SOME EFFECTS OF CARBONATION

ON

KEEPING QUALITIES, FLAVOR, AND SANITARY QUALITY

OF

DAIRY PRODUCTS

A Major Thesis

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INTRODUCTION

In view of the fact that during the last few years the consumption of dairy products has materially increased and that the quality requirements of these products are more rigid than in previous years, it is pertinent that the dairy industry develop and employ such methods of processing and storing as will enhance the quality and prolong the freshness of dairy products.

Also at present, the dairy industry is in dire need of a means whereby dairy products can be preserved over long periods of time with little or no deterioration resulting. Many dollars are lost to the industry because of the rapid spoilage so characteristic of milk and milk products.

The presence of oxygen is one of the chief direct and indirect causes of spoilage of dairy products. Rancidity and tallowy flavor in butter, spoilage of products due to production of lactic acid, and abundant growth of microorganisms causing deterioration, are all attributed to the presence of oxygen. Should some method be devised which would prevent atmospheric oxygen from coming in contact with milk and milk products produced under sanitary conditons, it seems logical to believe that the deterioration of these products would be greatly reduced.

The displacement of oxygen contained in the product with inert gases is a suggested means for maintaining the quality of the product over a longer period of time. It is the purpose of this work to determine the influence of carbon dioxide as an oxygen replacing agent, and to study the effects of the application of carbon dioxide in the solid and gaseous forms.

REVIEW OF LITERATURE

Carbon dioxide gas for years has been dreamed of as a means of preserving food. Many efforts have been made by investigators to determine the inhibitive and antiseptic properties of carbon dioxide as applied to milk. In some cases it has met with success and in others it has failed.

Among the investigators who have found that inert gases enhance the keeping qualities of food products are the following:

Bethell (1) patented a process for preserving milk in 1848 which consisted in first boiling the milk to expel the air contained in it, and then saturating the liquid with carbon dioxide gas. The milk when so treated remained fresh for a long time after being exposed to the air.

Leone (2) from his work concluded that carbonation effectively retards germ life in water and exterminates the great majority of microbes, very few having sufficient vitality to withstand its action.

Koser and Skinner (3) state that the carbonation of beverages exerts a distinctly destructive effect upon members of the colon-typhoid group, and their period of viability in carbonated water is much shorter than in plain tap water.

Jones (4) in stating the results of his work, reports that the carbonation of beverages actually causes a reduction in numbers of any colon-typhoid bacteria present.

Washburn (5) states, "Repeated tests of (carbonated) ice cream show clearly that carbonation will bring out the flavor and increase the tastiness of the product and that the presence of this gas (carbon dioxide) destroys bacteria and prevents other bacteria from growing".

Heis and Engel (6) working with the inert gas nitrogen, report that when the air in cream was displaced by an inert gas (nitrogen) and the butter was churned and stored in such gas, the keeping quality of the butter at low temperatures of storage was about three times as good as that for butter stored in air. At higher temperatures the influence of the inert gas (nitrogen) was negligible; hence, cold storage can not be displaced by storage in gas but higher temperatures might be used economically. Butter stored in air at 0°F. kept as well as butter stored with nitrogen gas at $5\text{-}6^{\circ}$F.

Allen and associates (7) conclude from a study of non-alcoholic carbonated beverages that carbon dioxide gas is germicidal to a decided extent, and that were it not for carbonation, the bacterial content of carbonated beverages would be enormously high.

Van Slyke and Bosworth (8) report the uncarbonated, pasteurized milk became sour in three days, while the carbonated portion kept nine days without souring, even at room

temperature. The milk used in these experiments was pasteurized at 185°F., separated, aged 24 hours, and then put into bottles under a pressure of 70 pounds of carbon-dioxide gas. Some untreated milk was kept for comparison. The same author reports that fresh, whole milk, pasteurized and charged with carbon dioxide gas under a pressure of 50 pounds developed practically no increase of acid in the course of about nine months. The milk was kept at a temperature varying from 40-60°F.

Prescott and Parker (9) conclude from experiments conducted with ice cream that while the apparent increase in the number of bacteria during the freezing of ice cream in air averaged 13.8%, comparative freezings of ice cream in an atmosphere of carbon dioxide gas showed an average reduction of 32.4% in the number of bacteria.

Prucha, Brannon and Ambrose (10) found that of twenty different cultures of bacteria isolated from ice cream, inoculated on agar, and subjected to an atmosphere of carbon dioxide gas for five days only two survived. However, they state that, "Entirely different results were obtained when these bacteria were subjected to carbon dioxide in milk. Not one of them was killed by the gas. Not only that, but in all cases they increased in numbers. It would seem that in milk the bacteria found certain protection against the action of the gas."

Probably the most extensive application of carbon dioxide gas is the employment of this gas as a retarder of microorganism growth in grape juice at Worcester, Africa (11). The grape juice at that plant is stored in 30 tanks, each of 11,000 gallon capacity. It is then saturated with carbon dioxide gas at 113.8 pounds pressure per square inch at 59°F. "Because of the inhibiting effect of the carbon dioxide gas on microorganisms, the grape juice can be stored as long as desired without changes occurring."

The work of some other investigators has not led them to conclusions so favorable to the action of carbon dioxide gas as the foregoing citations. Some of the conflicting results follow:

Valley and Rettger (12) make the following statement after having experimented with carbon dioxide. "The carbonation of ice cream under ordinary atmospheric pressure is a negligible factor in the reduction of numbers of viable bacteria in the stored product. Carbon dioxide treatment of milk at the same pressure likewise results in little or no bacteria inhibiting or destroying action."

Sabrazes and Bazin (13) concluded from results obtained from their study that carbon dioxide gas under a pressure of 60-70 atmospheres has a very little if any aseptic qualities.

Macy (14), after a very exhaustive study of some of the factors influencing the growth of molds in butter, states,

"It may be possible to replace the oxygen (of butter) by some inert gas, but experiments are necessary to demonstrate the practicability of such a plan."

Prucha, Brannon and Ambrose (15) found that the bacteria in milk under a pressure of 60 pounds of carbon dioxide gas increased from 35,000 to 359,000,000 in four days. However, this increase was not so rapid as that of the bacteria in milk treated with carbon dioxide gas at lower pressures or in untreated milk.

Hunziker (16), after extensive experiments with butter using the Heath method of carbonization, makes the following statements, "Carbonating does not enhance the quality of butter. Carbonated butter keeps no better than butter not carbonated. The pretended superiority of carbonated butter does not exist."

Some of these conflicting reports might be reconciled through the explanation that the same technique and experimental conditions were not employed by all workers. Most of the workers employing low storage temperatures (40°F. and below) with or without high pressures of carbon dioxide gas report favorable results from the use of this inert gas. Similarly, those workers storing the products studied at high temperatures (above 40°F.) using carbon

dioxide gas under low pressures or no pressure at all usually report that there are no benefits to be derived from the use of this inert gas.

It must also be mentioned that none of the above named workers studied the effects on the ultimate product of pasteurizing milk or cream in an atmosphere of carbon dioxide gas, nor do the available reports and records studied give any information as to the results obtained when dairy products are stored in contact with solid carbon dioxide.

METHODS OF PROCEDURE AND EQUIPMENT USED

Since the results of some of the previous experimental work with carbon dioxide gives promise of carbonation as a method of improving the quality of milk and milk products, the original object of this investigation was to study the effects of carbonating milk and milk products on a small commercial scale.

Due to the fact that only special equipment is capable of withstanding vacuum and pressure, some difficulty was encountered in devising such equipment as was necessary for this study.

A - List of Equipment

The numbers used in this list refer to parts of equipment so numbered on the diagram on the following page.

No. 1 - Motor which operated the vacuum pump.

No. 2 - Pressure equalizer used to maintain an even suction and to prevent the condensation of moisture in the vacuum pump.

No. 3 - Vacuum pump used for producing a vacuum on equipment.

No. 4 - Pasteurizer vat in which milk or cream could be processed in an atmosphere of carbon dioxide. A specially constructed rubber gasket between the cover of the vat and the top surface of the rim of the vat made it possible, with the use of "C" clamps, to make the vat tight enough to withstand 15 inches of vacuum and ten pounds of pressure.

No. 5 - Vertical pasteurizer coil through which the heating or cooling medium was pumped.

No. 6 - Pressure-vacuum gauge which indicated pressure conditions in the pasteurizer vat.

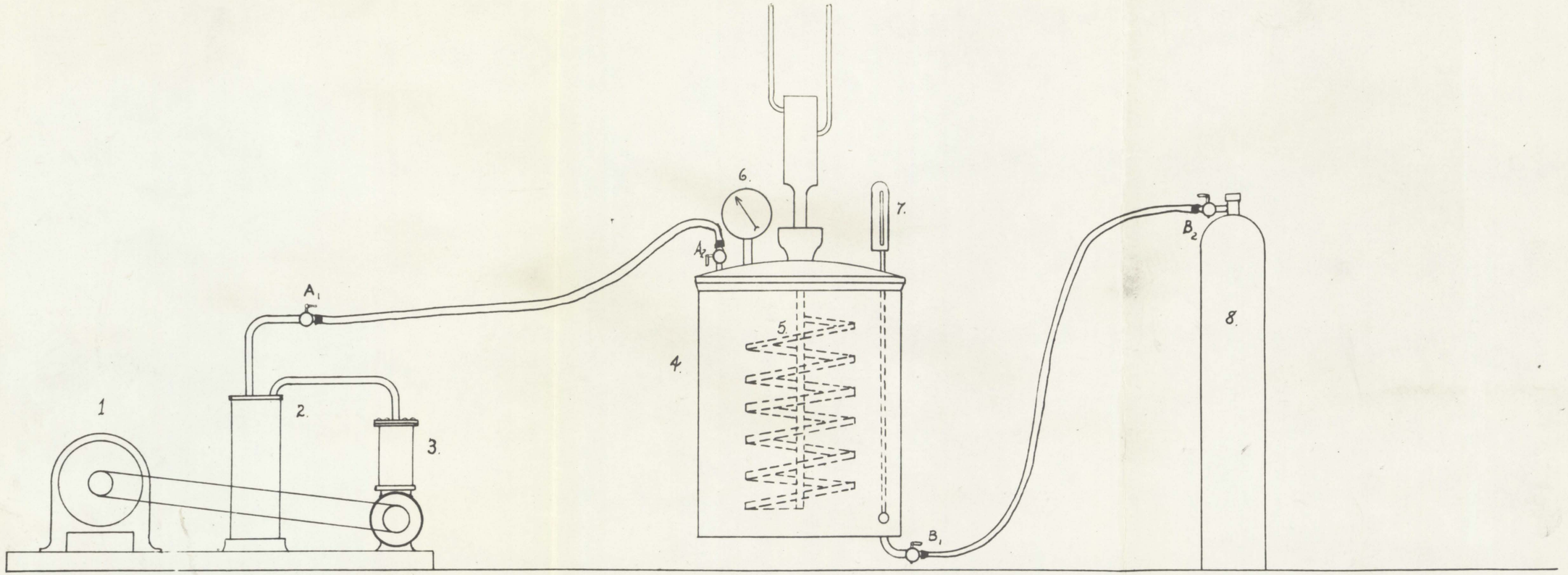
No. 7 - Thermometer used to indicate the temperature of the fluid in the pasteurizer vat.

No. 8 - Drum containing gaseous carbon dioxide under pressure.

A_1 and A_2 - Stop cocks used to regulate the vacuum drawn into the pasteurizer vat.

B_1 and B_2 - Stop cocks used to regulate the flow of carbon dioxide into the pasteurizer vat.

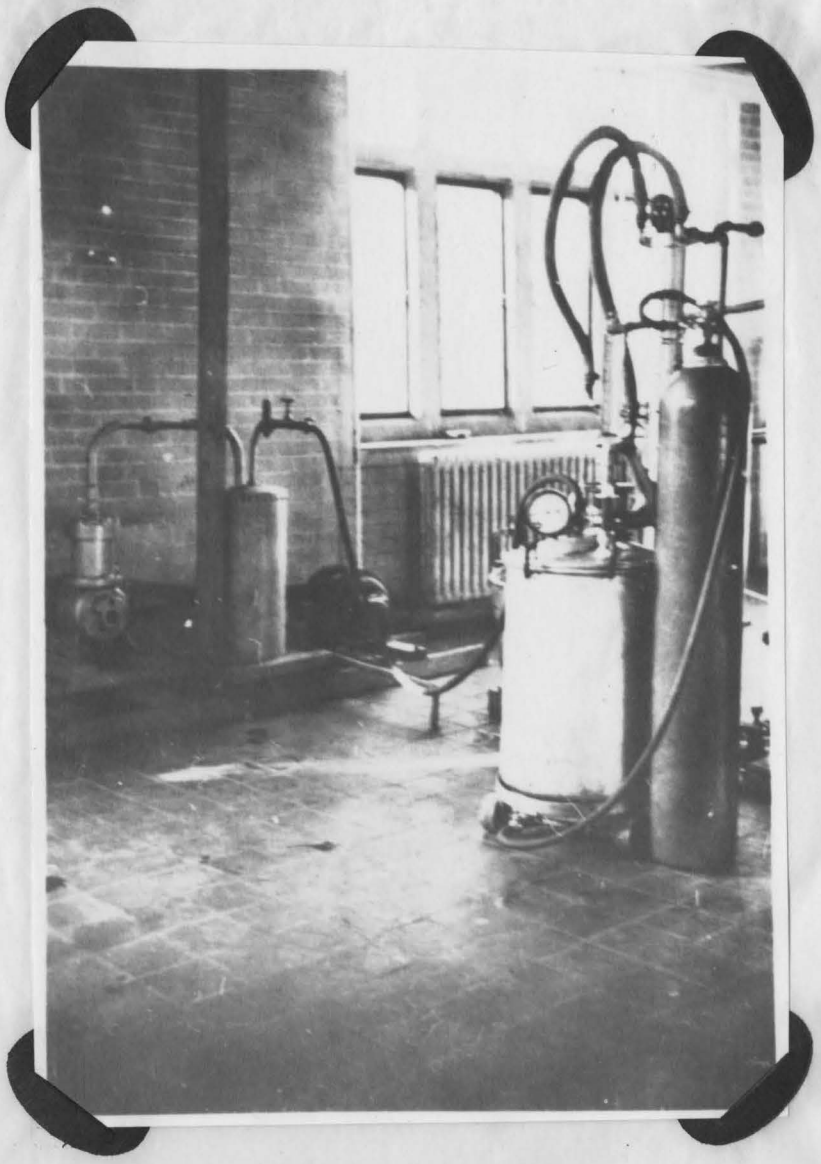
The picture on page 11 is of the pasteurizing equipment. On page 12 is a picture of the equipment used in the separation experiments.



Equipment for Carbonation During Pasteurization

Plate No. 1

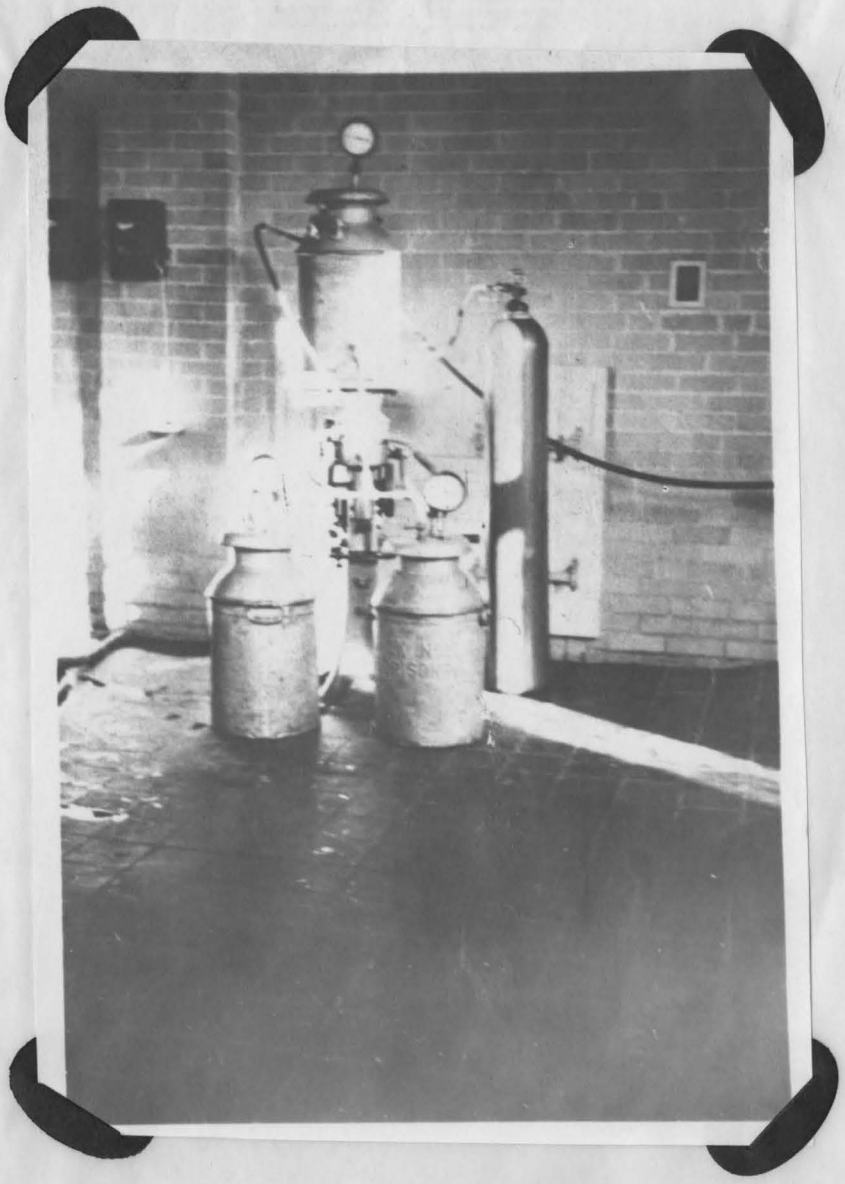
Equipment used for pasteurizing milk and cream in an atmosphere of carbon dioxide.



The pasteurizer was an altered 20 gallons, Vertical, Jenson Utility Series, Model 20. The vat could withstand 15 inches of vacuum and 10 pounds of pressure.

Plate No. 2

Equipment designed to separate milk in an atmosphere of carbon dioxide.



This equipment maintained the fluids in an atmosphere of carbon dioxide from the time the milk was drawn into the supply tank until the resulting cream and skim milk were in the catch receptacles.

B - Methods of Procedure

Wherever it was possible to do so, standard methods of procedure were employed. Where standard methods of procedure could not be used, methods believed to be most practical were used.

1 - Carbonation

A variety of methods of carbonation were used in the research work which follows. The method of carbonation used in any experiment is discussed in that particular experiment.

2 - Storage of Samples

Samples stored at temperatures below 70°F. were kept in well insulated, ammonia cooled rooms which were automatically maintained at constant temperatures. As a check on temperatures, a recording thermometer manufactured by the Consolidated Ashcroft-Hancock Company, Inc. was used. The bulb of this instrument was submerged in a jar of water located beside the samples in storage.

Samples stored at 100°F. were kept in an electrically heated bacteriological incubator. The temperature in this incubator was automatically controlled by a thermostat.

3 - Acidity Determinations

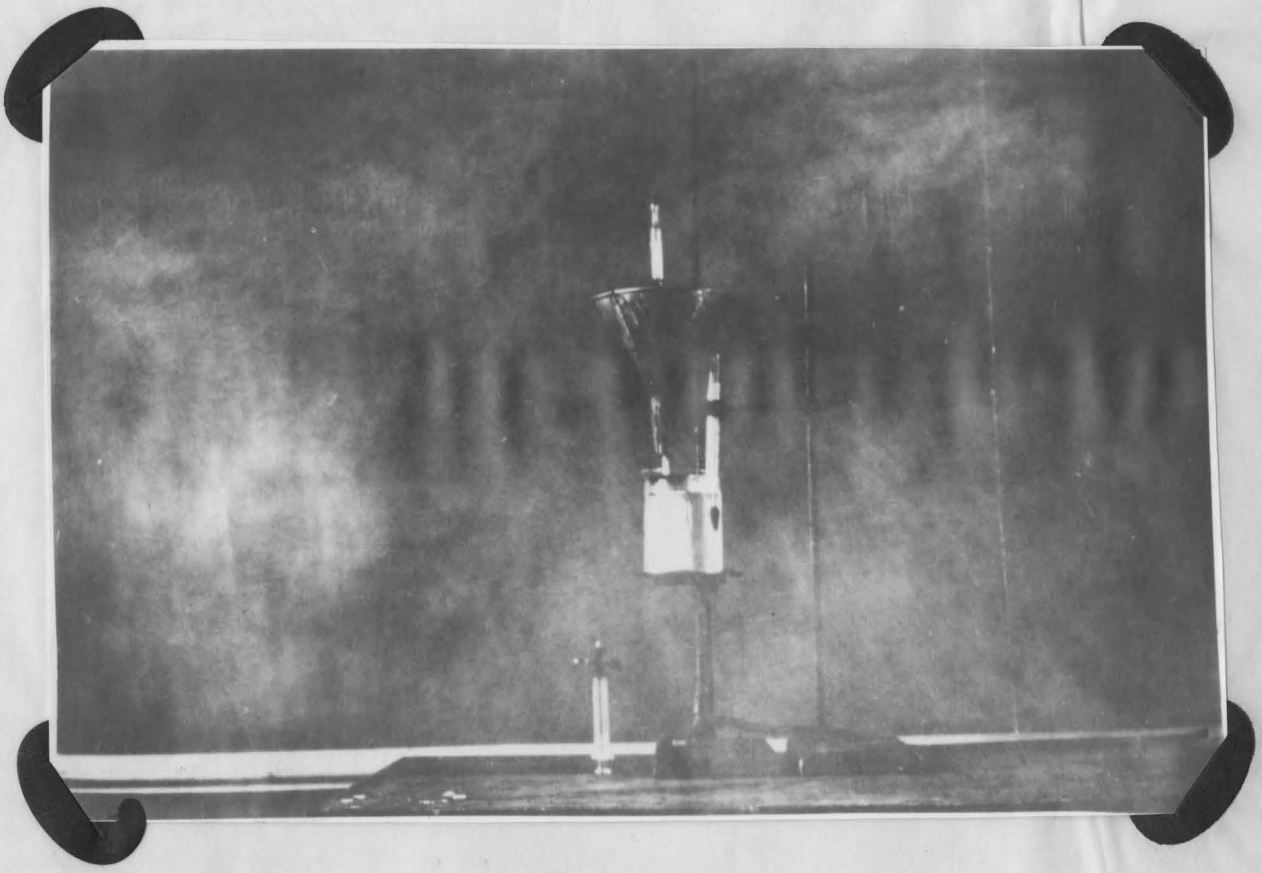
For the determination of titratable acidity, the Mann's Acidity Test, involving the use of phenolphthalein and tenth normal sodium sodium hydroxide, was used. In most instances, it was necessary to warm the samples gently to bring them to the desired temperature, 60-70°F.

4 - Gas Analysis

The picture on the following page shows the apparatus employed for making gas analyses. Samples to be used for gas determinations were placed in sample jars graduated to contain 100 c. c. of the fluid. Immediately before the gas test was to be made, distilled water was added to the sample jars to bring the contents of the jar flush with the bottom of the one hole rubber stopper which was then inserted. This stopper held the glass tube which contained the gas driven off. The sample jar was then placed in a 1000 c. c. beaker in which was sufficient brine ($CaCl_2$) to completely surround the sample jar. When this water was heated by a Brunsen burner to 222°F., the gases in the sample were driven off and collected in the glass tube above. The upper orifice of this glass tube was covered with a rubber ampule stopper. Through this rubber

Plate No. 3

Apparatus used for making gas analyses of milk and cream.



When the milk was heated to 222°F. the gases of the fluid were driven off into the glass tube above the funnel of water. From this tube the gases were drawn into a medical syringe by inserting the needle of the syringe through the rubber ampule stopper and producing a vacuum in the syringe.

covering a hypodermic needle could be inserted without an interchange of gases. By raising the plunger of the glass medical syringe, the gases could be drawn into the barrel of the syringe. The barrel being calebrated allowed the volume of gases to be easily read. Potassium hydroxide (100 gms. per 1000 c. c. of water) was then drawn into the syringe. This reagent absorbed the carbon dioxide from the enclosed gases. The difference in the volume of total gases in the syringe and the volume following absorption by KOH was then recorded as the volume of carbon dioxide present.

5 - Bacteria Determinations

The agar plate method as given in the sixth edition of Standard Methods of Milk Analysis was employed to determine the bacterial counts of samples. This work was done in the college Bacteriology Laboratory under the direction of the college Bacteriologist.

6 - Sterilization

All sample jars, jar tops, jar-top interliners and cork stoppers used in this study were sterilized in an autoclave at 15 pounds pressure per square inch for 15 minutes immediately previous to use.

The equipment used was washed after and steamed previous to each time used.

As milk at times came in contact with the rubber hose extending from the carbon dioxide drum to the carbon dioxide inlet in the bottom of the pasteurizer vat, this hose was washed, steamed and filled with a chlorine solution after each time it was used. The chlorine solution remained in the hose until it was to be used again.

MILK SEPARATION EXPERIMENT

The following experiment was set up to determine the effects on the bacteria of cream when the entire separation process was carried out in an atmoshpere of carbon dioxide.

Procedure: An eight gallon batch of raw milk was heated to 100°F. Four gallons of this milk was drawn by a vacuum pump into a specially designed supply tank of the No. 16, Delaval Separator. Gaseous carbon dioxide was then bubbled through this milk for two minutes.

The catch receptacles for the skim milk and cream and also the milk outlet at the float were so adapted that an atmosphere of carbon dioxide would surround the milk from the time it left the supply tank unlil it was desired to remove the lids from the skim milk and cream receptacles.

Separation was carried out in the usual way except that carbon dioxide was bubbled at two minute intervals through the milk in the supply tank and that carbon dioxide was kept in contact with the liquid throughout the process. The cream from this separation was dispensed to

sample jars, the caps of the jars were screwed tightly and the samples were stored in a refrigerated room maintained at 45°F.

The other four gallon portion of milk was drawn into the supply tank by means of the vacuum pump and separated in the ordinary manner. The cream from this separation was dispended to sample bottles, the tops of the jars screwed on tightly and the sample jars stored in the refrigerated room maintained at a temperature of 45°F. throughout the experiment.

The results of this experiment are given in Table 1.

On the sixth day after storage, those samples which had been separated in natural atmosphere showed numerous gas bubbles. Nine days after the date of storage, these samples of cream separated in an atmosphere of carbon dioxide showed gas bubbles on the surface.

Discussion: These data show the increase in the number of bacteria in those samples having been carbonated during separation to be much slower than that of samples having been separated in natural atmosphere.

Table 1

Milk Separation

Cream Carbonated During Separation vs. Cream Separated in Natural Atmosphere

Stored at 45°F.

Sample No.	Separation Atmosphere	Days in Storage	Acidity %	Bacterial Count
1	CO_2	0	0.167	140,000
2	Natural	0	0.145	210,000
3	CO_2	1	0.155	6,410,000
4	CO_2	1	0.150	7,300,000
5	Natural	1	0.150	13,350,000
6	Natural	1	0.160	13,800,000
7	CO_2	3	0.220	10,950,000
8	CO_2	3	0.220	13,050,000
9	Natural	3	0.250	35,000,000
10	Natural	3	0.240	37,000,000
11	CO_2	6	0.315	6,700,000
12	CO_2	6	0.310	6,900,000
13	Natural	6	0.260	9,000,000
14	Natural	6	0.250	10,800,000
15	CO_2	9	0.300	6,750,000
16	CO_2	9	0.320	6,300,000
17	Natural	9	0.295	20,000,000
18	Natural	9	0.270	21,000,000

The milk immediately previous to separation possessed 250,000 bacteria per c. c. and an acidity percentage of 0.162.

It is known that certain bacteria reduce their numbers by rapid growth and production of waste products. Under certain conditions when one species of microorganisms reduce their numbers in such a way as this, another species will predominate and multiply rapidly. Such a phenomena is probably responsible for the decrease of bacteria on the sixth day and the increase in numbers on the ninth day. Only those samples that were not carbonated during the separation showed such a decrease with a subsequent increase in numbers of bacteria.

The acidity of the carbonated cream was very little higher than the acidity of the cream not carbonated.

MILK PASTEURIZATION EXPERIMENT

Van Slyke and Bosworth experimenting with carbon dioxide as applied to the preserving of milk (9) reported that the carbonating of this fluid retards bacterial growth and also enhances the flavor of the product. The following experiment was undertaken to study the effects of carbon dioxide upon milk during pasteurization and storage.

Procedure: Four gallons of raw milk were thoroughly stirred and divided into two equal portions. One portion was pasteurized for 30 minutes at 145°F. Immediately previous to the pasteurization a ten inch vacuum was produced in the pasteurizer and replaced with carbon dioxide gas. During the pasteurizing period a pressure of from two to five pounds of carbon dioxide was maintained. After the thirty minutes of pasteurizing in an atmosphere of carbon dioxide gas, the milk was cooled to 45°F. by circulating ice cooled water throughout the pasteurizer coil. One-half of this portion of milk was placed in tube top jars (type shown in Plate 5). These samples were carbonated by attaching the hose from the vacuum pump to the tube in the jar top, producing a ten inch vacuum, and building up a five pound pressure with gaseous carbon dioxide.

The vacuum was removed from the jar tube and the opening in the tube tightly closed with a cork stopper. One-half of these samples were stored at 45°F. and the other half stored at 70°F. The remainder of this portion of milk was placed in plain top sample jars and likewise distributed to the 45°F. and 70°F. storage rooms.

The remaining two gallons of milk was pasteurized in a natural atmosphere for 30 minutes at 145°F. and then cooled to 45°F. This portion of milk after cooling was handled in identically the same way as the previous portion.

The results of this experiment are shown in Table 2.

Discussion: From the results of this experiment it is evident that carbonation during pasteurization can not supplant cold storage; however, this inert gas plus cold storage can be used to effectively maintain the bacterial count at a lower figure than can be maintained by the use of cold storage alone.

Even when the milk received no more carbon dioxide than that applied immediately before the samples were taken to the refrigeration rooms, a small reduction in the number of bacteria was noted.

Table 2

Milk Pasteurization

Milk Pasteurized in Carbon Dioxide vs. Milk Pasteurized in Natural Atmosphere

Sample No.	Atmosphere During Past.	Storage Atmosphere	Days in Storage	Bacterial Count 45°F.	Bacterial Count 70°F.
1	CO_2	CO_2	1	80	44,500
2	CO_2	Natural	1	250	45,000
3	Natural	CO	1	340	330,000
4	Natural	Natural	1	520	650,000
5	CO	CO	2	120	4,200,000
6	CO	Natural	2	100	4,600,000
7	Natural	CO	2	260	27,600,000
8	Natural	Natural	2	860	27,000,000
9	CO	CO	3	120	6,450,000
10	CO	Natural	3	80	8,250,000
11	Natural	CO	3	460	54,000,000
12	Natural	Natural	3	1,020	51,000,000
13	CO	CO	4	90	130,000,000
14	CO	Natural	4	280	126,000,000
15	Natural	CO	4	600	223,000,000
16	Natural	Natural	4	1,210	430,000,000
17	CO	CO	5	160	160,000,000
18	CO	Natural	5	170	240,000,000
19	Natural	CO	5	1,010	258,000,000
20	Natural	Natural	5	2,400	450,000,000

The flavor of the carbonated milk was decidedly off, and it is scarcely probable that such a flavor would ever be greatly in demand by the public.

Milk was used in several of the later experiments regardless of the off flavor produced by the carbon dioxide, for it was thought that this gas would have approximately the same effects upon cream as upon milk.

CREAM PASTEURIZATION EXPERIMENT

Investigators have studied the influence of applying carbon dioxide to cream during the churning process, but to the author's knowledge none have studied the effects of pasteurizing cream in an atmosphere of carbon dioxide.

Procedure:- Eight gallons of sweet cream, testing 43% butterfat, was thoroughly agitated and then divided into two equal portions. One portion was pasteurized at 145°F. for 30 min. Immediately previous to the heating of the cream, a 10 in. vacuum was secured and replaced by gaseous carbon dioxide. A pressure of from 2 to 5 lbs of carbon dioxide was maintained inthe pasteurizing vat throughout the pasteurizing period. Following the pasteurization period the cream was cooled to 45° F. by circulating ice cooled water through the pasteurizer coil. This cream was placed in sample jars preparatory to storage. To obtain an atmosphere of carbon dioxide approximately 15 gms. of carbon dioxide was introduced into the sample jars. This introduction supplied an atmosphere of carbon dioxide during the storage period. The jar tops were then screwed on tightly. A slight

pressure was produced due to the sublimation of the carbon dioxide. Although the jar tops were fitted with parafined interliners and screwed down tightly, the pressure developed in the jars did not exceed 27 lb. per square inch, due to the small escape of gas around the jar tops. Twenty-five minutes following the introduction of carbon dioxide into the sample jars, the pressure was negligible and would not register on a pressure guage; however, a small pressure was maintained during the storage period which was evidenced by the escaping gas at the time of loosening the tops.

The results of this experiment are given in Table 3.

Discussion:- The results of this experiment show solid carbon dioxide superior to gaseous carbon dioxide as a preserving agent when the gaseous carbon dioxide is applied in such quantities and by such methods as was employed in previous experiments. However, the bacterial count of this cream was low previous to the introduction of the solid carbon dioxide.

It has been supposed for some time that the saturating of a product with carbon dioxide causes an increase in acidity, but the available records do not give infor-

Table 3

Cream Pasteurization

Cream Pasteurized in an Atmosphere of Carbon Dioxide as Compared With Cream Pasteurized in Natural Atmosphere

Sample No.	Atmosphere During Past.	Storage Atmosphere	Days in Storage	Acidity % 45 F.	Bacterial Count 45°F.	Acidity % 55°F.	Bacterial Count 55°F.
1	CO_2	CO_2	3	0.400	0	0.340	500
2	CO_2	Natural	3	0.275	500	0.337	200
3	Natural	CO_2	3	0.300	350	0.245	14,000
4	Natural	Natural	3	0.130	61,000	0.170	1,300,000
5	CO_2	CO_2	6	0.325	0	0.285	0
6	CO_2	Natural	6	0.322	0	0.315	0
7	Natural	CO_2	6	0.227	0	0.222	960,000
8	Natural	Natural	6	0.140	1,080,000	0.295	2,000,000
9	CO_2	CO_2	9	0.280	0	0.350	0
10	CO_2	Natural	9	0.260	0	0.385	0
11	Natural	CO_2	9	0.225	0	0.360	13,500
12	Natural	Natural	9	0.250	2,250,000	0.350	3,350,000

nation as to what the increase is with regards to milk and cream. The conjecture has been made that the acidity developed when milk was inoculated with carbon dioxide was great enough to inhibit the brewth of microorganisms. The above results do not substantiate such a surmise. The highest acidity recorded from this experiment was 0.400%. Such an acid content is not high for cream and would probably not inhibit bacterial growth. Also as this reading was made on the third day of storage and was not superseded by any readings on the following days, this acidity at the beginning of the experiment may be apparent acidity and not actual acidity. This appears to be the case as the acidity readings from the carbonated samples decrease as the experiment progresses. The decrease in acidity probably means that there is a slow escape of carbon dioxide from the cream.

The off flavor of the carbonated cream was not nearly so noticeable as that of the carbonated milk in the previous experiments.

Butter made from this cream possessed a very nutty and desirable flavor which was superior to the flavor of butter made from the same cream but not carbonated.

Also the mold count of the butter from the cream carbonated during churning and storage was 400 as compared with 52,000 for butter which had received no carbon dioxide treatment of any kind.

STUDY OF EFFECTS OF CARBON DIOXIDE UPON SERRATIA MARCESCENS

Since the reduction of bacteria was so great in those instances where cream was processed in an atmosphere of gaseous carbon dioxide and stored in contact with solid carbon dioxide, the next few experiments were made in an attempt to learn more of the characteristics and properties of this preserving agent as related to its influence upon milk.

Carbon dioxide at ordinary temperatures as found naturally is in the gaseous state with one liter weighing 1.9768 gms. At 15°C. and under ordinary pressure one volume of water dissolves one volume of gas. It is rather easily condensed to a colorless solid, which under atmospheric pressure evaporates without melting at -78.5°C.

The first of this series of experiments was made to determine the influence of carbon dioxide upon a specific species of bacteria in milk.

Procedure:- 2,500 c.c. of skim milk were autoclaved for 15 minutes at 15 pounds pressure per square inch.

A plating was then made on this batch of milk to determine its bacterial count. The results showed it to be sterile. This milk was then inoculated with a pure culture of B. Serratia Marcescens. This organism was selected because of its red color which would allow easy detection of contamination. After the milk was thoroughly agitated, it was dispensed to sterile sample jars---100 c.c. portions to the jar. The caps were screwed down tightly, the jars shaken, and a one cubic centimeter portion taken from each sample to determine the bacterial count on each individual jar at the time of solid carbon dioxide introduction. In the table this count is referred to as Initial Bacterial Count. Twelve grams of carbon dioxide was introduced into each jar, the tops screwed tightly again, and the jars distributed to the constant temperature rooms. The results of this experiment are given in Table 4.

Discussion:- Of the samples carbonated and stored at 30°F., there was an increase in the number of bacteria in only one instance, that of sample number 5. Of the samples stored at 100°F., none of those carbonated gave rise to as great increase in bacterial growth as did those samples not carbonated. Twenty-four hours after storage the samples stored at 100°F. that were not carbonated had about one

Table 4

Effect of Carbon Dioxide upon Serratia Marcescens

Stored at 30°F.				Stored at 100°F.			
Sample No.	Initial Bacterial Count	Days in Storage	Final Bacterial Count	Sample No.	Initial Bacterial Count	Days in Storage	Final Bacterial Count
1	9,300	1	6,000	1a	27,400	1	14,000,000
Check	6,300	1	18,300	2a	8,000	1	8,000,000
2	22,000	2	10,400	Check	14,000	1	211,000,000
Check	15,600	2	150,000	3a	30,000	2	22,000,000
3	11,600	3	2,000	4a	12,000	2	20,000,000
Check	7,300	3	100,200	Check	10,000	2	50,000,000
4	2,000	5	2,000	5a	120,000	3	3,940,000
Check	10,200	5	3,020,000	6a	600	3	500,000
5	15,000	6	18,000	Check	16,000	3	107,000,000
Check	11,800	6	660,000				
6	16,000	7	10,000				
Check	12,000	7	2,500,000				

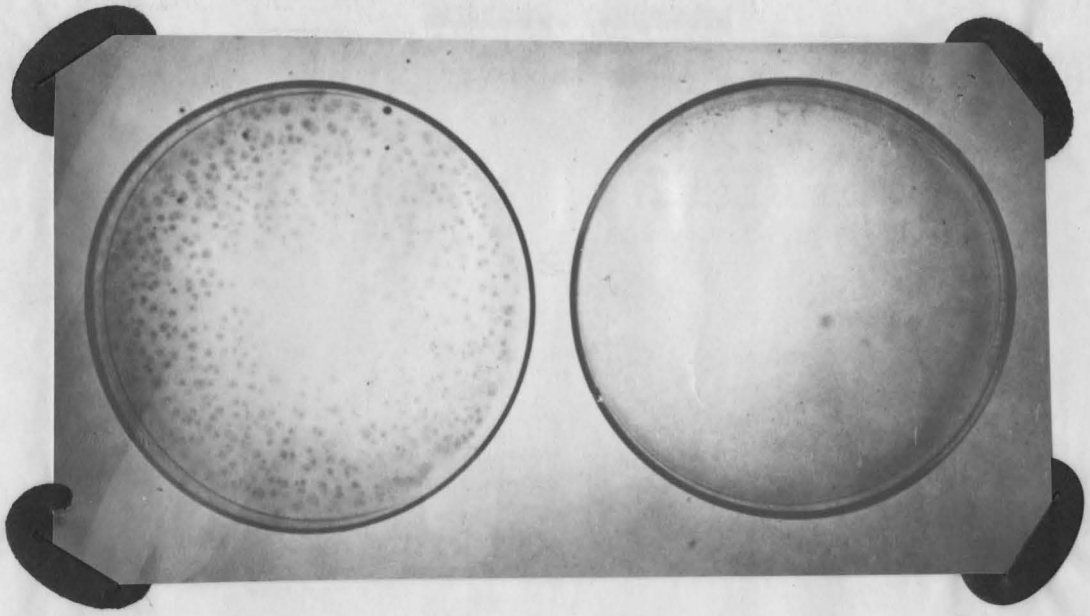
All samples indicated by numbers 1,2,3,4, etc. were carbonated. All "checks" received no carbon dioxide treatment.

quarter inch of whey on the surface while those samples that were stored at the same temperature and carbonated showed no signs of whey or coagulation. Seventy-two hours after storage the milk stored at 100°F and not carbonated had practically all turned to whey while the carbonated samples at the same temperature had a smooth coagulum with no whey.

The photograph on page 35 shows the comparative bacterial growth in carbonated and non-carbonated milk after six days storage at 45°F.

Plate No. 4

Petri plates showing numbers of bacteria in 1-10,000 dilutions of milk. The milk had been sterilized, then inoculated with a culture of Serratia marcescens, and stored at 30°F. for six days.



The sample of milk from which the petri plate on the left was poured received no carbon dioxide treatment. The sample of milk from which the petri dish on the right was poured received 12 grams of solid carbon dioxide immediately previous to storage.

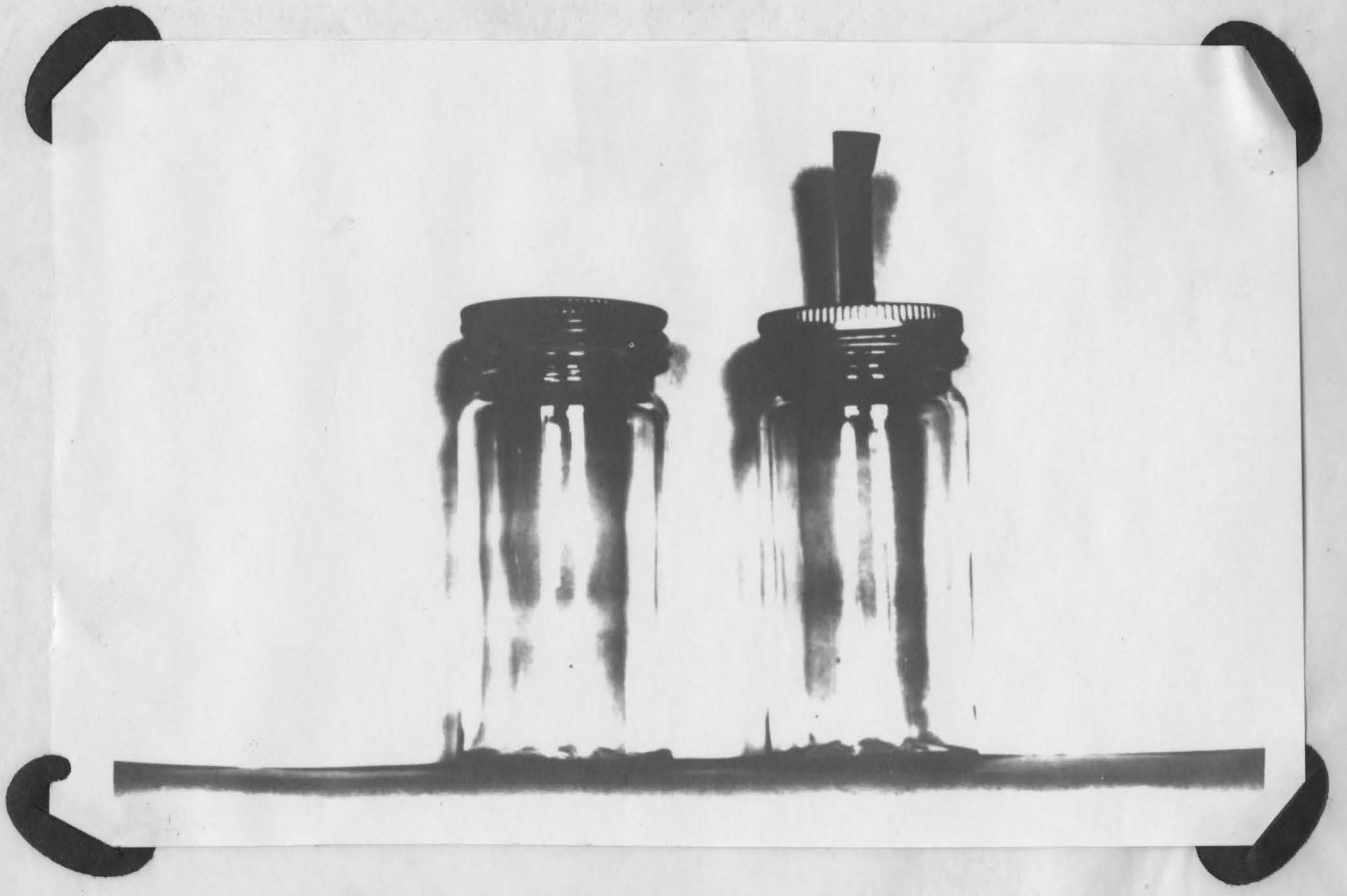
PRESSURE STUDIES

In the preceding experiment it was found that solid carbon dioxide introduced into a sample or milk did build up a pressure during sublimation. In that experiment the pressure amounted approximately to 27 pounds immediately after the sublimation process had begun. Twenty minutes after the carbon dioxide introduction, the pressure had decreased to such a point as to cause no deflection of the hand on the pressure guage. Later, when the jars were opened for examination, in every case some gas would escape when the jar tops were loosened.

In the following experiment two types of jars were used, a solid top jar and a tube top jar (pictures of jars on next page). Pressure could be and was produced in the solid top jars. No pressure could be built up in the tube top jars due to the fact that cotton plugs in the ends of the tubes allowed all pressure to escape but allowed no bacteria to enter the sample jar. The latter fact was established by running as checks, samples of sterile carbohydrate broth stored in tube top jars in exactly the same way as were the samples. At the termination of the experiment the carbohydrate broth was plated and was found to have remained sterile.

Plate No. 5

Types of sample jars used to determine the effects of the pressure produced by subliming carbon dioxide upon bacterial growth.



Subliming carbon dioxide built up a 27 pound pressure in the solid top jar. No pressure could be produced in the tube top jar.

Procedure:- Sixty cubic centimeters of pasteurized milk were placed in each of the sterile sample jars. The jars were then shaken and 1 c.c. removed from each to determine the initial bacteria count. Column 2 of table 5 gives these figures. Five grams of solid carbon dioxide were then introduced into each sample jar and the tops screwed down tightly.

Pressure tests run on samples of this experiment, showed that a pressure of approximately 20 pounds per square inch was produced in each of the jars having solid tops. The pressure at the end of 10 minutes was so small as to cause no response from the pressure guage. When the tops were released at the end of the storage periods, gas could be heard escaping, denoting that a small pressure had remained during the entire experiment.

Seven minutes were required for the carbon dioxide to completely sublime in the tube jars. At the end of that time, the temperature was 50°F. The carbon dioxide in the solid top jars sublimed in nine and one half minutes, at the end of which time the temperature was 50°F.

Table 5

Effects of Carbon Dioxide Pressure on Bacterial Content of Milk Stored at 45°F.

Sample No.	Initial Bacterial Count	Days in Storage	Final Bacterial Count
*T1	6,000	1	700
2	7,000	1	1,400
Check	5,100	1	5,500
T3	4,100	2	50
4	4,000	2	50
Check	3,200	2	8,800
T5	4,500	3	100
6	3,700	3	100
Check	4,400	3	11,400
T7	7,800	4	0
8	5,200	4	200
Check	28,400	4	120,000

*T preceding any number indicates that sample to have been stored in a tube top jar. Those numbers with no letter preceding them represent samples stored in solid top jars.

The samples were stored at 45°F. Untreated samples were stored as checks.

The results of this study are given in Table 5.

Discussion:- As is shown in the table, the carbon dioxide pressure formed in a solid top jar, when solid carbon dioxide is introduced into a sample, has no effect upon the aseptic powers of this gas. Work done by other authors (11) demonstrates that pressure must be much higher than that produced in this experiment to inhibit bacterial growth.

Apparently the amount of carbon dioxide absorbed by 60 cubic centimeters of milk from the sublimation of 5 grams of solid carbon dioxide inhibits the growth of bacteria to the same extent regardless of whether or not a small pressure is allowed to develop during the sublimation of the carbon dioxide.

METHODS OF CARBONATION

The following work was undertaken to study and compare results obtained from two methods of carbonation.

Procedure:- One hundred cubic centimeters of raw milk were placed in each of the sterile sample jars. Fifteen grams of solid carbon dioxide was then added to each jar. The tops were screwed down tightly and the samples taken to the storage room.

To carbonate milk with gaseous carbon dioxide, the liquid was placed in the 20 gallon pasteurizer tank and gaseous carbon dioxide vigorously bubbled through it for two minutes. One hundred cubic centimeters samples were then secured from this milk by means of a stop cock fitted in the bottom of this tank. The tops were then screwed down tightly and the samples taken to the storage room. Untreated samples were stored as checks.

Pasteurized milk received the same treatment as did the raw milk with the exception that the samples receiving solid carbon dioxide received 5 grams instead of 15 grams of carbon dioxide. Untreated pasteurized samples were stored as checks. The results of these studies are given in Tables 6 and 7.

Table 6

Solid vs. Gaseous Carbon Dioxide

Raw Milk Stored at 45°F.

Sample No.	Storage Atmosphere	Days in Storage	Acidity %	Bacterial Count
1a	Solid CO_2	1	0.492	1,000,000
2a	Gas CO_2	1	0.220	24,000,000
3a	Natural	1	0.170	40,000,000
4a	Solid CO_2	3	0.440	1,250,000
5a	Gas CO_2	3	0.220	48,000,000
6a	Natural	3	0.180	60,000,000
7a	Solid CO_2	5	0.430	1,330,000
8a	Gas CO_2	5	0.420	181,000,000
9a	Natural	5	0.460	96,000,000
10a	Solid CO_2	7	0.420	85,000,000
11a	Gas CO_2	7	0.665	90,000,000
12a	Natural	7	0.790	89,000,000

This milk immediately previous to treatment contained 7,000,000 bacteria per cubic centimeter and an acidity content of 0.150%.

Table 7

Solid vs. Gaseous Carbon Dioxide
Pasteurized Milk Stored at 45°F.

Sample No.	Atmosphere During Past.	Storage Atmosphere	Days in Storage	Acidity %	Bacterial Count
1	CO_2	0	0	0.350	120
2	Natural	0	0	0.150	2,000
3	CO_2	Solid CO_2	1	0.455	25
4	CO_2	Gas CO_2	1	0.350	20
5	CO_2	Natural	1	0.340	22
6	Natural	Solid CO_2	1	0.335	1,260
7	Natural	Gas CO_2	1	0.165	2,800
8	Natural	Natural	1	0.152	3,000
9	CO_2	Solid CO_2	3	0.410	15
10	CO_2	Gas CO_2	3	0.315	20
11	CO_2	Natural	3	0.310	25
12	Natural	Solid CO_2	3	0.352	4,000
13	Natural	Gas CO_2	3	0.175	6,000
14	Natural	Natural	3	0.160	33,200
15	CO_2	Solid CO_2	5	0.440	10
16	CO_2	Gas CO_2	5	0.250	15
17	CO_2	Natural	5	0.300	100
18	Natural	Solid CO_2	5	0.320	720,000
19	Natural	Gas CO_2	5	0.170	1,500,000
20	Natural	Natural	5	0.170	1,600,000
21	CO_2	Solid CO_2	7	0.405	0
22	CO_2	Gas CO_2	7	0.250	80
23	CO^2	Natural	7	0.270	120
24	Natural	Solid CO_2	7	0.320	2,400,000
25	Natural	Gas CO_2	7	0.190	3,000,000
26	Natural	Natural	7	0.175	2,600,000

This milk previous to pasteurization contained 7,330,000 bacteria per cubic centimeter and an acidity content of 0.150%.

Discussion of results from raw milk experiments:-
At the seventh day of storage, sample number 10a was sour but not nearly so curdled as were samples numbers 11a and 12a.

It appears from the results in Table No. 6 that 15 grams of solid carbon dioxide in 100 cubic centimeters of milk is far superior to 2 minutes of gaseous treatment.

Again the bacterial counts are low in those instances where acidity percentages are high. However, from later experiments, it was found that the percentage of acidity immediately following carbonation was in direct proportion to the amount of carbon dioxide retained in the milk. It is most likely that the number of bacteria that survive the treatment is determined by the carbon dioxide present rather than by the acidity, for the highest recorded acidity at the beginning of the experiment was 0.492 percent. However, the decrease in numbers of bacteria on the seventh day and the slight increase in numbers of bacteria on the fifth day over the third day may be due to the toxic products, including acidity, formed by the large

numbers of bacteria.

Later experiments show the amounts of carbon dioxide retained by the milk to be 32 cubic centimeters in the samples receiving the two minute gaseous treatment, and 112 cubic centimeters in samples which had received 15 grams of the solid carbon dioxide.

Discussion of results from pasteurized milk experiment:- All of the processed milk was sweet when examined after one, three, five and seven days which was as long as it was kept in storage. The carbonated flavor was not nearly so intense at the end of the experiment as at the beginning.

The benefits derived from the 2 minute treatment with gaseous carbon dioxide following processing seem as great as those from the use of 5 grams of solid carbon dioxide, except at the fifth and seventh day platings. The differences in results at these platings are not great enough to be very significant. The aseptic powers of gaseous carbon dioxide in this experiment are greater than in the experiment in which tube jars containing milk samples were filled with carbon dioxide gas and cork stoppers used to prevent escape

of the gas from the sample jars. See experiment on milk pasteurization.

Apparently there is a gradual replacement of the carbon dioxide in the milk with oxygen of the air, as is suggested by the decrease in acidity and the increase in numbers of bacteria. Should the milk be thoroughly saturated with carbon dioxide during processing and then sotred in air tight containers at relatively low temperatures, it seems logical to believe that few bacteria would survive. However, should milk or cream be carbonated during processing on a practical commercial scale as it was in this experiment, the bacterial count would be maintained at a low figure for a comparatively long period of time.

Processing in an atmosphere of carbon dioxide gives lower bacterial counts than does the carbonating of milk after processing.

WHAT IS ECONOMICAL AMOUNT OF CARBON DIOXIDE TO USE

Previous experiments have suggested that carbonation might be a very practical sanitary measure for dairymen to use. The following work is an attempt to determine the economical amount of solid carbon dioxide to use in preserving raw milk.

Procedure:- *Three day old raw milk was dispensed to sterile sample jars, 90 cubic centimeters being placed in each of the jars that were to be stored at 45°F and 35 cubic centimeters being placed in each of the jars that were to be stored at 100°F. Bacterial platings were made on each individual sample. The counts from these platings are listed in the tables which follow under the heading "Initial Bacterial Count". One third of the samples were then treated with 2 grams of solid carbon dioxide, one third with 4 grams of solid carbon dioxide, and the remaining third were left untreated and used as checks. The tops were screwed tightly on each sample jar and the samples distributed to the storage rooms.

The results of this study are tabulated in Table 8.

*It was through error that old raw milk was used in this experiment.

Table 8

What is Economical Amount of Carbon Dioxide to Use in Raw Milk Stored at 45°F.

Sample No.*	Gms. CO_2 Used	Days in Storage	Initial Bacterial Count	Final Bacterial Count
1	0	1	72,600,000	124,000,000
2	2	1	78,000,000	78,000,000
3	4	1	73,800,000	44,000,000
4	0	2	72,000,000	76,000,000
5	2	2	72,400,000	57,000,000
6	4	2	80,000,000	13,000,000
7	0	4	88,000,000	11,000,000
8	2	4	96,000,000	7,540,000
9	4	4	90,000,000	3,350,000
10	0	5	66,000,000	4,740,000
11	2	5	80,000,000	3,460,000
12	4	5	75,600,000	2,626,000

*90 c.c. of milk in each sample

Raw Milk Stored at 100°F.

Sample No.*	Gms. CO_2 Used	Days in Storage	Initial Bacterial Count	Final Bacterial Count
1	0	1	90,000,000	58,000,000
2	2	1	78,000,000	9,000,000
3	4	1	81,600,000	7,000,000
4	0	2	90,000,000	55,000,000
5	2	2	60,000,000	47,000,000
6	4	2	72,000,000	15,000,000
7	0	4	81,000,000	54,000,000
8	2	4	50,000,000	55,000,000
9	4	4	90,000,000	43,000,000
10	0	5	61,000,000	21,000,000
11	2	5	14,400,000	57,000,000
12	4	5	60,000,000	17,000,000

35 c.c. of milk in each sample

Discussion:- After one day in storage, the numbers of bacteria in the carbonated samples stored at 45°F. were higher with a consistent decrease in numbers throughout the remainder of the experiment. After four days in storage, these samples were coagulated. At the 45°F. storage temperature, the results obtained from the use of four grams of solid carbon dioxide showed a greater inhibitive action on bacteria than did those results obtained from the use of two grams of carbon dioxide.

All samples stored at 100°F. showed a decrease in numbers of bacteria after having been in storage one day. As the period of time in storage lengthened, the effects of the carbonation evidently were not great enough to inhibit the growth of bacteria where only two grams of carbon dioxide per sample was used. The decrease in bacterial numbers in samples receiving four grams of carbon dioxide was consistently greater than in those samples receiving two grams or no treatment with carbon dioxide. Apparently, the reduction in numbers of bacteria was partially caused by the toxic products formed by the rapid growth of large numbers of these microorganisms.

This experiment may not be a true test of the merits of the carbonation of raw milk as the milk used

was three days old at the beginning of the experiment.

A comparison of the figures representing the final bacterial counts of those samples stored at 45°F. with those figures representing the final bacterial counts of the samples stored at 100°F. may be misleading as 90 c.c. of milk was used in each sample stored at 45°F. while 35 c.c. of milk was used in each sample stored at 100°F.

The results obtained from further studies of the effects from the use of varying amounts of carbon dioxide in milk are presented in Table 9.

THE INFLUENCE OF VARYING AMOUNTS OF ABSORBED CARBON DIOXIDE UPON BACTERIA AND ACIDITY

This experiment was designed to determine the amount of carbon dioxide absorbed by milk when varying methods of carbonation were used and to also show the influence of varying amounts of absorbed carbon dioxide upon percentage acidity and bacterial counts. Raw milk and pasteurized milk were used.

Procedure:- One hundred cubic centimeters of the milk was placed in each of the sterile graduated sample jars. One, two, four, six, ten, and fifteen grams of solid carbon dioxide were introduced into the sample jars so that the effects of each amount used might be studied. Also gaseous carbon dioxide was bubbled through milk for one, three and five minutes. The milk that was treated with gaseous carbon dioxide was placed in an open top pasteurizer tank. The carbon dioxide was then bubbled through the fluid for the periods of time specified. A top-cock fitted in the bottom of the pasteurizing tank admitted the carbon dioxide.

The pressure produced by the subliming of the solid carbon dioxide was determined by the use of a vacuum-pres-

Table 9

Influence of Absorbed Carbon Dioxide Upon Percentage Acidity and Bacterial Count of Pasteurized Milk Stored at 45°F.

Sample No.	Days in Storage	CO_2 Treatment	Acidity	Total Gas	CO_2 Gas	Temp. Change	Pressure Produced	Bacterial Count
			percent	c.c.	c.c.	°F.	pounds	
1	0	1 gram*	0.27	19.90	17.8	-3	14	23,550
2	0	2 "	0.31	38.55	35.2	-1	17	22,200
3	0	4 "	0.38	50.60	48.9	-1.5	23	22,860
4	0	6 "	0.45	64.00	62.0	-2	27	23,550
5	0	10 "	0.49	93.00	90.7	-4	27	24,000
6	0	15 "	0.64	111.1	110.	-8	27	25,000
7	0	1 minute	0.27	26.20	23.3			27,300
8	0	3 "	0.35	44.20	42.8			26,700
9	0	5 "	0.40	69.20	68.1			18,300
Check	0	0 "	0.17	4.00	1.00			29,550
10	5	1 gram	0.26					295,000
11	5	2 "	0.27					280,000
12	5	4 "	0.37					50,000
13	5	6 "	0.39					9,100
14	5	10 "	0.43					3,800
15	5	15 "	0.60					2,350
16	5	1 minute	0.25					200,000
17	5	3 "	0.32					140,000
18	5	5 "	0.40					135,000
Check	5	0	0.18					930,000
19	9	1 gram	0.22					4,880,000
20	9	2 "	0.29					3,150,000
21	9	4 "	0.23					2,230,000
22	9	6 "	0.33					1,760,000
23	9	10 "	0.46					1,180,000
24	9	15 "	0.52					1,200,000
25	9	1 minute	0.25					13,200,000
26	9	3 "	0.32					3,750,000
27	9	5 "	0.37					3,800,000
Check	9	0	0.19					120,000,000

*The figure preceding the designation "gram" refers to the number of grams of solid carbon dioxide used in those samples. The figure preceding the designation "minute" refers to the number of minutes that gaseous carbon dioxide was bubbled through that sample.

Table 10

Influence of Absorbed Carbon Dioxide Upon Percentage Acidity and Bacterial Count of Raw Milk Stored at 45°F.

Sample No.	CO_2 Treatment	Days in Storage	Acidity Percent	Bacterial Count
1a	1 gram*	3	0.540	14,500,000
2a	2 "	3	0.595	8,000,000
3a	4 "	3	0.585	7,000,000
4a	6 "	3	0.575	7,000,000
5a	10 "	3	0.620	6,500,000
6a	15 "	3	0.665	7,000,000
7a	1 minute*	3	0.680	13,500,000
8a	3 "	3	0.670	10,000,000
9a	5 "	3	0.665	11,000,000
Check	0	3	0.680	14,500,000
10a	1 Gram	7	0.680	44,500,000
11a	2 "	7	0.630	36,400,000
12a	4 "	7	0.785	25,000,000
13a	6 "	7	0.785	24,700,000
14a	10 "	7	0.830	16,700,000
15a	15 "	7	0.825	16,850,000
16a	1 minute	7	0.815	35,000,000
17a	3 "	7	0.820	26,000,000
18a	5 "	7	0.825	17,000,000
Check	0	7	0.830	10,000,000

*The figure preceding the designation "gram" refers to the number of grams of solid carbon dioxide used in that sample. The figure preceding the designation "minute" refers to the number of minutes that gaseous carbon dioxide was bubbled through that sample.

sure guage. A floating type dairy thermometer was used to determine the temperature changes caused by the subliming solid carbon dioxide.

Discussion:- The results given in Table 9 of the tests run on pasteurized milk immediately following carbonation show a consistent increase in percentage of acidity in direct proportion to the amount of solid carbon dioxide used. These results give no indication that the bacterial content had been affected by the short time that the milk was in the carbon dioxide atmosphere. After 5 days in storage at 45°F., the pasteurized milk showed a decrease in numbers of bacteria in proportion to the amount of carbon dioxide absorbed by the milk. After nine days in storage, all of the pasteurized milk showed an increase in numbers of bacteria over counts made at the beginning of the experiment. This milk remained sweet through the ninth day of storage.

After three days in storage at 45°F., the carbonated raw milk samples showed a decrease in the numbers of bacteria present as compared with the numbers of bacteria present in the untreated samples. After seven days in storage, those samples having absorbed the largest amounts of carbon dioxide showed the greatest decrease in numbers of bact-

eria. The raw milk was sour twenty-four hours after it had been stored.

The carbonated flavor was not so intense at the end as at the beginning of the experiment.

The raw and pasteurized milk used in this experiment were old at the beginning of the experiment. Should good quality milk have been used the results from these samples in which carbon dioxide was used might have been more favorable.

SUMMARY

Milk and cream were carbonated during the following processes: separation, pasteurization, and during storage. Solid and gaseous carbon dioxide were used in these treatments. Untreated milk and cream were processed and stored as checks. Various methods of carbonation were employed. The different tests and determinations that were made on the samples were as follows: acidity percentage; pressure developed by subliming solid carbon dioxide; influence of solid carbon dioxide upon temperature; amount of gases absorbed by the fluids when different methods of carbonation were used; effects of carbon dioxide upon the number of bacteria; and the influence of carbon dioxide on the flavor.

It was found that the quality of the dairy products studied was improved when these products were treated with carbon dioxide. The degree of improvement was in direct proportion to the amount of carbon dioxide absorbed by the product.

Carbonation imparts an intense off flavor to milk. The intensity of this off flavor decreases as the storage period of the milk lengthens.

CONCLUSIONS

The results obtained from the carbonation treatment as applied to dairy products in this study give foundation for certain conclusions. These conclusions follow.

1. The carbonation of milk during separation does retard bacterial growth in the resulting cream. The acidity of cream this treated is slightly increased.
2. The numbers of bacteria in cream pasteurized and stored in carbon dioxide are materially reduced and in some cases the cream is rendered free of bacteria. To obtain such results the cream must be stored at a temperature of 55°F. or below.
3. Butter churned and stored in an atmosphere of carbon dioxide and made from cream pasteurized in an atmosphere of carbon dioxide possesses a superior nutty flavor and supports less mold growth than does untreated butter.
4. The flavor imparted to cream during the carbon dioxide treatment is scarcely perceptible and is not objectionable.

5. The carbonation of milk does not destroy its creaming ability.
6. Carbonated milk has a decidedly off flavor.
7. The inhibiting effects of solid carbon dioxide upon bacteria in cream is not due to the lowered temperature nor to the small pressure produced during sublimation, and as the reduction in bacteria numbers become greater as the storage period lengthens, the inhibiting effects of the gas is most likely attributable to the carbon dioxide absorbed by the cream.
8. The pasteurization of milk or cream in an atmosphere of carbon dioxide has a marked inhibitive effect upon the bacteria in these fluids, but pasteurization should be supplemented by storage in an atmosphere of carbon dioxide if the milk or cream is to be held for any length of time.
9. Where the initial acidity is known, the percentage of acidity following the carbonation of a product can be used as a rough index of the amount of carbon dioxide which has been absorbed by the product.
10. For processing purposes, gaseous carbon dioxide is more practical and convenient to use than solid carbon dioxide, while solid carbon dioxide is more

practical for storage purposes unless special storage receptacles are used.

11. Processing and storage equipment suitable for carbonation is practical and would most likely prove profitable.

12. Carbonated dairy products stored at recommended temperatures in an atmosphere of carbon dioxide may be kept for comparatively long periods with but little deterioration.

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