to evaluate definitely the curd strength of evaporated milk as commonly used.

During 1941, more than six billion pounds of milk were utilized in the production of 3,165,906,000 pounds of evaporated milk, representing a 50 percent increase over the past five years, according to Professor Marquardt. Because of the increased use of evaporated milk, research was undertaken in the Station dairy laboratory on the curd strength and other properties of the product at the request of the Evaporated Milk Association.

In these studies it was demonstrated that re-made evaporated milk as generally used as a food for infants has a curd strength that complies with the standards set by the American Medical Association for the curd strength for soft curd milk. In fact, re-made evaporated milk was found to have a softer curd than other types of milk designed for infant feeding, such as homogenized milk and so-called special milks.

Re-made evaporated milk will not separate into layers of varying composition upon standing as does whole untreated milk. This is regarded as an important advantage as it insures uniformity when used in the home. Through the cooperation of the Evaporated Milk Association, samples of evaporated milk of comparable composition were obtained from many sections of the United States, thus aiding greatly in the conduct of the experiments.