THE EFFECT OF CARBON DIOXIDE REMOVAL UPON THE FREEZING POINT OF VACUUM TREATED MILK

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Vacuum treatment of fluid milk by non-steam injection flavor removal equipment has been shown to elevate the freezing point of milk when the equipment is operated at low levels of flash-cooling. This study indicates that the loss of carbon dioxide, at the reduced pressures employed for the vaporization of volatile flavor components, is a prime factor responsible for the freezing point elevation. The implications of the effects of carbon dioxide loss on the freezing point of milk are discussed.

The use of non-steam injection vacuum treatment equipment in conjunction with high-temperature, short-time (HTST) pasteurizers has been shown *(2, 4)* to result in an elevation of the freezing point of milk when processing occurred at low levels of treatment or degrees of flash-cooling. The loss of dissolved gases, resulting from the reduced pressures used in this type of vacuum equipment to boil off the undesirable flavor components, has been thought to be *(2, 6)* the primary cause of the freezing point alteration.

The effects of processing upon the gas content of milk have been noted by several investigators. Frayer (1) and Van Slyke and Keeler (11) discovered that the heating of milk during pasteurization to 143°F. for 30 minutes reduced the carbon dioxide content. Van Slyke and Baker *(10)* reported it was possible to remove the carbon dioxide completely from milk by laboratory vacuum exhaustion. This was verified by Noll and Supplee (5), who were able to effect significant changes in the gas content of milk by subjecting it to vacuum treatment, flushing with other gases, or a combination of these techniques. It is apparent that the gas content of milk is constantly shifting as a result of processing. The low partial pressure of carbon dioxide in the atmosphere, and the comparatively higher concentration of this gas in milk could account for this readjustment.

The present study was undertaken to determine the relationship between carbon dioxide loss and the freezing point elevation of milk resulting from vacuum processing of fluid milk.

EXPERIMENTAL

Mixed herd milk was received every-other-day from producers with bulk tanks and processed the following day. The milk was pasteurized at 172°F. for 16 seconds in a HTST unit engineered for 80% regeneration and a capacity of 3400 pounds per hour. It was homogenized at 2200 psi. after vacuum treatment, but before the final heating section. Vacuum treatment was accomplished with a non-steam injection single chamber unit[2] located after raw regeneration. The vacuum unit was operated as explained in a previous publication *(4)*.

Eight trials representing 11 different levels of treatment or flash-cooling degree increments were studied. Raw milk samples collected from the balance tank and vacuum treated milk samples obtained from a valve in the line after the final cooling section were checked for freezing point and carbon dioxide content. The freezing points were determined in duplicate using a model F Fiske cryoscope.[3] The carbon dioxide content was determined with a Van Slyke blood gas manometric apparatus using the method as described by Van Slyke and Peters *(9)*, but substituting 20% lactic acid for sulfuric acid *(10)*.

The following is a description of several terms used in this study: *(a)* the degrees of flash-cooling refers to the difference in temperature between the milk entering and leaving the vacuum chamber; *(b)* the percentage concentration of milk was calculated from degrees of flash-cooling using the formula $Y = -0.270 + 0.109\ X$, where Y represents the percentage of milk loss, and X the degrees of flash-cooling (7); (c) the freezing point difference was the difference between the freezing point of the vacuum treated milk and the raw milk control; and *(d)* the freezing point elevation was the rise in the freezing point of the laboratory vacuum treated sample over the raw sample.

In the experiments performed in the laboratory, an apparatus, as shown in Figure 1 was designed to facil-

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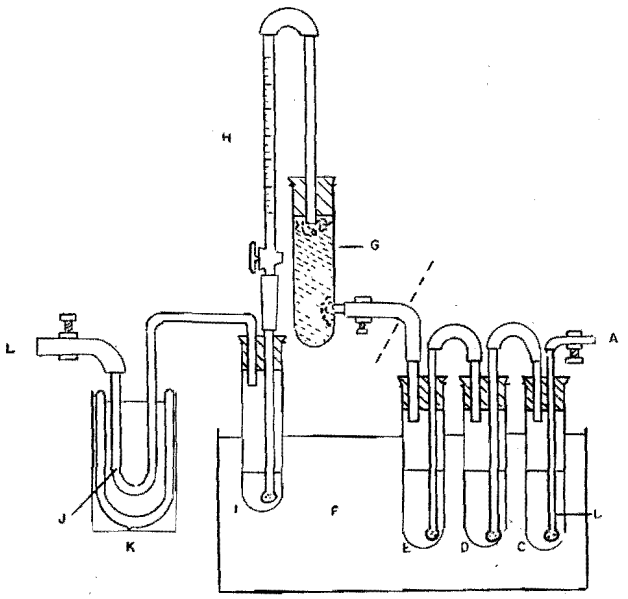

Figure 1. Apparatus for laboratory removal of carbon dioxide from milk: (A) Air intake; (B) Gas washing device; (C) Sulfuric acid; (D, E,) Sodium hydroxide; (F) Water bath; (G) Anhydrous calcium sulfate; (H) Burette; (I) Milk sample; (J) U-tube vapor trap; (K) Dewar flask; (L) Water aspirator.

itate and regulate the removal of carbon dioxide from a raw milk sample. Carbon dioxide-and moisture-free air was aspirated at a uniform rate through 10-ml milk samples for varying lengths of time (a few seconds to an hour) so that the resulting samples contained various levels of carbon dioxide. Moisture-free air was obtained by drawing laboratory air *(A)* through a series of 35 mm. x 250 mm. gas washing test tubes and a moisture absorbent. The air was drawn *(B)* through 100 ml of concentrated sulfuric acid *(C)*, then through two tubes each containing 100 ml of 0.1 *N* sodium hydroxide *(D, E)* and finally through a column of anhydrous calcium sulfate with an indicating band *(G)*, before bubbling slowly into the milk sample *(I)*. The tubes containing sulfuric acid, sodium hydroxide and the milk sample were immersed above their content level in a water bath *(F)* thermostatically maintained at 30°C. To prevent loss of water vapor which would result in a depression of the freezing point of the milk sample, a vapor trap *(J)* was installed in the exhaust line from the sample tube. The vapor trap consisted of a U-tube submerged in a liter Dewar flask containing dry ice and isopropyl alcohol *(K)*. After the desired aspiration time the tubing was closed at all closures to prevent entrance of air into the system and the U-tube assembly was removed from the Dewar flask. The milk sample then was flushed into the vapor trap and back to the sample tube by inverting the assembly. This flushing was done three times to insure recovery of all water collected in the U-tube. The sample then was drawn up through the gas washing device by applying suction at the point indicated by the broken line in Figure 1, and into a 5-ml burette *(H)* installed in the line directly above the sample tube. This sampling burette then was used to deliver samples to the manometer and the cryoscope. The carbon dioxide determination requires the sample to be delivered into the manometer cup above the extraction chamber under a measured amount of lactic acid. This feature plus the fact that a sealed-off rubber tubing was attached to the upper end of the burette prevented exposure of the sample to atmospheric carbon dioxide.

The studies on the effect of laboratory removal of carbon dioxide on the freezing point of milk required protection of the sample from atmospheric carbon dioxide during the freezing point determination. A 2-ml sample was delivered from the burette (Figure 1) to a test tube which had been flooded with nitrogen. When the sample was introduced into the test tube, nitrogen was allowed to flow continuously into the test tube and the space above the sample was flooded prior to applying a rubber stopper. These samples were stored in ice water until the freezing point determinations were made. When the stopper was removed and the probe assembly was introduced for operation of the cryoscope and during the entire freezing point determination, a constant flow of nitrogen was maintained over the test tube.

The method of least squares was used for determining the relationship between freezing point elevation and carbon dioxide removal. The standard error of estimate was used as a measure of accuracy of the regression equation *(8)*. Simple and partial coefficients of correlation were used to indicate the significance of the data *(8)*.

RESULTS

The effect of a single vacuum chamber located after raw regeneration upon the carbon dioxide content and freezing point of milk is shown in Table 1. The data indicate a loss of carbon dioxide due to vacuum treatment and an elevation of the freezing point at low levels of flash-cooling. There appears to be a linear relationship between freezing point difference and concentration of the milk, which agrees with previous findings *(4)*. There does not appear to be a similar relationship between carbon dioxide loss and freezing point difference. It may be postulated that with increasing degrees of flash-cooling there should be a decrease in carbon dioxide content because of the increasing amount of vacuum applied to the milk. This does not appear to be true. The variable carbon dioxide content of the raw milk (2.42 to 5.15 volume

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percent) probably results in its variable removal at a given amount of vacuum. This may explain the non-uniform pattern of the carbon dioxide loss.

The correlation coefficient between freezing point difference and carbon dioxide loss calculated from Table 1 is $r = 0.62$. The partial correlation between freezing point difference and carbon dioxide loss after adjusting for the concentration variable resulted in a correlation of 0.51, and after adjusting for the variable raw milk carbon dioxide values resulted in a correlation of 0.38.

Since the correlation coefficients between freezing point difference and carbon dioxide loss in the dairy plant experiments were not high and because of the variable concentration of milk in the study, the results were not considered conclusive. Thus, a laboratory study, using apparatus described under Experimental, whereby the concentration factor could be eliminated, was undertaken to determine the relationship between carbon dioxide loss and freezing point difference in vacuum treated milk. The raw milk samples used in the laboratory study were equivalent in age to the milk used in the plant experiment.

The results of the laboratory study on the effect of carbon dioxide removal upon the freezing point of milk are shown in Table 2. The upward trend of freezing point elevation with increasing volume percent carbon dioxide loss was $Y = -0.0021 + 0.000077X$ with a standard error for the regression equation of 0.00007°C. and a correlation coefficient of 0.72, where Y denotes the freezing point elevation, and X the carbon dioxide volume percent loss. The correlation coefficient was not materially improved by calculations based on a curvilinear trend. The partial correlation between freezing point elevation and carbon dioxide loss after adjusting for the variable raw milk carbon dioxide values resulted in a correlation of 0.72. The freezing point elevation did not appear to become apparent until approximately 25% of the carbon dioxide had been removed from the milk. This may be explained by the sensitivity limitations of the cryoscope (±0.001°C.).

The results clearly indicate that laboratory removal of carbon dioxide from milk by vacuum treatment will elevate the freezing point. The effect appears to be of sufficient magnitude to account, at least in part, for the freezing point elevation which occured in the plant study (Table 1), when operating the vacuum equipment at low levels of flash-cooling.

The removal of carbon dioxide by vacuum processing should vary with the carbon dioxide content of the original milk. The carbon dioxide content of the raw milk in the plant study varied from 2.42 to 5.15 volume percent (Table 1) and in the laboratory study from 2.80 to 6.33 volume percent (Table 2). Since carbon dioxide removal elevated the freezing point of milk, it would seem logical that the degree of elevation at a given level of vacuum processing would depend upon the carbon dioxide content of the raw milk as indicated by the partial correlation calculated from the data shown in Table 1. Thus, the accuracy of regression equations *(4)* for estimating water adulteration of vacuum treated milk would be contingent

TABLE 1. THE EFFECT OF A SINGLE VACUUM CHAMBER LOCATED AFTER RAW REGENERATION UPON THE CARBON DIOXIDE CONTENT AND FREEZING POINT OF MILK

Flash-cooling	Concentration of milk	Freezing point difference	Carbon Dioxide volume percent		Carbon dioxide loss
			Raw milk	Treated milk	
(°F)	%	(°C)	(%)	(%)	(%)
6	0.38	+0.004	5.15	1.10	78.6
7	0.49	+0.005	5.15	0.95	81.6
8	0.60	+0.002	5.15	0.76	85.2
9	0.71	+0.002	2.86	0.96	66.4
9	0.71	+0.001	3.14	1.06	66.2
10	0.82	−0.002	3.15	1.06	66.3
11	0.93	0.000	2.95	0.67	77.3
13	1.15	−0.005	3.15	0.94	70.2
13	1.15	0.000	2.95	0.53	82.0
14	1.26	−0.004	3.44	1.17	66.0
16	1.47	−0.005	2.42	0.76	68.6
18	1.69	−0.008	3.21	1.30	59.5
19	1.80	−0.009	2.86	0.84	70.6

TABLE 2. THE EFFECT OF LABORATORY REMOVAL OF CARBON DIOXIDE UPON THE FREEZING POINT OF MILK

Volume percent Carbon dioxide		Carbon dioxide loss	Freezing point elevation
Raw milk	Treated milk		
(%)	(%)	(%)	(°C)
4.63	3.63	21.6	0.000
4.63	3.44	25.7	0.001
4.89	3.18	35.0	0.002
4.71	2.99	36.5	0.001
4.89	2.84	41.9	0.002
4.97	2.31	53.5	0.001
4.71	2.10	55.4	0.001
4.35	1.66	61.8	0.001
4.45	1.64	63.1	0.002
4.03	1.34	66.7	0.003
4.45	1.01	77.3	0.004
4.35	0.96	77.9	0.002
3.37	0.55	83.7	0.002
4.03	0.55	86.4	0.006
4.97	0.53	89.3	0.002
3.51	0.36	89.7	0.004
6.33	0.64	89.9	0.002
4.97	0.46	90.7	0.006
4.03	0.37	90.8	0.008
2.80	0.17	93.9	0.008
2.80	0.13	95.4	0.009
6.33	0.18	97.2	0.004
3.37	0.01	99.7	0.007

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upon the carbon dioxide content of the original milk. This would probably make borderline cases of watering in milk treated at low levels of vacuum difficult to detect.

The variation in carbon dioxide content (2.42 to 6.33 volume percent) of the raw milk supplies as received at the dairy plant in this study is in agreement with observations of other investigators *(1, 5)*. However, the carbon dioxide content of milk immediately after milking has been reported as 10 volume percent *(3)*. The present-day trend toward longer storage of raw milk and methods involved in handling such milk, apparently results in a lowering of the carbon dioxide content of fluid milk as it is received at dairy plants. Since this study indicates that carbon dioxide loss elevates the freezing point, the trend toward longer storage would have the effect of elevating the −0.550°C. standards for milk. Futhermore, the variable carbon dioxide content of milk may account, in part, for the normal freezing point range for milk.

REFERENCES

1. Frayer, J. M. The Dissolved Gases in Milk and Dye Reduction. Vt. Agr. Expt. Sta. Bull., 461. 1941.
2. Henningson, R. W., and Lazar, J. T. Effect of Vacuum Pasteurization on the Freezing Point of Milk. J. Assoc. Offic. Agr. Chem., **42**: 417. 1959.
3. Jenness, R. and Patton, S. *Principles of Dairy Chemistry*. John Wiley and Sons, Inc., New York. 1959.
4. Moore, J. M., and Smith, A. C. Observations on the Freezing Point of Vacuum Treated Milk. J. Milk and Food Technol., In Press.
5. Noll, C. I., and Supplee, G. C. Factors Affecting the Gas Content of Milk. J. Dairy Sci., **24**: 993. 1941.
6. Roberts, W. M. The Practical Application of Steam Injection Systems to Fluid Milk and Cream Operations. Am. Milk Rev. **19** (12): 44. 1957.
7. Smith, A. C., and Glazier, L. R. Influence of Vacuum Treatment Upon Milk Loss. J. Dairy Sci. **42**: 1234. 1959.
8. Snedecor, G. W. *Statistical Methods*. 5th Ed. Iowa State College Press, Ames. 1956.
9. Van Slyke, D. D., and Peters, J. P. *Quantitative Clinical Chemistry*. Vol. II, Methods. The Williams and Wilkens Co., Baltimore, 1932.
10. Van Slyke, L. L., and Baker, J. C. Carbonic Acid and Carbonate in Cow's Milk. New York (Geneva) Agr. Expt. Sta. Tech. Bull., 69. 1919.
11. Van Slyke, L. L., and Keeler, R. F. The Carbon Dioxide Content as a Basis for Distinguishing Heated from Unheated Milk. New York (Geneva) Agr. Expt. Sta. Tech. Bull., 78. 1920.

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TO: MEMBERS OF THE INTERNATIONAL ASSOCIATION OF MILK AND FOOD SANITARIANS

A number of problems arose concerning the scheduled meeting of the IAMFS at Jekyll Island, Georgia. Without detailing those problems, the officers of the Georgia Society of Sanitarians advised the Executive Board of the IAMFS that it would be advisable to hold the meeting at some other location. The Iowa Affiliate has agreed to be host to the annual meeting this year instead of 1963. The dates will be August 14th-17th and detailed information concerning reservations, etc., will be sent to each of you.

If any of you had made reservations at Jekyll Island, the reservations are cancelled and refunds will be made to those who made deposits.

It is with regret that all of the various changes have been made but we want to thank the Iowa Affiliate for helping arrange the meeting upon short notice and hope that all of you will support this meeting by your attendance.

We want to thank the Georgia Society of Sanitarians and its members who worked so hard to promote the meeting in the Southeast. The change of meeting place does not reflect in any manner upon the management of the Wanderer Motel, the officers of the Georgia Society of Sanitarians, or the many members who contributed so much to the planning of the meeting.

Let's all be in Des Moines, August 14-17.

JOHN J. SHEURING

President, IAMFS

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