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Carbon dioxid or carbonic acid gas, is a transparent gas under ordinary atmospheric pressure and temperature. It is readily soluble in water and the solubility is increased under pressure. Water charged with this gas is known as soda water, or as carbonated water. Such water has a pleasant, acid, pungent taste, and on account of this many of the soft drinks are charged with it.

The use of carbon dioxid in dairy products is not a new idea. The ice cream soda, which consists largely of ice cream suspended in carbonated water, has been known for many years. Some of the fermented drinks, such as kumis and kefir, become charged with carbon dioxid as a result of fermentation, and these drinks have been known for generations. In 1906 Van Slyke and Bosworth[1] conducted some experiments on carbonated milk. They concluded that it makes a pleasant beverage and may find practical use as a healthful drink.

From the sanitary standpoint, the use of carbon dioxid in foods and in drinks is of special interest. It is a well known fact that in general carbon dioxid atmosphere is injurious to animals and to plants. It has also been known that under certain conditions this gas is harmful to certain bacteria. On these accounts, various claims have been made as to the sanitary quality of carbonated drinks and carbonated foods.

This circular is a preliminary report of experiments to determine the influence of carbon dioxid on the germ life in carbonated dairy products. It includes a brief summary of four experiments: (1) effect of carbon dioxid on germ life in carbonated ice cream; (2) effect of carbon dioxid on typhoid bacteria in carbonated ice cream; (3) behavior of bacteria in carbon dioxid atmosphere; (4) effect of carbon dioxid on bacteria in milk under pressure.

The plate method was used in counting the bacteria. In sampling the hardened ice cream, a long cylindrical plug of the ice cream was taken by means of a cheese trier and put into a glass bottle. After it had melted, the desired dilutions were made from it and it was then plated. The medium used for the plating was lactose agar having the standard composition. The plates were incubated

[1]L. L. Van Slyke and Alfred W. Bosworth. Effect of Treating Milk with Carbon Dioxid Gas Under Pressure. N. Y. (Geneva) Agr. Exp. Sta. Bul. 292. 1907.

4 days at room temperature and one day at 86 degrees Fahrenheit. The counts as given are averages of two or more plates in each case.

The plates from the material with typhoid bacteria were incubated only one day at 100 degrees Fahrenheit.

EXPERIMENT 1

EFFECT OF CARBON DIOXID ON GERM LIFE IN CARBONATED ICE CREAM

In this experiment seven 12-gallon lots of ice cream were prepared. Each lot was divided into two portions; the first was frozen in the usual way, and the second was frozen in carbon dioxid atmosphere. A 50-quart Perfection Dreadnaught Brine freezer was used. The following procedure was used in freezing the ice cream in carbon dioxid: A rubber tube was fastened to the nozzle of a carbon dioxid tank, and the loose end of the tube was inserted into the freezer thru the fruit hopper. The gas was then blown into the freezer for about 30 seconds. The ice cream mix was then poured into the freezer. After the freezer was run for a few minutes, the carbon dioxid was again blown in for about 30 seconds, the loose end of the rubber tube being inserted under the surface of the mix and the gas allowed to bubble thru it. As soon as the ice cream was frozen, it was put into two-gallon storage cans which were placed in the hardening room and kept there during the experiment. The temperature of the hardening room averaged about two degrees below zero Fahrenheit, varying between ten below and eight above.

Samples for the bacteriological examination were taken, first, from the mix prior to the freezing, and then from the ice cream, both the plain and the carbonated, immediately after it was frozen. Subsequent samples were taken at frequent intervals during the experiment. The results of the bacteriological examinations are given in Table 1.

In comparing the bacterial counts in the plain and in the carbonated ice cream as shown in Table 1, we are forced to the conclusion that the carbon dioxid gas did not cause any appreciable reduction in the number of bacteria in the carbonated ice cream. If it did destroy any bacteria, the number was so small that our method did not detect it. One of the lots of ice cream which is not included in this table was kept in the hardening room for six months. Even after this long period of time the carbonated ice cream had just as many bacteria as the plain ice cream.

TABLE 1.—EFFECT OF CARBON DIOXID ON THE BACTERIA IN CARBONATED ICE CREAM

Bacteria per cc. of ice cream

LOT 1		
Samples	Plain	Carbonated
Mix	*620,000*	
Freshly frozen	703 000	765 500
1 day old	356 000	928 000
2 days old	789 000	832 000
4 days old	640 000	644 000
6 days old	963 000	1 070 000
8 days old	581 000	780 000
11 days old	474 000	616 000
14 days old	686 000	725 000
18 days old	1 249 000	518 000
22 days old	578 500	502 000
26 days old	647 000	578 000
30 days old	534 000	585 000
34 days old	607 000	409 000
40 days old	472 000	432 000
49 days old	536 000	710 000
58 days old	530 000	742 000

LOT 2		
Samples	Plain	Carbonated
Mix	*341,000*	
Freshly frozen	534 000	408 000
1 day old	629 000	506 000
2 days old	620 000	683 000
3 days old	622 000	571 000
4 days old	480 000	598 000
7 days old	666 000	510 500
9 days old	867 000	559 000
11 days old	420 000	435 000
14 days old	341 000	526 000
17 days old	436 000	555 000
20 days old	442 000	429 000
24 days old	565 000	450 000
27 days old	388 000	340 500
32 days old	794 000	552 000
34 days old	258 000	303 000
42 days old	492 000	445 000
51 days old	415 000	419 000

LOT 3		
Samples	Plain	Carbonated
Mix	*715,000*	
Freshly frozen	1 255 000	910 000
1 day old	1 004 000	777 000
2 days old	699 000	1 017 000
3 days old	750 000	708 000
4 days old	694 000	670 000
6 days old	607 000	891 000
9 days old	836 000	787 000
12 days old	207 000	212 000
14 days old	730 000	731 000
17 days old	677 000	558 000
20 days old	523 000	536 000
24 days old	388 000	455 000
28 days old	537 000	505 000
31 days old	477 000	330 000
39 days old	629 000	715 000

LOT 4		
Samples	Plain	Carbonated
Mix	*26,325,000*	
Freshly frozen	36 725 000	28 600 000
1 day old	22 430 000	21 450 000
2 days old	6 370 000	23 800 000
3 days old	17 880 000	13 980 000
5 days old	23 700 000	19 930 000
7 days old	29 250 000	37 700 000
10 days old	16 250 000	16 250 000
13 days old	16 420 000	15 600 000
16 days old	14 625 000	14 300 000
19 days old	8 410 000	9 610 000
21 days old	13 000 000	10 820 000
24 days old	17 550 000	16 250 000
51 days old	2 060 000	3 130 000

LOT 5		
Samples	Plain	Carbonated
Mix	*13,800,000*	
Freshly frozen	15 930 000	14 950 000
1 day old	10 550 000	15 600 000
2 days old	18 200 000	15 280 000
3 days old	8 800 000	11 380 000
5 days old	18 530 000	17 930 000
7 days old	15 330 000	18 200 000
9 days old	8 450 000	8 450 000
11 days old	11 050 000	14 300 000
13 days old	18 200 000	16 250 000
17 days old	10 070 000	6 500 000
28 days old	8 440 000	10 988 000
32 days old	14 300 000	11 400 000
40 days old	10 500 000	16 250 000
53 days old	3 340 000	3 480 000

LOT 6		
Samples	Plain	Carbonated
Mix	*98,000*	
Freshly frozen	56 500	121 000
2 days old	51 000	65 000
3 days old	52 000	52 000
4 days old	58 000	50 000
5 days old	40 000	46 000
6 days old	55 000	54 000
7 days old	52 000	84 500
8 days old	56 000	45 500
10 days old	7 000	6 150
11 days old	55 000	51 500
12 days old	43 000	35 000
13 days old	35 000	53 500
14 days old	34 000	54 000
15 days old	33 500	52 500
16 days old	5 000	32 000
17 days old	43 500	34 000
18 days old	45 000	53 500
22 days old	35 000	43 000
24 days old	44 500	76 000
33 days old	18 000	22 500
46 days old	30 000	50 000

EXPERIMENT 2

EFFECT OF CARBON DIOXID ON TYPHOID BACTERIA IN CARBONATED ICE CREAM

It was seen in Experiment 1 that the bacteria in the carbonated ice cream were not appreciably harmed. However, these bacteria were not pathogenic. Now it is a well known fact that the pathogenic bacteria, as a rule, are more sensitive to environmental conditions than the non-pathogenic. It was therefore necessary to determine how the pathogenic bacteria would behave in carbonated ice cream. Typhoid bacteria were selected for this purpose.

About four quarts of ice cream mix were sterilized and then heavily inoculated with the typhoid bacteria. Half of the mix was frozen plain and half was frozen in carbon dioxid. A one-gallon White Mountain freezer was used for this purpose.

Samples were taken from the mix prior to freezing and then from both the plain and the carbonated ice cream immediately after freezing. The ice cream was then stored in the hardening room and samples were again taken five and twelve days later. The results of the examination of these samples are shown in Table 2.

TABLE 2.—EFFECT OF CARBON DIOXID ON TYPHOID BACTERIA IN CARBONATED ICE CREAM

Bacteria per cc. of ice cream

Samples	Plain	Carbonated
Mix	*24,700,000*	
Freshly frozen	50 050 000	50 700 000
5 days old	8 180 000	9 380 000
12 days old	10 465 000	6 896 000

The samples from the freshly frozen ice cream gave much larger counts than the sample from the mix, altho the interval of time between them was only about one hour. This apparent increase was probably due to the breaking up of the bacterial clumps during the process of freezing. The samples taken after the ice cream was kept in the hardening room for five days showed a sharp reduction in the number of typhoid bacteria. Similar reduction, however, took place in both the plain and the carbonated ice cream and could not be attributed to the carbon dioxid. The samples taken after the ice cream was in storage for twelve days showed but a slight change in

the counts. There were fewer bacteria in the carbonated ice cream than in the plain at this time. Whether or not this was due to the effect of the carbon dioxid is not known but will be determined when this experimental study is completed. This is certain, there were still plenty of living typhoid bacteria in the ice cream after twelve days of storage.

From the above two experiments, it seems very evident that the incorporation of the carbon dioxid gas into the ice cream during the process of freezing does not guarantee the ice cream to be a safe product.

EXPERIMENT 3

BEHAVIOR OF BACTERIA IN CARBON DIOXID ATMOSPHERE

In the process of making the carbonated ice cream, the carbon dioxid gas is blown into the freezer in order to replace the air by the gas. Carbon dioxid and air mix readily in all proportions and it is to be expected that what is incorporated into the ice cream is not pure carbon dioxid but a mixture of this gas and air. The effect that such a mixture would have on the bacteria may be quite different from that produced by the pure gas.

This experiment deals with the effect of pure carbon dioxid gas on bacteria. Twenty different cultures of bacteria from milk and ice cream, and also two pathogenic bacteria were inoculated into milk and on the surface of agar jelly. They were placed in an air-tight vessel and the air was replaced by carbon dioxid gas. They were kept in this condition at room temperature for five days. The examination of these cultures at the end of five days showed that on the agar jelly only two of the twenty different bacteria could grow in carbon dioxid atmosphere. The remaining eighteen showed no visible signs of growth. These agar cultures were then taken out and were left in the open air for a few days to determine whether or not the bacteria were killed by the carbon dioxid gas. All of them except three resumed their growth, indicating that the gas did not kill them. The three cultures that were killed were the two pathogenic bacteria and one of the non-pathogenic.

Entirely different results were obtained when these bacteria were subjected to the carbon dioxid gas in milk. Not one of them was killed by the gas. Not only that, but in all cases they increased in number. It would seem that in milk the bacteria found certain protection against the action of the gas.

EXPERIMENT 4

EFFECT OF CARBON DIOXID ON BACTERIA IN MILK UNDER PRESSURE

Since the bacteria could survive and even grow in the milk when they were kept in pure carbon dioxid gas, it was decided to go a step further and note the effect on bacteria when the milk was charged with the gas under pressure. For this purpose one-quart soda-water bottles were used. The carbon dioxid was first blown, thru the bottles to replace the air by the gas. The bottles were then filled with the milk and the gas was admitted under pressure while the bottles were being shaken vigorously. The pressures of ten, twenty, forty, and sixty pounds were tested. The bottles were kept at room temperature. Each day samples were taken from the bottles for bacteriological examination; the pressure was tested and any decrease in pressure was adjusted. In no case, however, did the pressure decrease more than one pound.

TABLE 3.—EFFECT OF CARBON DIOXID ON BACTERIA IN MILK UNDER PRESSURE

Age of milk	Untreated	Under 10 lbs. pressure	Under 20 lbs. pressure	Under 40 lbs. pressure	Under 60 lbs. pressure
Freshly frozen	35 000	35 000	35 000	35 000	35 000
1 day old....	1 606 000 000	93 000 000	34 000 000	3 740 000	1 270 000
2 days old....	.....	630 000 000	1 044 000 000	292 000 000	175 200 000
4 days old....	.....	.....	.....	428 000 000	359 000 000
	Soured in 30 hours	Soured in 48 hours	Soured in 3 days	Soured 5th day	Soured in 9 days

The treatment of milk with carbon dioxid under pressure delayed the souring of the milk. The untreated milk soured in thirty hours. Under 10 pounds pressure it soured in forty-eight hours, under 20 pounds pressure in three days, under 40 pounds pressure in five days, and under 60 pounds pressure in nine days.

In Table 3 are given the bacteriological results from one of the series in this experiment. It is seen that the bacteria grew under all the pressures tested. Under 60 pounds pressure they increased in four days from 35,000 to 359,000,000 per cc. of milk.

In another series of this experiment, the milk was first sterilized and then inoculated with typhoid bacteria. The milk was then sub-

jected to 10 and to 20 pounds pressure. The typhoid bacteria were not killed under these conditions. In four days under 20 pounds pressure they increased from 47,000,000 to 153,000,000 per cc. of milk.

In these preliminary experiments, the authors were concerned only with the sanitary aspects of carbonated milk and carbonated milk products. The process may have many commercial advantages and it may be of decided benefit to the dairy industry; however, the results of these experiments point to the conclusion that the process cannot be relied upon as a means of insuring the sanitary quality of dairy products.

NOTE.—The terms *carbonated ice cream, carbonated milk,* etc., as used in this circular, refer to laboratory products made by laboratory methods, and not to products made by commercial processes patented or otherwise.