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SOME PHYSICAL EFFECTS OF FREEZING UPON MILK AND CREAM*

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Many observations on the physical effects of freezing upon pure sols have been reported and an interesting review of some of these studies is given by Jones and Gortner (3). An investigation of the effects of freezing upon milk and cream is complicated by the heterogeneous character of the material. The constituents of milk with which this study is chiefly concerned are the casein and the fat.

A hydrophilic sol which has been frozen will repeptize upon thawing whereas a hydrophobic sol similarly treated will precipitate when the frozen mass is melted. The casein of milk, being weakly hydrated retains its normal degree of dispersion under the usual conditions of freezing and thawing. The fact, however, has been noticed that when milk is held in the frozen state for considerable periods of time the casein gradually becomes insoluble (1, 2, 5). Evidently its hydrophilic properties are altered during storage in a frozen condition.

The fat is present in milk as an emulsion and is surrounded by adsorbed protein. If milk or cream has been frozen slowly free fat separates or oils off during thawing, especially when the thawing is conducted at high temperatures. If the product is frozen rapidly enough destruction of the fat emulsion can largely be prevented.

It is to be noted that while a destruction of the colloidal character of the caseinate system during freezing involves a considerable storage period, the effects harmful to the fat emulsion have occurred by the time the material is completely frozen.

A change in the distribution of the constituents when milk is partially frozen has been reported (6, 7, 11, 14). The results show a concentration of constituents in the unfrozen liquid which is to be expected since the solid to separate is pure ice. A condensing process utilizing this principle has

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recently been developed, the milk being concentrated by removing the frozen ice crystals in a centrifuge (10).

An extensive investigation of the effects of freezing upon many physical constants of milk and upon the distribution of the different constituents in the frozen mass has been reported by Cvitl (4). Other papers also present data upon phase distribution (7, 8, 9). In general the results show a concentration of the fat phase in the upper portion of the frozen mass. a higher percentage of protein in the middle or lower portions and the greatest concentration of lactose in the center or last frozen portion.

The effect of freezing upon milk and cream in relation to the marketability of these products has received much attention. A number of investigators have studied the use of frozen cream in ice cream manufacture and detailed directions for the successful handling and storage of frozen cream have been made available. Reports of this work may be found in the trade journals or in Chemical Abstracts.

EXPERIMENTAL

Samples were frozen in air in a cold room maintained at $-16°$ to $-18°$ C. (3.2° to $-0.4°$ F.). Unless otherwise noted the containers used were either tins of 180 cc. capacity or Babcock cream test bottles graduated for 9 gram samples. The material in the tins froze completely in six to seven hours while that in the test bottles required only 50 to 60 minutes to freeze.

The degree of destruction of the fat emulsion in cream was measured as follows: 9 grams of cream were weighed into a 9-gram Babcock test bottle and placed in the $-17°$ C. (1.4° F.) room for 24 hours. The samples were then thawed at 40° C. and water was added to bring the surface of the mixture near the highest graduation on the bottle. They were next placed in a 40° C. bath for 15 minutes, then whirled in a warm (40° C.) Babcock tester for 30 minutes, removed and held in a cold box (15° C.) overnight. The warming and whirling were repeated next day, after which time the length of the column of clear fat was read in the usual manner. Holding the cream cold between whirlings was found necessary to give a clear fat column. Control tests were always run on the unfrozen samples which generally showed about 0.5 to 1.0 per cent fat separation. These figures were subtracted from the readings obtained on the frozen samples. Correction was thus made for the quantity of fat which the method of testing caused to separate and for the action of homogenization in retarding the rise of very small globules into the neck of the test bottle.

Relative viscosity measurements on the frozen and thawed milks were made at 30° C. with an Ostwald type viscosimeter which measured the time required for 24 cc. of milk to flow through one of three different sized

capillary tubes. The water rate for each of the tubes was ascertained and the results are expressed as relative viscosity.

Coagulation and heat stability tests were conducted by sealing the samples in pyrex test tubes and sterilizing them at 120° C. in a glycerine bath.

SOME EFFECTS OF FREEZING UPON THE DISPERSION OF THE CALCIUM CASEINATE SYSTEM

Reference has been made to the gradual change of the calcium caseinate to an insoluble form when milk is held frozen. During this work a progressive decrease in the dispersed state of the casein was produced by freezing.

The effect of freezing upon the heat stability of skim milk was investigated. Data from a representative experiment are given in table 1. Skim

TABLE 1

Effect of freezing at −18° C. (0.4° F.) upon the heat stability of skim milk. Freezing time 6½ hours in cans)

TIME FROZEN	TIME OF COAGULATION AT 120° C.	
	9% S. N. F.	18% S. N. F.
Weeks	Min.	Min.
Not frozen	204	102
7	215	105*
12	225*	95**
17	235**	0
33	205	

* First distinct separation of casein.

** Clear serum could be removed by thawing the frozen mass on a filter at room temperature.

milk of 9 per cent solids was unchanged in heat stability after storage for 33 weeks in a frozen condition. All the samples of this milk were used after the termination of this time, but from the appearance of the milk it was believed that its stability would have dropped to zero in a few more weeks. The milk of 18 per cent solids did not possess any stability toward heat on the 17th week.

Visible separation of the casein in both 9 per cent and 18 per cent milks began to take place early in the storage period. When the precipitated casein first appeared it redispersed during heating but as the time of storage increased more and more casein remained in an insoluble state during sterilization. The precipitated casein did not at first appear to affect the stability of the remaining colloidal particles. Separation of the casein

finally became very marked and if the frozen mass was allowed to melt on a filter, a clear filtrate could be obtained. The calcium caseinate from the 9 per cent milk which remained on the filter when the 12-week sample was thawed possessed remarkable heat stability. Mixed with water in place of serum, its heat stability was 195 minutes; and when the serum from the 18 per cent solids sample was used as a continuous phase, the calcium caseinate from the 9 per cent sample showed a stability of 366 minutes.

The effect of freezing upon the dispersed state of casein in skim milk is shown in a different way through some representative data plotted in Fig. 1. Duplicate cans of skim milk were sterilized at 120° C. for different

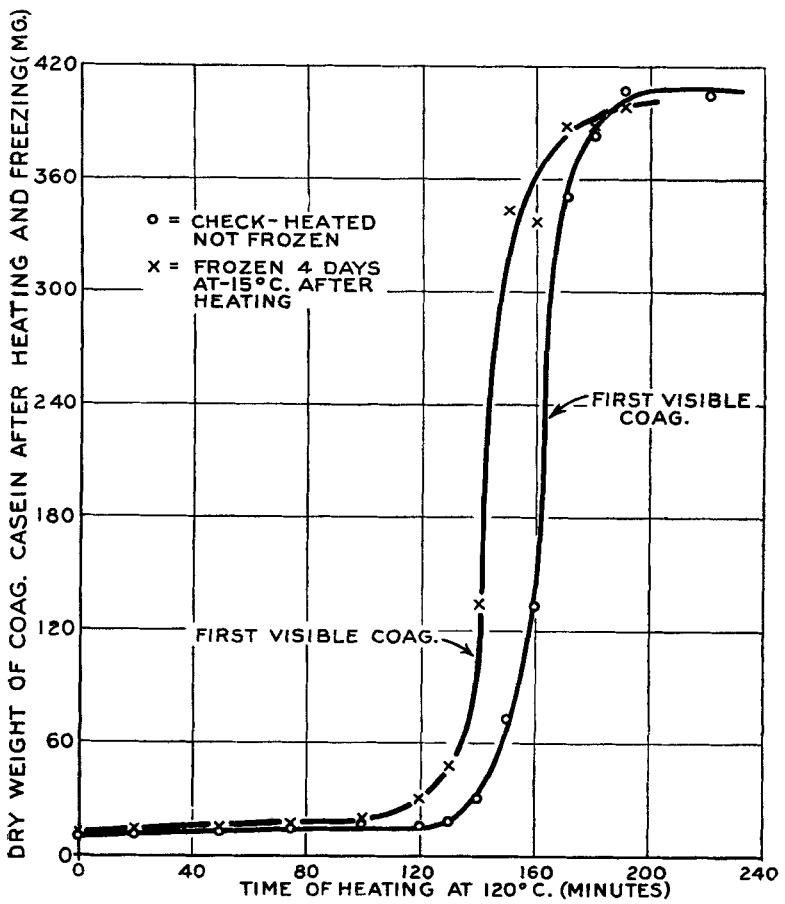

FIG 1. THE EFFECT OF FREEZING UPON THE PRECIPITATION OF CASEIN IN SKIM MILK HEATED TO 120°C. FOR DIFFERENT LENGTHS OF TIME.

periods of time up to 220 minutes. One set of milks was frozen at – 15° C. (5° F.) for four days, the other set acting as the unfrozen control. After thawing the frozen samples at room temperature 10 cc. of milk from each

can was measured into a weighed centrifuge tube. These were centrifuged at a high speed for half a hour, the supernatant liquid decanted and the precipitated casein dried and weighed. The curves of Fig. 1 are of the same type but the freezing process has caused a considerable increase in the amount of heated casein which was found precipitated. The change after a few days freezing is so slight it cannot be detected by visual examination of the sol or by comparing its heat stability before and after freezing. Since this decrease in the degree of dispersion of the casein occurred within a few days after freezing, it indicates that denaturation of the caseinate system during freezing proceeds in a very limited but progressive manner.

SOME EFFECTS OF FREEZING UPON THE FAT EMULSION

While freezing milk over a long period gradually produces an increase in precipitated protein its effect upon the fat phase is much more rapid.

The amount of destruction of the fat emulsion in frozen milk and cream is dependent upon several variable factors. The most important of these appear to be the freezing point of the aqueous phase, the protection afforded the emulsified fat by adsorbed protein and the size of the globules themselves.

The proportion of fat "oiling off" or separating from frozen creams of different fat percentages was measured by means of the test previously described. Large differences in the amount of fat separating after freezing were found. Generally about 25 to 50 per cent of the fat present in 20 to 40 per cent cream and 40 to 60 per cent or more of the fat present in creams over 40 per cent was freed from its normal emulsoid state by freezing. Different creams of the same fat content often showed considerable differences in the amount of fat which separated. In rare instances a cream sample was obtained in which the fat globules were apparently very well protected since only 2 or 3 per cent fat could be separated by the above test after freezing.

Two methods were employed to lower the freezing point of cream, one adding cane sugar and the other increasing the milk-solids-not-fat. Cane sugar in quantities ranging from 5 to 25 per cent added to cream before freezing was found to retard markedly the oiling off of the fat after thawing. The larger quantities of sugar almost entirely inhibited fat separation.

An increase in percentage of milk-solids-not-fat retarded the destruction of the fat emulsion in cream and a decrease in solids favored fat separation. Representative data are presented in table 2.

As the percentage of solids-not-fat of the cream was decreased the test readings for free fat increased from 5.5 to 27 per cent which, on the basis of the total fat present, corresponds to a destruction of the fat emulsion from 12 to 54 per cent. The protective action of high solids can probably

TABLE 2

Effect of variation in solids-not-fat upon the fat separation in frozen and thawed cream thinned from cream containing 60%–63% fat. (Freezing time 60 minutes at –18° C.) Figures represent fat column readings.

MIXTURE NUMBER	THINNING INGREDIENT	COMPOSITION WHEN FROZEN		FAT SEPARATION AFTER THAWING
		Fat	S. N. F.	
		%	%	%
1	Condensed milk	45.5	12.14	5.5
2	Water and condensed milk	46	9.81	10.5
3	Skim milk	45	4.86	15.0
4	Skim milk	40.5	4.05	15.0
5	Water	50	2.62	27.0

be attributed partly to the protection afforded by the protein but chiefly to the lowering of the freezing point caused by the lactose and salts.

The degree of dispersion of the fat slightly influences its capacity to undergo freezing without undue fat separation. The effect of homogenization upon the stability of the fat emulsion was studied. The data of table 3 represent the average figures from three series of experiments. Homog-

TABLE 3

Effect of homogenization upon fat separation in cream during freezing. (Freezing time 60 minutes at –18° C.) Figures represent fat column readings.

FAT IN CREAM	FAT SEPARATION AFTER THAWING		
	Not homogenized	Homogenized at	
		1500 lbs.	3000 lbs.
%	%	%	%
10	1.4	1.1	0.7
20	7.9	5.7	3.6
30	10.5	16.1	18.5
40	25.6	22.2	24.9

enization of 10 per cent and 20 per cent creams helped to decrease fat separation during freezing but in 30 per cent and 40 per cent creams this inhibiting influence was not evident. The amount of clumping caused by homogenization of low fat creams is small but becomes much greater as the fat content of the cream is raised. The clumps are easily broken up by the freezing process and the fat emulsion is destroyed.

The results appear to indicate that the fat emulsion in ice cream remains intact for at least three reasons. The high percentage of cane sugar lowers the freezing point of the aqueous phase; homogenization of the mix inhibits fat separation and increases the amount of protein and gelatin adsorbed; and the formation of countless small ice crystals in the freezer prevent the growth of large and destructive crystals.

Interesting evidence which shows the extent to which freezing destroys the fat clumps in homogenized cream was obtained. Homogenization is known to lower to a striking degree the heat stability of cream. The lowered stability is considered to be due to the formation of fat clumps during homogenization, these clumps acting as nuclei around which coagulation may proceed (13). Data resulting from a study of the heat stability of homogenized creams before and after freezing are presented in table 4.

TABLE 4

Effect of freezing for 24 hrs. at −18° C. upon the heat stability of cream heated to 80° C. and homogenized at 2500 lbs. pressure prior to freezing.
(Freezing time 6½ hours in cans.)

FAT	TREATMENT	HEAT STABILITY AT 120° C.	
		Before freezing	After freezing
%		min.	min.
10	Not homog.	122	121
	Homog.	102	120
20	Not homog.	135	137
	Homog.	75	137
30	Not homog.	142	142
	Homog.	2	146

Freezing restores to the cream the heat stability which it possessed before large fat clumps were formed by homogenization. The clumps are apparently disintegrated during freezing and cannot therefore initiate a general flocculation of the caseinate system during heating. Accordingly a homogenized cream which feathered in coffee because of excessive fat clumping could be freed of this defect by freezing. However the free fat which would oil off on the coffee after the addition of frozen cream would be undesirable.

PRACTICAL APPLICATION OF STUDIES ON THE EFFECTS OF FREEZING ON MILK AND CREAM

An application of the foregoing study to manufacturing problems has yielded some interesting data and the possibility of obtaining new products which may be of value either to research workers or to the industry itself.

A concentrated frozen milk (12) may be produced by pasteurizing fresh whole milk, condensing it to one-third of its weight, sealing it in containers and subsequently freezing it in an ice box maintained at about −17° C. Such a milk, if held at a temperature below −13° C. (8.6° F.) may be thawed and easily reconstituted with cold water. It will yield a normal fresh milk at any time up to the 4th week of storage. This product is an excellent substitute for market milk where the latter is expensive or not easily available. It is essential that the milk be handled in equipment constructed of metals other than copper since contamination with this metal will cause a metallic flavor to develop in the product after a storage period of approximately one week.

The usual fat separation which occurs during the thawing of slowly frozen whole milk does not take place in milk condensed to a 3:1 ratio before freezing. The high solids-not-fat content of the product prevents fat separation in the milk just as it does in the creams referred to in table 2.

The denaturation of the casein during freezing is more readily noticed when the solids-not-fat content of the milk is raised. If the milk is condensed to less than one half its weight, the casein concentration is sufficiently great to produce a gel structure as denaturation in the frozen state sets in.

The relation between concentration, fat separation and protein denaturation or gel formation in frozen milks condensed to various degrees is shown by the data given in table 5. A milk evaporated to three times its

TABLE 5

Relation between concentration, fat separation and gel formation in frozen milk. (Freezing time 6½ hours in cans.)

CONCENTRATION NORMAL = FAT 4% / SNF 9%	FAT SEPARATION		TIME TO FORM GEL AT −15° C.
	18 hrs.	2 mos.	
½ N (% fat × 2)	2.0	3.0	No gel. Ppt. in 3 mos.
N	1.0	1.5	"
2 N	trace	trace	3 mos.
3 N	0	0	5 wks.
4 N	0	0	5 days

normal concentration was found to be the most satisfactory for freezing. The data of table 5 may be considerably varied by changing manufacturing conditions and temperatures. High or long heat treatment shortens the storage period in which the milk is free from casein precipitation. High storage temperatures produce the same effect.

The development of viscosity in concentrated frozen milk during storage at two different temperatures is shown by the data plotted in Fig. 2.

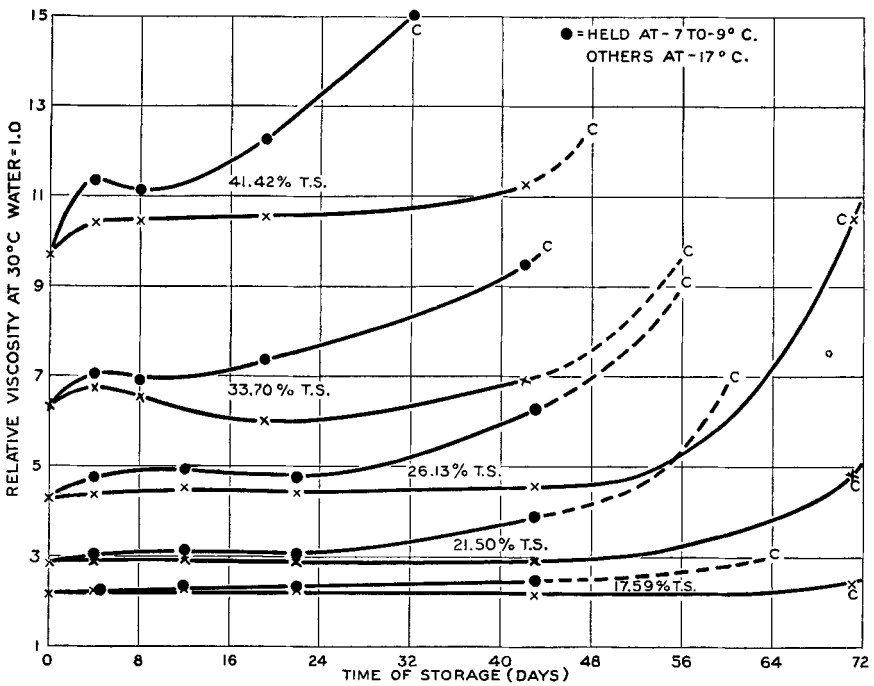

FIG. 2. THE EFFECT OF TIME OF STORAGE IN THE FROZEN STATE UPON THE RELATIVE VISCOSITY OF CONDENSED MILKS OF DIFFERENT CONCENTRATION. "C" AT THE END OF EACH CURVE INDICATES THE TIME WHEN CASEIN COAGULATION WAS FIRST FOUND.

Whole milks condensed to different percentages of solids were used. In most cases when the slight coagulation which precedes gel formation set in, viscosity measurements could no longer be accurately made. In these cases the probable course of the curves is indicated by dotted lines. The importance of low temperatures of storage in preventing the development of high viscosities is apparent.

The ability of the homogenization process to increase protein adsorption and fat clumping in cream was utilized in a method developed to separate some of the milk constituents. It was observed that when homogenized frozen cream was thawed at a temperature below the melting point of the fat, the serum could be drained from the thawing mass, leaving behind a mixture of fat and adsorbed casein. If the melting temperature was high enough to liquefy the fat the material melted as cream and no separation was possible. The fat-casein mixture could be washed with ice water without loss to remove traces of serum.

Data are given in table 6 which are representative of the composition of the fat-casein mixture obtained from creams of 10, 20 and 30 per cent

TABLE 6

Composition of the fat-casein mixture secured from frozen and thawed cream. (Freezing time 10 to 12 hours in 1 gal. cans at −18° C.)

FAT IN ORIGINAL CREAM	WATER	FAT (BABCOCK)	PROTEIN (TOTAL N × 6.38)	LACTOSE AND ASH (BY DIFFERENCE)	CLARITY OF SERUM REMOVED
%	%	%	%	%	
10	53.61	35.0	7.12	4.27	very turbid
20	36.60	55.5	6.59	1.31	turbid
30	20.51	74.0	5.29	.20	clear

fat. A clear separation of serum was obtained only with creams above 25 per cent fat. When the fat content of a cream was lower than this figure there was insufficient fat present to adsorb and hold all the casein. Under such conditions some of the casein escaped with the serum.

The fat-casein mixture was found to be of some practical value. If water was added to replace the serum and the mixture warmed, much of the fat separated by oiling off from the casein. A cream separator removed all but about 0.5 to 1.0 per cent fat. The resulting casein dispersion appeared to possess all of its original characteristics. It was almost tasteless in the absence of the serum and developed very little cooked flavor or brown color after heating to sterilization temperature. Its heat stability remained about the same as that of normal milk. The product provided a normally dispersed casein which should be of value in studies of this protein in its native state. It has been used to advantage in heat stability studies in these laboratories.

The fat-casein mixture was used to raise the milk-protein solids of ice cream mixes without also increasing their lactose content. When the fat for the mix was obtained entirely from frozen cream, the procedure for using only the fat and casein of the frozen cream was very simple. The homogenized frozen cream was thawed upon a fine wire netting suspended over a receiver for the serum, the operation being conducted in an ice box held at 5° to 15° C. (41° to 59° F.). After thawing about 24 hours the fat-casein mixture was added to the mix before pasteurization. For small quantities the most rapid method of removing the serum from the thawed cream was by means of a Büchner funnel using suction. Through use of the fat-casein mixture the protein solids of the mix were increased as much as 1.5 per cent in this manner without increasing the lactose or salts.

Low temperature thawing of homogenized frozen cream provided a simple means of obtaining milk serum in large quantities. By careful handling, a serum equal in clarity to that obtainable by ultra-filtration methods was secured. When the serum was obtained as a by-product in the preparation of the fat-casein mixture for ice cream, it was used to advantage in milk sherbet, giving it a distinctive and pleasing milk flavor.

SUMMARY

1. Slow freezing of milk or cream caused a gradual precipitation of the caseinate system and an immediate destruction of the fat emulsion.

2. Freezing did not alter the heat stability of skim milk until the product had been held frozen for several months at −18° C. (0.4° F.) or below. Freezing caused an immediate increase in the amount of casein which could be centrifuged from milks heated before freezing. Freezing, therefore, caused a slow and gradual increase in the size of the casein aggregates but the change was not noticeable until the freezing period was well advanced.

3. The destruction of the fat emulsion in cream during slow freezing was lessened by adding cane sugar or increasing the solids-not-fat content of the cream before freezing. Homogenization slightly retarded fat separation when low fat creams were frozen. Freezing destroyed the fat clumps formed in cream by homogenization and restored to the cream the heat stability which it possessed before processing.

4. Fresh whole milk was pasteurized, condensed to $\frac{1}{3}$ its weight, canned and frozen without any detrimental effects to the body or flavor of the product. This milk when held frozen at a low temperature and reconstituted at any time within a four-week period by the addition of cold water, yielded a product which often could not be distinguished from fresh market milk. Its use where fresh market milk is expensive or not available was suggested.

5. A process for the preparation of large quantities of normal undenatured casein and of milk serum was developed. Frozen homogenized cream was thawed at a temperature below the melting point of the fat; clear milk serum was collected from the melting mass and the residual mixture of fat and casein was utilized in the preparation of normal casein or to raise the protein solids of ice cream mix.

REFERENCES

(1) ANDERSON, E. O., AND PIERCE, R. L. Some chemical changes in frozen milk occurring in storage. Milk Dealer, 18, No. 12, p. 60, Sept. 1929.

(2) BISCHOFF. Über Eismilch. Archiv. f. Hygiene 47: 68 (1903).

(3) JONES, I. D., AND GORTNER, R. A. Free and bound water in elastic and non-elastic gels. J. Phys. Chem. 36: 387 (1932).

(4) CVITL, JOSEFA. Einfluss des Gefriersens auf einige Bestandteile und Eigenschaften der Milch. Milchwirtsch. Forschgn. **12**: 409 (1931).

(5) FULD, E., AND WOHLGEMUTH, J. Über eine neue Methode zur ausfällung des reinen Caseins aus der Frauenmilch durch Säure und Lab sowie über die Natur der labhemmenden Wirkung der Frauenmilch. Biochem. Zeit. **5**: 118 (1907).

(6) KASDORF, OTTO. Eis und Kälte im Molkereibetrieb. Leipzig, 1904.

(7) KOENIG, W. Gefrorene und ausgefrorene Milch. Molkerei-Zeitung. Hildesheim, **17**: 289 (Feb. 1930).

(8) MANTOVANI, A. Una neuva industria del frio Leche solidificada. De la "Rivista de la Facultad de Quimica Industrial y Agricola." Vol. III (1933).

(9) MUNKWITZ, R. C., BERRY, M. H., BOYER, W. C. Some effects of freezing on the physical and nutritional properties of milk. Md. Agr. Expt. Sta. Bul., 344 (1933).

(10) TUFFT, J. E. Milk concentrated by cold hydrating process. Dairy World 12, No. 1, p. 6 (June 1933).

(11) VIETH, P. Mitteilungen ans dem Laboratorium der Aylesbury Dairy Company in London. Milchztg. **15**, 131 (1886).

(12) WEBB, B. H. Process for preserving fresh whole milk. U. S. Patent 1,964,279 (1934).

(13) WEBB, B. H., AND HOLM, G. E. The heat stability and feathering of sweet cream as affected by different homogenization pressures and different temperatures of forewarming. J. Dairy Sci. **11**, 243 (1928).

Effect of single and double homogenization of cream upon coagulation by heat and rennet and upon separation of the fat. Ibid **14**, 508 (1931).

(14) WINTER, V. V. The separation of the components of frozen milk by fractional melting. Chemical News **138**: 321 (1929).