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#### ABSTRACT

Freezing points were determined for a series of concentrates prepared from skim milk by three methods. Methods were reconstitution of powder, evaporation of fresh milk, and concentration of fresh milk by reverse osmosis. Refractive index-concentration data were developed so that concentration could be monitored. The freezing point-concentration data were described by empirical equations. Statistical comparison of the equations showed the correlation for the reconstituted milk different from the two fresh milks. Over the range .5 to 3.3°C/min rate of cooling did not affect freezing point depression.

#### INTRODUCTION

We are studying the freeze concentration of skim milk. A method under study, described as "indirect", employs chilling on a refrigerated surface. The refrigerant does not contact the milk, and its temperature is determined by the freezing point of the concentrate. In the other method under study, "direct" freezing is achieved by exposing the concentrate to a vacuum. In the former case, freezing point data for concentrates are required to control the process; in the latter vapor pressure-concentration data are needed. Because freezing point is a colligative property, we can use it to estimate all other colligative properties. For example, osmotic pressure can be calculated by the formula (6):

$$\pi = \Delta F \Delta H_F / v_1 T_1 T_s$$

where  $\pi$  = osmotic pressure,  $\Delta F$  = freezing point depression,  $T_1$  = absolute freezing temperature

of the pure solvent,  $T_s$  = absolute freezing temperature of the solution,  $\Delta H_F$  = heat of fusion of the solvent, and  $v_1$  = partial molal volume of the solvent. Additionally, activity  $A_w$  can be calculated from the freezing point depression by the formula (5):

$$-\ln A_w = 9.6934 \times 10^{-3} \Delta F + 4.761 \times 10^{-5} \Delta F^2.$$

Because  $A_w = P/P_0$  where  $P_0$  is the vapor pressure of the pure solvent at the given temperature, the vapor pressure of the solution can be calculated. From the activity the boiling point of the solution can be calculated by the formula (5):

$$-\ln A_w = (L_B - G T_B) / RT - (1/T_B) + (G \ln T_B / T) / R$$

where  $L_B$  is the latent heat of evaporation at the boiling point of pure water ( $T$ ),  $G$  is the difference of the molal heat capacities of water as a vapor and as a liquid, and  $T_B$  is the boiling point of the solution. The osmotic pressure and boiling point data of skim milk concentrates are needed in studies in this laboratory on reverse osmosis and evaporation.

Skim milk concentrates from three sources were in this investigation: reconstituted skim milk powder, evaporated fresh skim milk, and fresh skim milk concentrated by reverse osmosis. All three may possess different properties because of the processes used to prepare them. For example, concentrates prepared by reverse osmosis may have significantly smaller freezing point depressions because of losses of lactose, minerals, or both through the membrane. Results will yield freezing point data needed to control the freeze concentration unit and to provide information on the effect of method of preparation on freezing point of the concentrates.

Freezing point determinations have been utilized rarely in the dairy industry except for

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TABLE 1. Skim milk analysis (evaporated, reverse osmosis, and nonfat dry milk).

Sample	Total solids	Moisture	Analysis % (MFB) <sup>1</sup>				Protein
			Lactose	Ash	Fat	TKN	
Nonfat dry milk low heat treated		4.05	51.80	8.46	.74	5.88	36.74
Evaporated milk	35.85		60.31	7.67		5.66	35.39
Reverse osmosis feed	9.41		56.75	8.18		5.95	37.19

<sup>1</sup> MFB, Moisture free basis; TKN, total Kjeldahl nitrogen.

detecting illegal addition of water and for ice cream mixes (9). Probably for this reason, few if any data can be found in the literature on the freezing points of skim milk 9 to 35% solids. The suitability of this method has not been established for skim milk and depends on lactose not precipitating. The measurement of a binary system's freezing point is more difficult than that of a pure liquid. If no precipitation of lactose occurs, skim milk concentrates can be considered a pseudo-binary, which further complicates determination of freezing points (2).

## MATERIALS AND METHODS

### Sample Preparation

The first series of experiments were on concentrates prepared from skim milk powder. The skim milk powder was obtained from the United States Department of Agriculture, Agricultural Stabilization and Conservation Service, Kansas City Commodity Office. This powder was a low heat-treated skim milk; all samples were from the same lot number. Concentrates were prepared by reconstituting the powder with distilled water to the desired solids content and were then refrigerated overnight. A typical experiment used ca. 3500 g of concentrate. The range of solids was from 9

to 35%.

The second series of experiments were on concentrates prepared by vacuum evaporation. Fresh pasteurized, homogenized, vitamin A + D, grade A skim milk was purchased from the A + P Dairy Center,<sup>2</sup> Fort Washington PA. The evaporator used to concentrate the skim milk was an A.P.V. recirculating batch vacuum evaporator manufactured by A.P.V. Company, Inc., Buffalo, NY with .71 m<sup>2</sup> of heat transfer surface. The evaporator was operated at an internal pressure of 6 kPa (39.4°C overhead vapor temperature), a constant pump speed of 124 rpm, and 27.8°C ΔT. The final concentrate, 35.85% solids (Table 1), was used as starting material for the second series of freezing point determinations; the concentrate was diluted to lower concentrations with distilled water.

The third series of experiments was on skim milk that was concentrated in a reverse osmosis unit. Feed for this series was the same as in the evaporator series.

The reverse osmosis unit was a DDS-20-Lab Module, manufactured by DDS-RO-Division, Nakskov, Denmark, a laboratory-scale reverse osmosis system. Our unit has .72 m<sup>2</sup> of membrane surface area. The three runs on the DDS-20-Lab Module were under the following conditions: 40 membranes (CA990) were used; the operating pressure was 6 MPa; and feed temperature varied from 12 to 20°C. When each desired concentration was reached, 7.6 liters of concentrate was removed for the freezing point determinations. Table 2 shows the analysis of all samples in this portion of the

<sup>2</sup> Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others not mentioned of a similar nature.

investigation. The first three samples (9A1, 10A1, 11A1), the second three (12A1, 13A1, 14A1), and the last one (15A1) were each a single run on the reverse osmosis unit. Analytical data for all three feeds are in Table 1.

#### Methods

Standard methods of the Association of Official Analytical Chemists (AOAC) were used to determine total solids, ash, and total Kjeldahl nitrogen (3). Moisture and fat contents of the nonfat dry milk were determined by methods outlined in the American Dry Milk Institute Bulletin (1). Lactose was determined by first hydrolysis with lactase, then measurement of the liberated glucose by the classical glucose oxidase method described by Della-Monica et al. (4).

All of the sample determinations were in duplicate. Ash and protein results were in the normal range for variability, whereas lactose results were slightly high for our fresh milk samples. We have no explanation for this but have complete confidence in our analytical methods.

The TP2 temperature programmer and Multicool system, manufactured by FTS Systems, Inc., were used to determine freezing points. The system consisted of a 1-liter steel insulated chamber (10.16 cm diameter × 13.97 cm deep) with a magnetic stirrer on the bottom, mechanically refrigerated to  $-20^{\circ}\text{C}$ , and a temperature programmer with heater. The Philadelphia differential thermometer, manufactured by Precision Scientific Co., Chicago, IL, was used to determine our final temperatures.

It was set to  $0^{\circ}\text{C}$  by a saturated ice-water bath. Precision differential readings can be taken to  $.01^{\circ}\text{C}$  with this thermometer that has a differential span of  $5^{\circ}$  in the range of  $-35$  to  $300^{\circ}\text{C}$ . The Antlia hand pump, manufactured by Schleicher & Schuell, Keene, NH, which was used for sampling, consisted of a 50-ml polycarbonate cylinder attached to a 50-mm filter holder. Samples were drawn into the cylinder through the filter holder. Only 3 to 5 ml was needed for a refractive index reading.

The freezing point was determined for each of a series of concentrations of skim milk. Each series of skim milk ranged from 9 to 35% solids. Freezing point was determined at four cooling rates (.5, 1.3, 2.2,  $3.3^{\circ}\text{C}/\text{min}$ ). Different rates were used to gain insight in the nucleation of ice crystals in the skim milk concentrates. As the temperature reached  $0^{\circ}\text{C}$ , the Philadelphia differential thermometer was used to determine the exact temperature reading down to  $.01^{\circ}\text{C}$ . Time and temperature readings were recorded for each sample. Experiments showed that when our samples froze, they held a constant temperature for 10 to 15 min and were at equilibrium; therefore, approximately 5 min after each sample was frozen, a sample was taken with the Antlia hand pump. Refractive index was measured for each sample with an AO Abbe Refractometer, Model 10450, manufactured by American Optical, Buffalo, NY, and used as an indication of the final concentration at that temperature.

During the freezing process, water was removed as it was frozen, increasing the equilibrium concentration at the freezing point.

TABLE 2. Analysis of reverse osmosis milk.

Sample	Total solids	Analysis % (MFB) <sup>1</sup>			
		Ash	Lactose	TKN	Protein
TPR-9A1	9.41	8.18	56.75	5.95	37.19
TPR-10A1	15.75	7.81	51.75	5.78	36.11
TPR-11A1	25.42	7.55	56.14	5.27	32.95
TPR-12A1	23.40	8.16	59.40	6.92	43.27
TPR-13A1	28.93	8.05	60.84	6.64	41.48
TPR-14A1	34.26	7.79	59.25	5.63	35.21
TPR-15A1	21.28	7.42	57.89	5.59	34.94

<sup>1</sup> MFB, Moisture free basis; TKN, total Kjeldahl nitrogen.

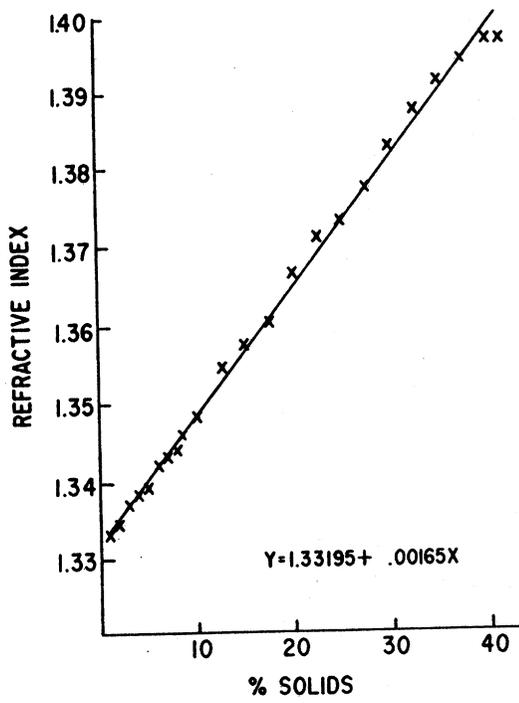


Figure 1. Refractive index versus concentration of reconstituted nonfat dry milk.

Instead of using the initial concentration as the concentration at the freezing point, samples were taken to find the true concentration. The Antlia hand pump was kept at  $-20^{\circ}\text{C}$  to prevent melting upon sampling with a warm hand pump. This would tend to give erroneously low refractive indices from the diluting effect of the melting ice.

**RESULTS**

We showed three things: 1) significant correlations can be developed for freezing points versus concentration (% solids) of skim milk; 2) difference was significant between samples from fresh milk and powdered milk; and 3) freezing points from our model equations can be used to calculate any other colligative properties needed in our research project, which will be treated in a separate publication on colligative properties.

Curves were developed for refractive index versus concentration for each method of preparation. The models in each case reflect

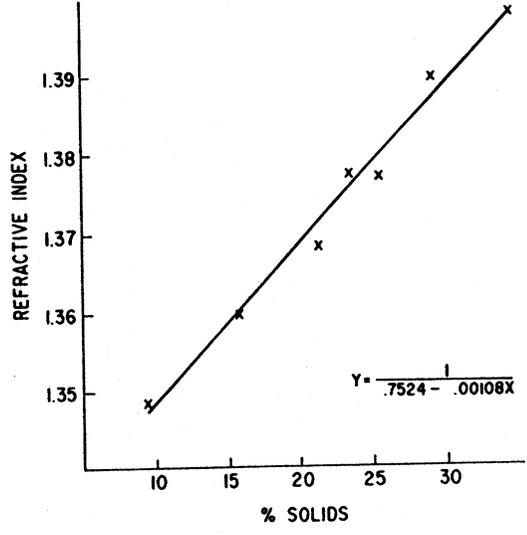


Figure 2. Refractive index versus concentration of skim milk concentrates prepared by reverse osmosis.

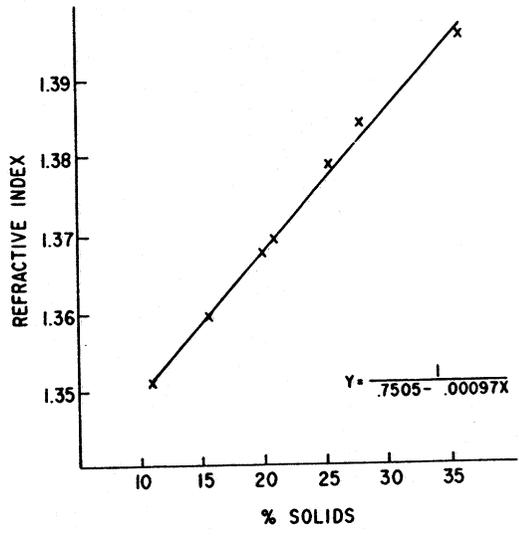


Figure 3. Refractive index versus concentration of skim milk concentrates prepared by evaporation.

the best fit of data by the regression coefficients. Figures 1 to 3 show correlations. Refractive index was measured for each sample that was used to determine freezing points.

We observed two types of cooling curves depending on cooling rate and concentration.

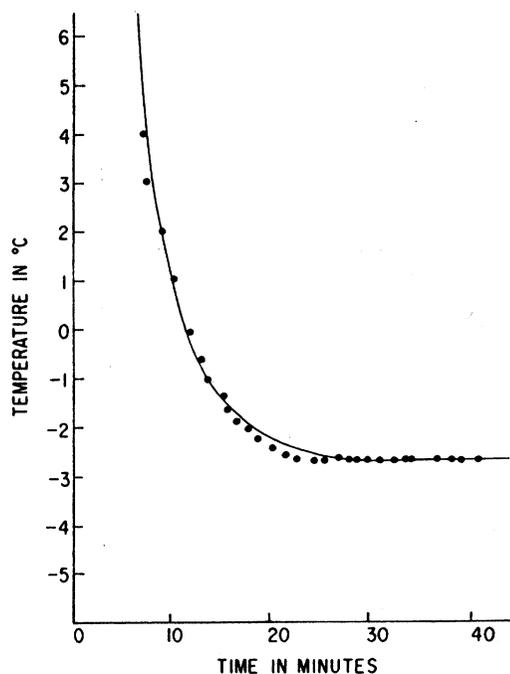


Figure 4. Cooling curve (time versus temperature) of reverse osmosis prepared milk (34.26% solids), 2.2°C/min.

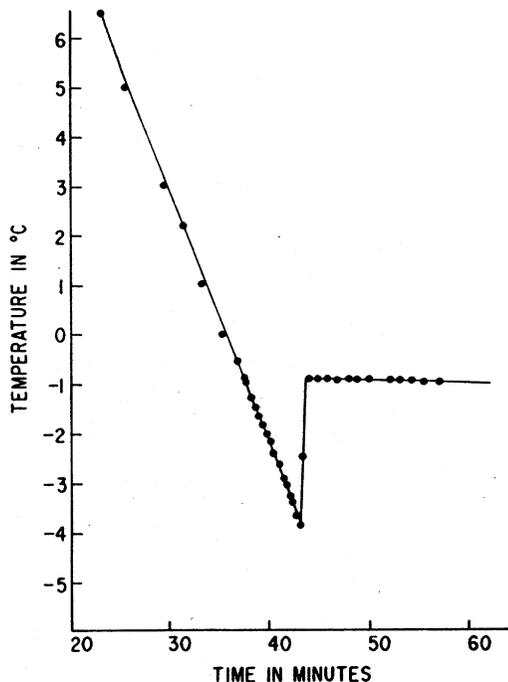


Figure 5. Cooling curve (time versus temperature) of reverse osmosis prepared milk (15.75% solids), .5°C/min.

Curves are similar to cooling curves obtained with and without seeding in a freeze-concentration crystallizer unit (8). The curve in Figure 4 is similar to the seeded type whereas that of Figure 5 resembles curves without seeding typically obtained in freezing point studies. In each case, a different type of nucleation is said to occur (8); crystal growth in the seeded curves is mainly due to secondary (contact) nucleation whereas in the other case crystal growth is said to be due to primary nucleation. The unseeded type cooling curves were observed at lower concentrations and lower cooling rates. At higher concentrations (above 30%), regardless of cooling rate, curves were the seeded type.

Results in Table 3 show that two sets of data exist for each method of preparation, the first being the one without sampling, initial concentrations, and the second the sampled data, equilibrium concentration. After correlating the data we decided sampling was not necessary because there was no significant difference in

the results. The nonsampled data (concentration taken directly from feed) resulted in better fits in all cases. The residual, in all cases, was less, although not significantly, for the nonsampled data. For nonfat dry milk the residual was  $8.6 \times 10^{-4}$  without sampling compared to  $1.41 \times 10^{-3}$  with sampling. The same was true with evaporated and reverse osmosis milks; evaporated milk residuals were  $3.4 \times 10^{-4}$  without sampling and  $6.1 \times 10^{-4}$  with sampling, and reverse osmosis milk residuals were  $1.48 \times 10^{-3}$  without sampling and  $2.43 \times 10^{-3}$  with sampling.

Our final model equations are graphed in Figures 6 to 8. The nonsampled data for the nonfat dry milk were pooled with a previous set of data run without sampling to yield one final model equation for nonfat dry milk (Figure 6). The nonsampled data from the reverse osmosis (Figure 7) and evaporated fresh skim milk (Figure 8) were treated as separate runs. The resulting model equations are: nonfat dry milk line  $Y = .151902 + 4.54666 X$ ,

TABLE 3. Results of freezing point depression ( $\Delta F$ ) versus concentration.

Rate of freezing (°C/min)	Type of treatment													
	Reverse osmosis						Evaporation						Nonfat dry milk	
	1 <sup>1</sup>		2		1		2		1		2		1	2
	$\Delta F(-^{\circ}C)$	X <sup>2</sup>	$\Delta F(-^{\circ}C)$	X	$\Delta F(-^{\circ}C)$	X	$\Delta F(-^{\circ}C)$	X	$\Delta F(-^{\circ}C)$	X	$\Delta F(-^{\circ}C)$	X	$\Delta F(-^{\circ}C)$	X
.5	.49	.1111	.49	.1213	.1172	.54	.1265	.54	.1172	.54	.1265	.56	.0940	.1111
1.3	.48	.1119	.48	.1181	.1179	.54	.1331	.54	.1179	.54	.1331	.61	.1060	.1247
2.2	.49	.1119	.49	.1168	.1179	.54	.1324	.54	.1179	.54	.1324	.59	.1036	.1163
3.3	.49	.1125	.49	.1181	.1179	.54	.1331	.54	.1179	.54	.1331	.55	.1071	.1103
.5	.91	.1850	.91	.1999	.1792	.82	.1912	.82	.1792	.82	.1912	.95	.1853	.1905
1.3	.89	.1836	.89	.1935	.1792	.84	.1919	.84	.1792	.84	.1919	.98	.1836	.1913
2.2	.90	.1836	.90	.1970	.1784	.84	.1855	.84	.1784	.84	.1855	.98	.1819	.1922
3.3	.89	.1836	.89	.1949	.1792	.84	.1864	.84	.1792	.84	.1864	1.01	.1827	.1922
.5	1.20	.2483	1.20	.2750	.2617	1.29	.2706	1.29	.2617	1.29	.2706	1.33	.2547	.2653
1.3	1.20	.2483	1.20	.2631	.2626	1.27	.2679	1.27	.2626	1.27	.2679	1.29	.2587	.2615
2.2	1.19	.2498	1.19	.2615	.2477	1.16	.2591	1.16	.2477	1.16	.2591	1.30	.2587	.2625
3.3	1.19	.2467	1.19	.2561	.2609	1.26	.2732	1.26	.2609	1.26	.2732	1.31	.2587	.2644
.5	1.64	.3187	1.64	.3289	.3481	1.72	.3552	1.72	.3481	1.72	.3552	1.74	.3470	.3637
1.3	1.62	.3212	1.62	.3289	.3481	1.73	.3622	1.73	.3481	1.73	.3622	1.76	.3492	.3602
2.2	1.61	.3221	1.61	.3342	.3481	1.72	.3481	1.72	.3481	1.72	.3481	1.70	.3470	.3682
3.3	1.62	.3238	1.62	.3376	.3441	1.70	.3492	1.70	.3441	1.70	.3492	1.67	.3514	.3546
.5	1.64	.3221	1.64	.3289	.4039	2.03	.4213	2.03	.4039	2.03	.4213	2.05	.4174	.4310
1.3	1.58	.3196	1.58	.3298	.4168	2.06	.4180	2.06	.4168	2.06	.4180	2.04	.4188	.4225
2.2	1.55	.3212	1.55	.3298	.4136	2.04	.4136	2.04	.4136	2.04	.4136	2.05	.4188	.4225
3.3	1.57	.3205	1.57	.3273	.4203	2.08	.4245	2.08	.4203	2.08	.4245	1.99	.4150	.4213
.5	2.22	.4341	2.22	.4360	.5404	2.70	.5442	2.70	.5404	2.70	.5442	2.38	.4905	.4945
1.3	2.22	.4320	2.22	.4489	.5404	2.71	.5494	2.71	.5404	2.71	.5494	2.38	.4852	.4972
2.2	2.23	.4300	2.23	.4480	.5316	2.62	.5328	2.62	.5316	2.62	.5328	2.33	.4919	.5097
3.3	2.23	.4292	2.23	.4360	.5456	2.68	.5494	2.68	.5456	2.68	.5494	2.33	.4865	.4919
.5	2.64	.5202	2.64	.5335								2.65	.5509	.5805
1.3	2.62	.5314	2.62	.5314								2.62	.5477	.571
2.2	2.64	.5279	2.64	.5279								2.72	.5610	.5743
3.3	2.59	.5270	2.59	.5516								2.62	.5463	.5610

<sup>1</sup>X = c/1-c, where c = concentration by weight.

<sup>2</sup>1 = Nonsampled (initial); 2 = sampled.

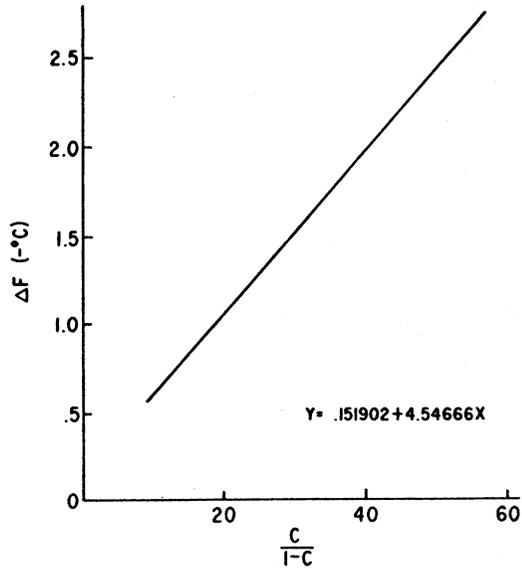


Figure 6. Freezing point depression versus concentration of reconstituted nonfat dry milk.

reverse osmosis milk line  $Y = -.077245 + 5.21085 X$ , evaporated milk line  $Y = -.066706 + 5.10335 X$ .

Using statistical tests from Neter and Wasserman (7) for the comparison of two regression lines, we compared the nonfat dry milk line with reverse osmosis and evaporated fresh skim milk lines. At the 95% confidence, the nonfat dry milk line was significantly different from both reverse osmosis and evaporated skim milk lines. The  $F^*$  for the nonfat dry milk versus the reverse osmosis milk was 51.53 whereas  $F(.95; 2; 79) = 3.89$ ; therefore, they are significantly different where  $F^* = [(SSE(R) - SSE(F))/2] / [SSE(F)/(n_1 + n_2 - 4)]$  and  $SSE(R)$  = residual sum of squares reduced model,  $SSE(F)$  = residual sum of squares full model, and  $(n_1 + n_2 - 4)$  = degrees of freedom. For the nonfat dry milk versus evaporated milk,  $F^* = 55.93$  where  $F(.95; 2; 75) = 3.13$ ; this is also significantly different at this confidence. The reverse osmosis line was also significantly different from the evaporated milk line. The  $F^*$  equalled 4.89 for reverse osmosis versus evaporated milk whereas  $F(.95; 2; 48) = 3.20$ ; therefore, they are significantly different at this confidence. When the regression for the nonfat dry milk line was compared with the reverse

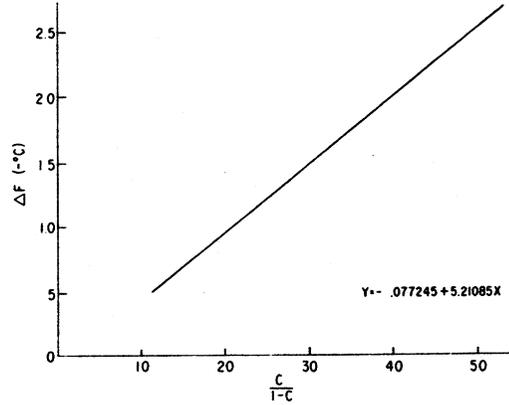


Figure 7. Freezing point depression of skim milk concentrates prepared by reverse osmosis. Concentration is plotted as  $c/1-c$ .

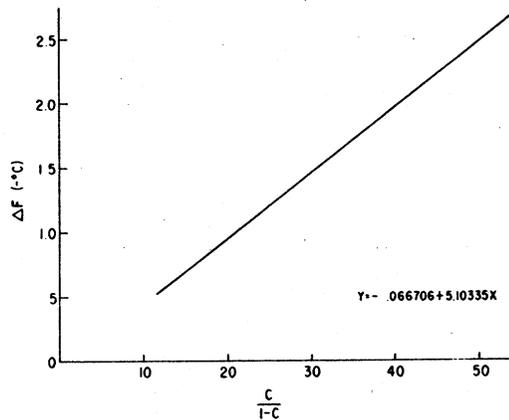


Figure 8. Freezing point depression of skim milk concentrates prepared by evaporation. Concentration is plotted as  $c/1-c$ .

osmosis and the evaporated fresh skim milk lines, slopes were significantly different at the 95% confidence. By the test for comparisons of regression statistics from Neter and Wasserman (7), the 95% confidence interval for the difference in slopes for nonfat versus reverse osmosis milk was  $-.6043 < b_1 - b_2 < -.3379$ , excluding no difference at 95%. For the nonfat versus the evaporated milk the interval was  $-.5051 < b_1 - b_2 < -.2692$ , which is also significantly different at the 95% as  $b_1 - b_2 = 0$  is not included in the interval. There is a significant difference between results of a

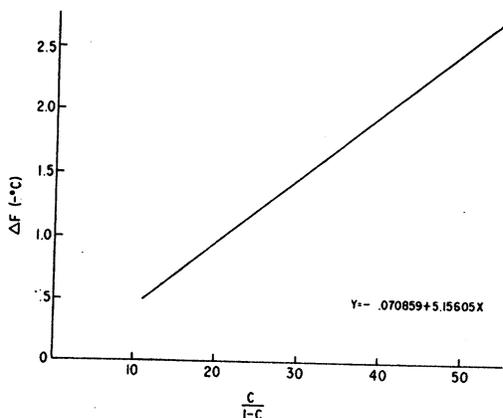


Figure 9. Freezing point depression versus concentration (pooled data of concentrates prepared by reverse osmosis and evaporation).

nonfat skim milk powder or a fresh skim milk sample. Nonfat dry milk samples had a smaller freezing point depression (lower slope) than fresh milk samples. We attribute this difference to composition of the milk. The analysis shows a lower lactose content in the nonfat powder milk than in our fresh skim milk (the reverse osmosis and evaporated), the mineral contents being about equal. Because the freezing point depression is principally due to lower molecular weight compounds, lactose, and minerals for milk, a smaller amount of lactose in the nonfat samples would have a significant effect on the freezing point depression. This in turn would make the slope of the nonfat dry milk line lower than that of the fresh skim milk (reverse osmosis and evaporated) line.

When slopes of the reverse osmosis and evaporated skim milks were compared, they were not significantly different at 95% confidence. The 95% confidence interval for the reverse osmosis versus the evaporated milks was  $-.2928 < b_1 - b_2 < .5078$ . Because  $b_1 - b_2 = 0$  is included in the interval, the slopes are not significantly different at 95% confidence. This means that in the range of solids for the investigation (9 to 35%), data for the reverse osmosis and evaporated skim milks can be pooled to give a common slope for our range. Figure 9 shows the pooled reverse osmosis and evaporated skim milk data. The resulting model equations, nonfat dry milk line  $Y = .151902 + 4.54666 X$ , fresh skim milk line (reverse osmosis

and evaporated)  $Y = -.070859 + 5.15605 X$ , where  $Y$  is the freezing point depression and  $X$  is  $[c/(1-c)]$ ,  $c$  being the concentration by weight of the skim milk, can be used to predict the freezing point depression for skim milk concentrates in the range of 9% to 35% solids.

As expected, rate of freezing did not affect freezing point depressions. Similar depressions were at all four rates. The amount of supercooling, in the cases where we had supercooling, was different for different rates of cooling. Of the four rates (.5, 1.3, 2.2, and 3.3°C/min), 2.2°C/min gave us the maximum supercooling in several cases, but this was not true of all samples that had supercooling.

Using the freezing points from our model equations and physical chemistry equations, we can calculate vapor pressures, osmotic pressures, and boiling point elevations for different concentrations of skim milk. If, however, each feed is of different composition, especially lactose and minerals, a new equation is required. This involves making a series of freezing point determinations in the desired range of solids, then correlating the data.

## CONCLUSIONS

It appears that the method of concentration had no effect on freezing point depression. Skim milk concentrated by either method will give similar results when its freezing point depression is measured.

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