JOURNAL OF DAIRY SCIENCE

Contents

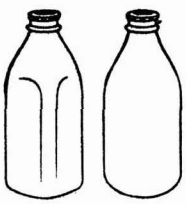
Vol. XXXIII, No. 11, November, 1950

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

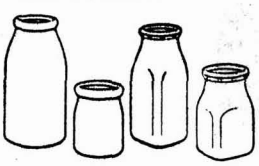
Handi-Squares—3 cases fit easily in space needed for 2 cases of round bottles. Available in all standard sizes and finishes, plain or with Applied Color Lettering.

It's Duraglas Milk bottles

TRADE MARK REG. U.S. PAT. OFF.



Square and Round Two Quart—used successfully for many years in certain markets. Consumers like their convenience.



Dairy Products Jars—ideal for cottage cheese and sour cream. Round or Handi-Square shapes. Available plain, private lettered or with ACL.

for down-to-earth economy and sky-high quality!

● Our concern for Duraglas bottle quality assures dairies of a truly low-cost milk container. Duraglas bottles are made to maximum strength for highest trippage. They resist impact, heat, cold, and caustic washing solutions.

Dairies benefit further from the latest advances in packaging, developed through continuing research at the Duraglas Center.

There you have it—complete service, top quality, and the down-to-earth economy of returnable Duraglas bottles. From half-pints to gallon jugs, *it pays dairies to let Owen-Illinois supply all their container needs.*

Duraglas Dairy Containers

TRADE MARK REG. U.S. PAT. OFF.

OWENS-ILLINOIS GLASS COMPANY

Toledo 1, Ohio • Branches in Principal Cities

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

F. E. NELSON, *Editor*
Ames, Iowa

Subscriptions. Price, $10.00 per volume in North and South America; $10.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to P. R. Ellsworth, The Ohio State University, Columbus 10, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

Advertising copy should be mailed to P. R. Ellsworth, The Ohio State University, Columbus 10, Ohio. Advertising plates or cuts should be mailed direct to the Business Press, Inc., N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Post Office Notices of undeliverable copies and changes of address should be sent to P. R. Ellsworth at the address above stated.

Entered as second-class matter April 13, 1934, at the postoffice at Lancaster, Pa. under the act of March 3, 1879.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXIII NOVEMBER, 1950 NUMBER 11

TOCOPHEROL, CAROTENOID AND VITAMIN A CONTENT OF THE MILK FAT AND THE RESISTANCE OF MILK TO THE DEVELOPMENT OF OXIDIZED FLAVORS AS INFLUENCED BY BREED AND SEASON

VLADIMIR N. KRUKOVSKY, FRANK WHITING[1] AND J. K. LOOSLI

New York State College of Agriculture, Cornell University, Ithaca

The work at this station dealing with deteriorative processes in milk and milk products involving ascorbic acid oxidation have shown that a relationship exists between the tocopherol content of milk fat and the ability of milk to resist the reactions which produce the oxidized flavors, and that both the tocopherols and the stability of milk are influenced by the type of hay and pasture fed to the cow (6, 7, 8). It has first been postulated and then shown on cream (7, 9) that the increase in the anti-oxidant activity of fat as determined by the tocopherol method resulted in the inhibition of development of oxidized flavors associated with deterioration of unstable lipids of the fat globule membrane and in the prolongation of the storage life of fat as determined by the re-emulsification test (5, 9).

Consequently, a study was made to determine the normal tocopherol content of milk produced by different breeds of dairy cows throughout the season and under standard feeding conditions commonly employed at the Cornell Station.

EXPERIMENTAL

Samples of morning milk were collected from cows of Holstein, Brown Swiss, Jersey and Guernsey breeds in the Cornell University Herd, in October, 1947, toward the end of pasture season; in March, 1948, after 5 mo. of barn feeding; and again in July, 1948, following 3 mo. of pasture feeding. Milk was pasteurized at 61.6° C. for 30 min., and the stability of milk was determined on the basis of its ability to resist the reactions which produce the oxidized flavors during 7 days storage at 0 to 5° C. A part of this milk was separated by gravity creaming. The cream was churned and the butter obtained was melted and centrifuged clear and the fat was analyzed for the fat-soluble vitamin content. Vitamin A, carotenoids and tocopherols were determined using Koehn and Sherman (3) and Quaife (10) methods, respectively.

RESULTS

The average values for tocopherols, carotenoids and vitamin A content of dif-

Received for publication Nov. 29, 1949.

[1] Now at Dominion Expt. Station, Lethbridge, Alberta, Canada.

ferent milk fat samples are presented in table 1. The data show large variations

TABLE 1

Tocopherol, carotenoid and vitamin A of the milk fat as influenced by breed and season

Breed	Date	No. of cows	Quantities per 100 g. of fat			
			Total tocopherols	Carotenoids	Vitamin A	Total vitamin A
			(μg.)	(μg.)	(μg.)	(*I.U.*)*
Holstein-Fresian	10/24/47	18	2253 ± 822	504 ± 249	546 ± 145	3020
	3/26/48	20	2011 ± 341	290 ± 109	398 ± 86	2076
	8/ 1/48	13	2492 ± 369	774 ± 276	908 ± 152	4922
	Av.	51	2220	489	580	3135
Brown Swiss	10/24/47	11	2860 ± 656	785 ± 221	703 ± 146	4120
	3/26/48	9	2149 ± 487	341 ± 165	383 ± 47	2100
	8/ 1/48	13	2567 ± 563	1019 ± 309	859 ± 131	5134
	Av.	33	2550	756	677	3968
Jersey	10/24/47	5	3036 ± 498	1236 ± 358	578 ± 123	4372
	3/26/48	4	1905 ± 361	341 ± 108	301 ± 26	1772
	8/ 1/48	7	2740 ± 508	1370 ± 109	631 ± 166	4807
	Av.	16	2623	1070	532	3911
Guernsey	10/24/47	9	3164 ± 462	1583 ± 237	381 ± 120	4162
	3/26/48	7	2329 ± 343	772 ± 169	312 ± 109	2534
	8/ 1/48	12	3346 ± 692	2484 ± 512	663 ± 259	6792
	Av.	28	3033	1766	485	4883
Av. of all breeds	10/24/47	43	2763	887	555	3698
	3/26/48	40	2087	391	370	2131
	8/ 1/48	45	2779	1394	785	5463
Grand Total Av.		128	2533	910	578	3828

* 0.6 micrograms of the carotene and 0.25 microgram of the vitamin A are equal each to 1 I.U. of vitamin A.

in tocopherol, carotenoid and vitamin A content of the fat between individual cows of the same breed, even though they were fed the same rations. There also are wide variations between different breeds and between seasons. As an average, the samples of fat obtained from Guernsey milk were higher in tocopherols, carotenoids and total vitamin A content than the milk fat of any other breed, and this difference held to some extent for any season, as it is shown in table 1.

Holstein milk fat samples were uniformly lower in tocopherols and carotenoid content, and Brown Swiss and Jersey samples were intermediate. During the pasture season the fat samples were 24 per cent higher in tocopherols, 55 to 71 per cent higher in carotenoids and 42 to 60 per cent higher in total vitamin A activity than during the barn feeding. As an average (grand total), the samples of fat contained 2533 μg. of tocopherols and 3828 I.U. of total vitamin A activity per 100 g. of fat. The total vitamin A activity was found to be only slightly below that reported by the U.S.D.A. butter survey committee (12), (14,098 I.U. and 15,529 I.U. per pound of butter, respectively).

The relationships between tocopherol, carotenoid and vitamin A content of the fat from four breeds of dairy cows as affected by both pasture and barn feeding are presented in figure 1. A highly significant correlation has been

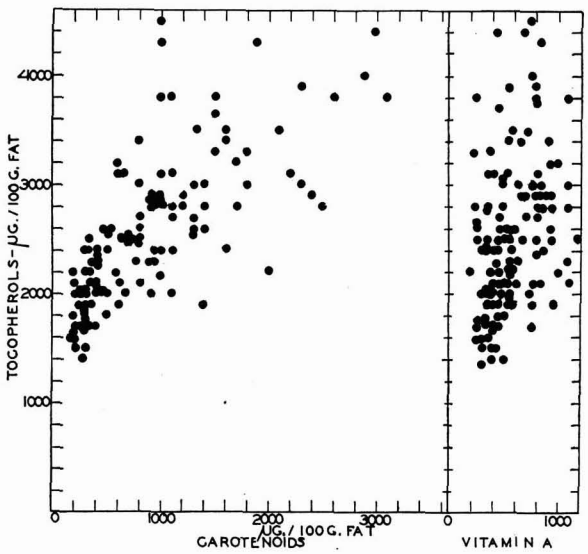


FIG. 1. *The relationship between the tocopherol and carotenoid and vitamin A content* of the milk fat from four breeds of dairy cows as affected by both pasture and hay feeding (128 samples of milk).

found between tocopherol and carotenoid content of the fat (+0.69, +0.63 and +0.68 for the three sampling periods, respectively). No significant correlation could be shown between tocopherol and vitamin A.

The data in figure 2 show the per cent distribution of tocopherols in samples of stable and unstable milks as affected by both pasture and barn feeding. They show again as before (7) that the stability of the fresh pasteurized milk was improved when its tocopherol content was increased to 3,000 μg. and above per 100 g. of fat.

DISCUSSION

Although the data presented in figure 2 were rather conclusive in showing that the anti-oxidant activity centered in the fat phase of the milk plays an important part in the inhibition of oxidized flavors associated with deterioration of the unstable lipid components (9) of the milk system, nevertheless it is necessary also to consider the related effect of the additional factors. This is evident from the observations showing that some of the samples of milk of low tocopherol content did not develop the oxidized flavors during 7 days storage at 0 to 5° C. This fact can be explained by the assumption that the type and quality of the roughages fed to the cow, together with the physiological response of the

cow may determine not only the fat constants and the assimilation and deposition of tocopherols into the milk fat, but also the catalytic properties of the milk with respect to its natural ability to promote ascorbic acid oxidation. This particular factor can be responsible either for too rapid or too slow rate of oxidation of ascorbic acid, thus delaying the onset of the coupled reactions which produce the oxidized flavors. In this connection it should be noted that the rate of ascorbic acid oxidation is an important factor in the promotion or retardation of oxidized flavors in milk (2, 4). Furthermore, the presence of more readily oxidizable substances than the unstable lipids of the milk may result in a selective and stepwise oxidation of the respective components of the milk system. In such a case, the deterioration of unstable lipids might be postponed or not have taken place at all, depending on the availability of ascorbic acid. Likewise, an

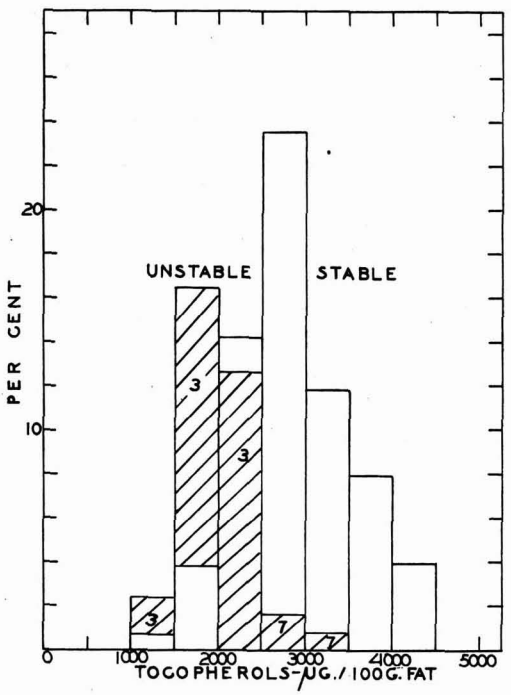


FIG. 2. The distribution of tocopherols in 128 samples of stable and unstable natural milks as affected by both pasture and hay feeding (seasonal variations). The numbers (3) and (7) indicate the days within which the oxidized flavor developed in unstable milk.

increase in the anti-oxidant activity of milk fat as estimated by the tocopherol determination may force the reaction to deviate from its course, resulting again in oxidation of other substances than unstable lipids of the fat globules membrane. This particular phenomenon will be discussed in a following paper.

These observations also are in good agreement with the data of Beck *et al.*

(1) on the relation of carotene in milk fat to the development of oxidized flavors. These investigators have found a relationship between the color intensity of milk fat and the inhibition of oxidized flavors. However, Beck *et al.* have supplemented the rations with carotene concentrates during the barn feeding. Our analysis of some of the carotene concentrates by molecular distillation methods (11) revealed that their total tocopherol content was exceptionally high (approximately 20,000 μg. per gram of concentrate).

The data we have presented are conclusive in showing that there is a relationship between the tocopherol and carotenoid contents of the milk fat as influenced by the roughages fed to the cow even though it might merely reflect parallel intakes of these two vitamins on the particular diet studied (13). It also has been shown that the promotion of oxidized flavors in milk products containing ascorbic acid, and which are associated with deterioration of milk fat, is apparently dependent on the stability of tocopherols and that the destruction of vitamin A and carotene follows that of tocopherols (9). Consequently, it would be logical to assume that the stabilizing effect on milk of carotene concentrate fed to the cows (1), largely was due to the increase in tocopherol content of the fat and not to that of carotene and that the latter is only a coincidental factor.

SUMMARY

The tocopherol, carotenoid and vitamin A content of cow's milk was determined for Holstein, Guernsey, Brown Swiss and Jersey cows during both pasture and barn feeding. Large variations were found in the tocopherol, carotenoid and vitamin A content of milk fat between individual cows of the same breed, between different breeds and between seasons.

As an average, the fat obtained from Guernsey milk was highest in tocopherol content with 3033 μg. per 100 g. of fat, and Holsteins was lowest with 2220 μg. Pasture milk contained more tocopherols than winter milk.

A significant positive correlation between the tocopherol and carotenoid content of milk was found but tocopherols and vitamin A were not correlated.

There is a relationship between the tocopherol content of the fat and the ability of milk to resist the oxidized flavors. A high proportion of samples of milk which contained less than 2500 μg. of tocopherols per 100 g. of fat were unstable and developed oxidized flavors during the storage tests.

ACKNOWLEDGMENT

The authors take pleasure in expressing their appreciation to J. W. Bratzler and D. A. Theokas for chemical analyses on some of the samples.

"This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number *278* in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army."

REFERENCES

(1) BECK, G. H., WHITNAH, C. H., AND MARTIN, W. H. Relation of Vitamin C, Lecithin and Carotene of Milk to the Development of Oxidized Flavor. J. Dairy Sci., **22**: 17–29. 1939.

(2) CHILSON, W. H., MARTIN, W. H., AND PARRISH, D. B. The Relation of Ascorbic Acid to the Development of Oxidized Flavor in Market Milk. J. Dairy Sci., **32**: 306–315. 1949.

(3) KOEHN, C. J., AND SHERMAN, W. C. The Determination of Vitamin A and Carotene with Photoelectric Colorimeter. J. Biol. Chem., **132**: 527–538. 1940.

(4) KRUKOVSKY, VLADIMIR N., AND GUTHRIE, E. S. Ascorbic Acid Oxidation a Key Factor in the Inhibition or Promotion of the Tallowy Flavor in Milk. J. Dairy Sci., **28**: 565–579. 1945.

(5) KRUKOVSKY, VLADIMIR N., GUTHRIE, E. S., AND WHITING, FRANK. The Stability of Fat and Fat Soluble Vitamins as Determined by the Re-Emulsification Test. J. Dairy Sci., **31**: 961–971. 1948.

(6) KRUKOVSKY, VLADIMIR N., AND LOOSLI, J. K. Unpublished data.

(7) KRUKOVSKY, VLADIMIR N., LOOSLI, J. K., AND WHITING, FRANK. The Influence of Tocopherols and Codliver Oil on the Stability of Milk. J. Dairy Sci., **32**: 196–201. 1949.

(8) KRUKOVSKY, VLADIMIR N., LOOSLI, J. K., AND THEOKAS, D. A. Preliminary Observations of the Effects of Ladino Pasture and Hay Feeding on Tocopherol Content of the Fat and Stability of Milk. J. Dairy Sci., **32**: 700. 1949.

(9) KRUKOVSKY, VLADIMIR, N., THEOKAS, D. A., WHITING, FRANK, GUTHRIE, E. S., YAGER, NAOMI, AND MATTUS, MARY ANN. The Effects of Nordihydroguaiaretic Acid, Salt and Temperature of Storage on the Stability of Fat and Fat Soluble Vitamins in Cream and Butter. J. Dairy Sci., **32**: 679–687. 1949.

(10) QUAIFE, M. L. Tocopherols (Vitamin E) in Milk: Their Chemical Determination and Occurrence in Human Milk. J. Biol. Chem., **169**: 513–514. 1947.

(11) QUAIFE, MARY LOUISE, AND HARRIS, PHILIP L. Chemical Assay of Foods for Vitamin E Content. Anal. Chem., **20**: 1221–1224. 1948.

(12) REPORT OF BUREAU OF DAIRY INDUSTRY. Vitamin A in Butter. U.S.D.A. Misc. Pub. No. 571. 1945.

(13) WHITING, FRANK, LOOSLI, J. K., KRUKOVSKY, VLADIMIR N., AND TURK, K. L. The Influence of Tocopherols and Cod-Liver Oil on Milk and Fat Production. J. Dairy Sci., **32**: 133–138. 1949.

ISOLATION OF OVA FROM THE LIVING BOVINE[1, 2]

ARTHUR E. DRACY[3] AND W. E. PETERSEN

Minnesota Agricultural Experiment Station, St. Paul

Transfer of sperm cells by artificial means has greatly enhanced the use of the good proven sire. Transfer of the egg from good proven cows likewise would enhance the dissemination of good germ plasm to the extent that the method would be successful and multiple ovulation could be induced. The first step in attaining such an objective is the recovery of the fertilized egg from the cow.

The potentialities and problems of ovum transfer have been known for some time. The possibility of transferring fertilized ova from one individual to a foster mother has been adequately demonstrated in rats (2, 3, 4) and rabbits (5). Each of the above mentioned experiments required sacrificing the donor which in itself defeated much of the purpose of the experiment. However, Allen (1) has successfully isolated unfertilized monkey ova by a combination of surgery and flushing the oviducts. A similar procedure has been described by Umbaugh (6) as a means of securing ova from the cow. Surgery, although not extremely difficult on cattle, is not entirely satisfactory. Thus, a series of surgical and nonsurgical experiments have been undertaken to find a practical means of securing ova for transfers.

EXPERIMENTAL PROCEDURES AND RESULTS

Study of the isolation of bovine ova falls into two parts. (a) To make the ovaries more accessible by translocation or transplantation and (b) to recover fertilized ova without surgical intervention.

Over a period of years a number of different techniques have been employed in attempts to obtain ova from developed ovarian follicles. The idea at first was that if the mature egg could be obtained, it could be fertilized *in vitro* before transfer to a recipient. However, this procedure recently has been shown not to be feasible. Since the ovary is located where it cannot be reached except by rather complicated surgery, the approach was to transplant or translocate the ovary so as to make it more readily accessible. The following surgical procedures were attempted: (a) transplantation into the neck, (b) subcutaneous translocation, (c) translocation into the vagina, and (d) resectioning of the uterine horn.

Received December 22, 1949.

[1] These data are taken from a thesis presented to the graduate school of the University of Minnesota by Arthur E. Dracy in partial fulfillment of the requirements for a Ph.D. degree.

[2] Published as paper no. 2607, scientific journal series of the Minnesota Agricultural Experiment Station.

[3] Present address: South Dakota State College of Agriculture, Department of Dairy Husbandry, Brookings.

Transplantation of the ovary into the neck. The first attempts to isolate ova from the living bovine were conducted by removal and transplantation of the ovary under the skin in the neck muscle of the animal. It was thought that eggs could be removed easily from developed follicles in such a preparation and subsequently fertilized before transplantation. None of these experiments was successful because the transplanted ovaries did not function. After several unsuccessful attempts, this method of trying to isolate living ova was discontinued.

Subcutaneous translocation of the ovary. Another attempt was made to place the ovary where it could be more easily observed. This was done by removing the broad ligament and ovary from the pelvic arch and placing it subcutaneously in the paralumbar fossa without interrupting its circulation. Such transplants did not ovulate, probably due to the reduced temperature in the new environment.

Translocation of the ovary into the vagina. Two attempts were made to translocate the ovary into the vagina. In one animal a laparotomy was performed and an incision was made in the anterior portion of the vagina, adjacent to the junction with the uterus. The ovary then was sutured into the vagina. On the second animal, however, the ovary was secured in the vagina without a laparotomy. This was accomplished by making an incision in the anterior portion of the vagina. The ovary then was moved into the vagina through this incision and sutured into place. From all physical appearances the animals withstood surgery very well. However, the ovaries did not remain translocated because of the violent vaginal contractions.

In the first animal the ovary slipped back into place indicating that the surgical technique was not adequate. In the second animal the translocated ovary was secured more substantially, but this ovary in turn pulled back into place, taking with it a fold of the vaginal wall which encased the ovary and grew together. This formed a pus-filled pocket about 3 in. in diameter around the ovary.

Exteriorlizing the resected uterine horn. Another attempt by the use of surgery to isolate living ova was undertaken by resectioning the horn of the uterus and bringing the cut end to the exterior surface with the view that the ovum could be recovered by flushing the stump of the uterine horn after descent of the egg. In spite of precautions taken, salpingitis resulted.

Non-surgical techniques. After the previous surgical experiments, it became obvious that some other technique had to be used for the isolation of ova from the cow. From unpublished data, it also became apparent that the ovum must be fertilized and pass through the oviduct, since *in vitro* fertilization was unsuccessful. Two different approaches were made. First, a tube was inserted through the cervix up to the orifice of the oviduct and, second, the uterus was flushed by entrance through the cervix.

Insertion of a rubber catheter up to the oviduct. A rubber catheter was inserted through the cervix and butted against the oviduct with the view of capturing the descending ovum in the catheter, from which it might be flushed after removal from the uterus. The catheter was inserted on the third day following ovulation and left until the end of the fourth day. In the first animal the

cervix appeared to be large and little difficulty was encountered in passing the rubber catheter through the cervix into the uterine horn. However, in subsequent animals the cervix was smaller and a dilating apparatus had to be devised for opening the cervix. This was done by inserting a 0.25-in. stainless steel probe over which was placed a sleeve to act as a cannula. With the hand in the rectum holding the cervix, the probe and cannula were guided past the cervical folds. The probe was removed and the catheter extended through the cannula into the uterine horn. The cannula then was removed, leaving the catheter in the uterine horn.

Another difficulty experienced in this method was that as soon as a foreign body entered the uterus, violent uterine contractions occurred which did not stop until the tube was forced out of the uterus. To keep the catheter in the uterus, a stiff wire was inserted inside its posterior end at the anterior end of the cervix. The wire was bent in an "S" curve just inside the cervix to hold the catheter in place. This helped some; however, several of the animals were able to force the catheter out of the horn and into the body of the uterus.

In these experiments seven attempts to isolate fertilized ova were conducted on four cows. Even though some of the cows were unable to force the catheter out of the uterine horn, fertilized ova never were recovered. One of the animals conceived, further substantiating the fact that ova did by-pass the tube. In view of these apparently insurmountable difficulties, the experiments were discontinued.

Flushing the uterus with a physiological solution. The principle involved in this method was to force a warm (about 100° F.) physiological solution into the uterine horn and then to recover the fluid containing the ovum. Numerous laboratory experiments with isolated ova suspended in a physiological saline solution proved that ova have a greater specific gravity than the solution. The increased specific gravity allowed the ova to settle quickly to the bottom of a French separatory funnel. This procedure seemed to have some possibilities for the separation of ova from large quantities of fluid.

The equipment for flushing the uterus was the following: a 0.5-in. stainless steel probe 36 in. long for dilating the cervix and a fitted stainless steel cannula 24 in. long. These instruments sufficed for normal animals. Smaller animals, such as heifers, required proportionally smaller dimensions, usually not smaller than a 0.25-in. probe.

The flushing part of the apparatus consisted of a tire pump, a 1-l. aspiratory flask to hold the fluid and a 0.125-in. Koroseal tube. One end of the Koroseal tube was fastened into the stoppered aspiratory flask. The other end was heated by a Bunsen burner and drawn to a point sealing the end of the tube. Holes then were made in the sealed end of the tube by holding a heated dissecting probe against the Koroseal tubing in such a manner that when fluid was forced out of the tube there was a backward action.

Because 4 days elapsed from the time the animal was in heat before the ovum reached the uterus, the seventh day was arbitrarily selected as the best time for attempted recovery of the eggs. Thus, when the animal was in heat, she was

bred naturally or artificially, and then on the seventh day the removal of the eggs was attempted.

The technique of using the instruments mentioned in a preceding paragraph was simple if certain steps were adhered to rather closely. The steps in isolating ova by flushing with a physiological solution were as follows: (a) The arm was inserted into the rectum and all the fecal material was removed. Ovulation was determined by the presence of one or more corpora lutea on an ovary. After this the uterus was palpated for any abnormalities. (b) The probe and cannula were inserted through the cervix by grasping the cervix with the hand in the rectum and guiding the instruments past the cervical folds similar to the rectal methods of artificial insemination. After the probe was through the cervix, it was directed to either horn by maneuvering the uterus to either side. The probe was removed and the cannula was left in the horn. (c) The Koroseal tube was inserted into the cannula and passed through it. The Koroseal tube was directed from the end of the cannula to the tip of the uterus by the hand in the rectum. When the Koroseal tube was in place, pressure was applied to the flask. As the pressure increased in the flask, fluid was forced through the Koroseal tube into the uterine horn and returned through the cannula by gravity and the aid of the contracting uterus. A receptacle was held at the external end of the cannula and all of the returning fluid collected. (d) After 1 l. of physiological solution was pumped into the uterus, the Koroseal tubing was removed and the returning solution caught in the receptacle. (e) After the fluid had been taken to the laboratory, it was transferred into a series of 125-ml. French separatory funnels and allowed to stand 20 min. This usually allowed ample time for the egg to settle to the bottom of the separatory funnel. However, there were experiments in which the ovum adhered to the sides of the glass. This possibility was reduced by swirling the funnel and allowing the fluid to resettle. (f) After the required time had elapsed, a few milliliters of the mucus plus liquid were withdrawn from the bottom of the separatory funnel and observed at 23 magnifications under the dissecting microscope. Since ova are more than 100 μ in diameter, identification was easy at this magnification. However, for further identification, the ovum was removed from the fluid by means of a fine capillary pipette, a hanging drop slide of it was prepared and it was observed under a high dry objective lens.

From observations using high-power magnification and from photomicrographs, the ovum appeared to be in the late blastula stage. Great care had to be taken not to confuse the ovum with tiny air cells that appeared in the liquid. By focusing up and down, air cells showed a reflection that was not observed when an ovum was under examination.

Table 1 shows the results obtained by flushing the uterus with a physiological solution. In this experiment, 12 cows were flushed 37 times. During these 37 trials, 41 ova were recovered. The table shows that cow 504 yielded ten ova at one time and cow 37E yielded two ova at one time, indicating superovulation. These two animals each were injected subcutaneously with 1,500 units of pregnant-mare serum to produce superovulation. At the time, 1,500 units of pregnant-mare serum induced the liberation of ova. However, about 2 mo. later

without a repeated injection, 37E liberated at least 20 ova at one ovulation which were recovered at one flushing.

DISCUSSION

The data presented on the ovary transplantation and translocation experiments indicate the impracticability of such methods for isolating ova from the living cow. Possibly too few experiments were conducted to prove that recovery could not be accomplished by these techniques. With improved surgical technique it may be possible to transplant and translocate the ovary with satisfactory results. However, with the available material and the techniques applied at the time the experiments were conducted, the impracticability of this method of approach was apparent.

TABLE 1

Ova recovered by flushing uterus with a physiological solution

No. of cow	Date of flushing	Ova recovered	No. of cow	Date of flushing	Ova recovered
E600	10–30–47	0	E598	2–18–48	0
	3– 3–48	0		2–23–48	0
	3–24–48	0		7–19–48	0
	4–28–48	0		8– 5–48	0
	7–21–48	0	479	2–21–48	0
E638	2–26–48	0		3–15–48	1
	5–31–48	0	504	10–14–48	0
	6– 7–48	0		11–11–48	1
813	3–24–48	0		12– 8–48	1
E608	9–15–47	0		1–17–48	10
	9–25–47	0		4– 1–49	1
	10–27–47	0	B10	1–29–49	1
	2–18–48	0	37E	2– 8–49	2
	3– 7–48	0		2–23–49	0
A53	8–16–47	1		4– 2–49	20
	4–12–48	0	34W	2–24–49	1
	6– 7–48	0		4– 2–49	0
E598	9–10–47	0	40E	4– 4–49	1
	10–16–47	1			

Attempts to recover ova in a catheter were unsuccessful. The fact that one cow became pregnant suggests that the ovum by-passed the catheter. The likelihood of capturing ova by this method seems remote. Also, as soon as the tube entered the uterus, violent uterine contractions occurred and continued until the tube was forced out. The contractions may have had a devastating effect upon the ovum coming down the oviduct into the tube. Even though one animal became pregnant, the possibility remained that some ova may fail to enter the uterine cavity. The possibility of obstructing the oviduct may be remote, yet it must not be overlooked as a cause of failure for recovery of ova.

From the data presented in table 1, regarding the flushing of the uterus with a physiological solution, eggs were recovered 12 times from 37 trials yielding a total of 41 ova. Of the 41 ova recovered, 32 were due to superovulation. In these experiments, four of the cows used never yielded an ovum in 14 attempts. In 23 attempts eight yielded ova 12 times. Thus, if the first four animals referred to were non-breeders, which could be possible because some of the

animals had been bred numerous times and because of repeated nonfertility were transferred from the college dairy herd to the experimental herd, the possibility exists that these animals could be sterile. The other eight animals were considered fertile, since fertilized ova were recovered. This being the case, ova were recovered 12 out of 23 trials, indicating that these cattle released ova for fertilization approximately 49.5 per cent of the time. Cow A53, which had yielded one ovum, later was sold from the herd as a sterile animal. This also was true of cow E598. When cow 479 yielded an ovum, she was returned to the college herd to produce a calf the following year. With improved techniques for obtaining and observing ova, possibly a larger percentage may be recovered. To further consider the number of ova that could in all probability be recovered, it should be kept in mind that according to data gathered from artificial insemination associations, 45–60 per cent of the cattle conceive on first service. If this is true and if the unfertilized ovum degenerates while traveling down the oviduct, the possibility of collecting nonfertilized ova is rare. Therefore, not more than 60 per cent of the recovery should be expected. However, there may be a few individual animals which would yield an ovum each time they were bred, the same as there are some cows that become pregnant on first breeding.

SUMMARY AND CONCLUSIONS

A series of experiments was conducted to determine the possibility of recovering ova from the living cow.

All surgical methods, such as transplantation of the ovary, resectioning the uterine horn and translocating the ovary subcutaneously, have yielded negative results.

The method of inserting a catheter into the uterus of a cow in order to recover fertilized ova has proved impractical.

Instruments and techniques for recovering fertilized bovine ova without injury to the donor's reproductive tract have been developed.

A grant from Babson Bros., Milker Co., Chicago, Illinois, that made this study possible is gratefully acknowledged.

REFERENCES

(1) ALLEN, E. An Unfertilized Tubual Ovum from *Macacus rhesus*. Anat. Record, 37: 351–356. 1928.

(2) HEAPE, W. Preliminary Note on the Transplantation and Growth of Mammalian Ova within a Uterine Foster Mother. Proc. Roy. Soc. (London), 48: 457. 1890.

(3) HEAPE, W. Further Note on the Transplantation and Growth of Mammalian Ova with a Uterine Foster Mother. Proc. Roy. Soc. (London), 62: 178. 1897.

(4) NICHOLAS, J. B. Development of Transplanted Rat Eggs. Proc. Soc. Exptl. Biol. Med., 30: 111–113. 1933.

(5) PINCUS, G. *Eggs of Mammals*. Macmillan Co., N. Y. 1936.

(6) UMBAUGH, R. E. Superovulation and Ovum Transfer in Cattle. Am. J. Vet. Research, 37: 295–305. 1949.

A COLORIMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF THE DEGREE OF LACTOSE HYDROLYSIS[1]

FRANK E. POTTER[2]

Bureau of Dairy Industry, Washington, D. C.

For the quantitative determination of a single sugar, numerous methods are available. However, most of these methods are not satisfactory when applied to a solution containing two or more sugars. In the hydrolysis of lactose three sugars, lactose, glucose and galactose, are involved. In certain methods of analysis, bacterial ferments or yeast enzymes are used to destroy one or more of the sugars in a mixture, but this is often time consuming.

In search of a method to follow the degree of acid hydrolysis of lactose, Ramsdell (6) used the following procedure. The sum of the two hexoses, glucose and galactose, was determined by Barfoed's modified reagent. Shaffer and Somogyi's procedure and their reagent no. 50 were used to measure the reducing power of the sugars before and after destruction of the glucose with bakers' yeast. From the results obtained, the quantities of glucose, galactose and lactose were calculated.

Another method which has been used to follow the hydrolysis of lactose is a modification (7) of the Willstaetter and Schudel procedure (8). This has been used for pure lactose in solution and in various dairy products.

The saccharimeter can be used to follow the hydrolysis of some sugars but with lactose it lacks sensitivity. It can be shown both experimentally and by calculation that for a 5 per cent solution of lactose an increase of less than 0.5 degree rotation occurs for every 10 per cent of lactose hydrolyzed.

Recently, Benham and Despaul (1) developed a quantitative colorimetric method for the determination of glucose. They measured the intensity of the blue color produced by the sugar in the presence of ammonium molybdate and potassium dihydrogen phosphate on heating. They also found the method suitable for the determination of glucose in the presence of moderate amounts of sucrose and recommended the procedure for the determination of other sugars. Later, Benham and Petzing (2) adapted this method to the quantitative measurement of maltose and mixtures of maltose and glucose.

It is this colorimetric method upon which the following study was conducted for the determination of the sugars obtained in the hydrolysis of lactose in milk products.

EXPERIMENTAL PROCEDURE

The molybdenum blue method of Benham and Despaul (1) was followed, except for slight modifications. To several 25-ml. volumetric flasks, 5 ml. of 0.02*M*

Received for publication May 4, 1950.

[1] This work was done with funds provided by the Research and Marketing Act of 1946.

[2] Present address: Department of Dairy Husbandry, Agricultural and Mechanical College of Texas, College Station.

potassium dihydrogen phosphate and 10 ml. of 7.5 per cent ammonium molybdate were added. Samples, whether of pure sugars or of mixtures, were added in quantities containing between 1 and 10 mg. of sugar and the contents made up to volume with distilled water. The flasks were stoppered, inverted several times to mix the contents and the stoppers removed. The flasks were covered with individual tin foil caps to prevent contamination from condensing steam and heated in a preheated autoclave at 100° C. for exactly 30 min. The flasks were removed and cooled at once in ice water to stop the reaction. The color intensity was determined with a Klett-Summerson photoelectric colorimeter using a colored glass filter to give a wave length of 640 mμ.

Aqueous solutions of pure sugars did not require any preliminary purification prior to analysis. However, with milk it was necessary to obtain a clear serum for analysis. Precipitating agents, such as trichloracetic acid, phosphotungstic acid and salts of heavy metals used for precipitating milk proteins in various chemical tests on milk products, interfered in the subsequent color production.

The method adopted for preparation of milk samples was as follows: To 50 g. of whole milk (20 g. of condensed skimmilk) in a 100-ml. volumetric flask, 5 ml. of 1*N* H_2SO_4 were added and made to volume with distilled water. The flask was stoppered, the contents mixed thoroughly and filtered through Whatman no. 2 filter paper. A 10-ml. aliquot of the filtrate was removed and placed in a 200-ml. volumetric flask. To this, 50 ml. of distilled water and five to six drops of phenolphthalein indicator were added. The contents then were neutralized to the phenolphthalein end point with 0.1*N* NaOH. The flasks were placed in a boiling water bath for 15 min. to coagulate the heat coagulable protein and cooled to room temperature in a cold water bath. To the contents of flasks five to six drops of methyl red were added and 0.1*N* H_2SO_4 acid was used to adjust the reaction to the methyl red end point. The flasks were made to volume with distilled water, contents mixed and filtered. A 10-ml. aliquot of the filtrate was placed in the 25-ml. color development flasks and the analysis completed as for sugar solutions.

The intensity of the blue color produced in the molybdenum blue reaction varies with the individual type of sugar. Results of preliminary investigations on sugar solutions containing 1 and 8 mg. of glucose, galactose, lactose and a mixture of glucose and galactose in equal parts are presented in table 1. The data show that the color produced with the glucose and galactose mixture is more than twenty times as great as an equal quantity of lactose. The small amount

TABLE 1

Klett-Summerson readings at 640 mμ for known quantities of sugar

	Scale readings	
	1.0 mg. sugar	8.0 mg. sugar
Lactose	< 5	20
Glucose	65	385
Galactose	140	too dark to read
Glucose and galactose (equal parts)	102	660

of color produced by the lactose can easily be corrected for by determining a blank value.

In order to determine unknown quantities of glucose and galactose, it was necessary to establish a standard curve using known quantities of these sugars. Three determinations were made on solutions containing from 0.5 to 8 mg. of glucose and galactose in equal parts. The average results of these determinations are presented in figure 1. This standard curve was used for calculating the results on unknown samples. The results of similar determinations on solutions of glucose, galactose and lactose also are presented in figure 1.

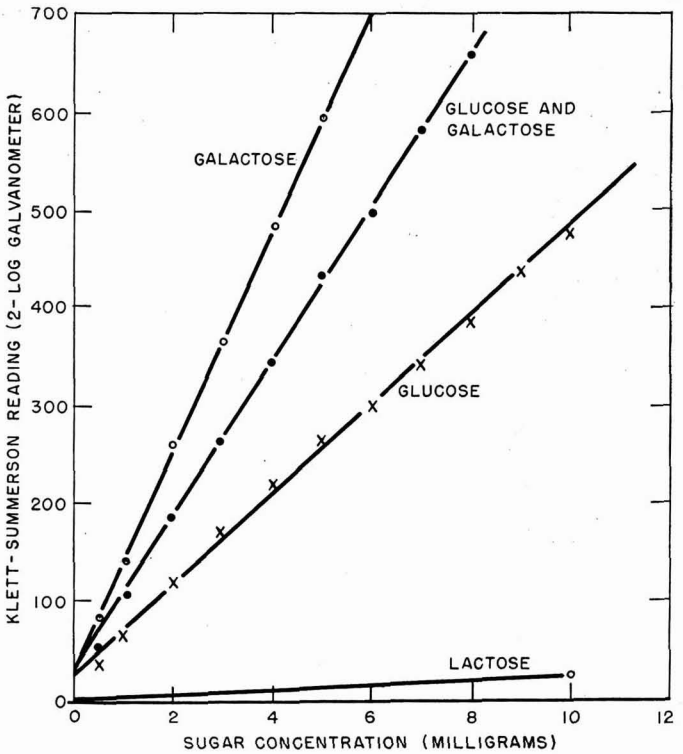


FIG. 1. Standard curves obtained from prepared solutions of known sugar content.

In analyzing an unknown sample of milk, the value obtained from the standard curve (mg. per aliquot) is converted to grams per 100 g. by a factor of 0.387 for whole milk. This factor is calculated by the method used by Hillig (3) for the quantitative determination of lactic acid.

To determine the accuracy of the molybdenum blue method when applied to milk, glucose and galactose in equal parts were added at the rate of 0.1 to 5.0 g. per 100 g. of whole milk. The results of seven trials in duplicate are presented in table 2.

TABLE 2

Recovery of glucose and galactose added in equal parts to fresh whole milk

Sample	Added	Recovered	Difference	Recovery
	(*g.*/100 *g.*)	(*g.*/100 *g.*)	(*g.*/100 *g.*)	(%)
1a	0.10	0.104	+0.004	104.00
b	0.10	0.116	+0.016	116.00
2a	1.00	0.956	−0.044	95.60
b	1.00	0.956	−0.044	95.60
3a	1.50	1.405	−0.095	93.66
b	1.50	1.405	−0.095	93.66
4a	2.00	1.989	−0.011	99.45
b	2.00	1.950	−0.050	97.50
5a	3.00	2.848	−0.152	94.93
b	3.00	2.980	−0.020	99.33
6a	4.00	3.870	−0.130	96.75
b	4.00	3.870	−0.130	96.75
7a	5.00	4.992	−0.008	99.84
b	5.00	4.938	−0.062	98.76
Av.				98.70

These data indicate that the method possesses a high degree of accuracy and reliability. Recovery of the sugars was within 0.1 g. for all but three of the samples and the percentage recovery usually was within 5 per cent. The average recovery for the 14 analyses was 98.70 per cent.

In a supplemental series of tests involving the enzymatic hydrolysis of a lactose solution, comparisons were made between the Willstaetter and Schudel modified method and the method presented in this paper. Table 3 shows the results

TABLE 3

The determination of glucose and galactose in a 5% solution of lactose at intervals during enzymatic hydrolysis

Sample	Willstaetter & Schudel modification[a]	Molybdenum blue method	Difference
	(*g.*/100 *g.*)	(*g.*/100 *g.*)	(*g.*/100 *g.*)
1	0.300	0.270	0.030
2	0.749	0.830	0.081
3	0.879	0.680	0.199
4	1.720	1.630	0.090
5	4.072	4.050	0.022

[a] These results obtained by G. Reed, Rohm and Haas Co., Philadelphia, Pa.

obtained by the two methods on five different samples. The quantity of glucose and galactose found by the two methods agreed within 0.20 g. per 100 g. for all samples and within 0.10 g. per 100 g. for all but one of the samples.

Since phosphomolybdic acid and ascorbic acid have been used for the determination of inorganic phosphate (4) and phosphomolybdic acid for determining ascorbic acid (5), experiments were conducted to determine the effect of ascorbic acid on this method of analysis. Fresh whole milk was divided into three lots and treated as follows: 1, control; 2 and 3, 50 and 100 mg. of ascorbic acid were added per liter of milk. Analysis of these samples (table 4) show that the addi-

TABLE 4

Effect of the addition of ascorbic acid and the heating of milk on the normal blank values for milk

Sample	Klett-Summerson reading	Glucose-galactose equivalent
Milk (control)	43	0.151
Milk + 50 mg. of ascorbic acid/l.	43	0.151
Milk + 100 mg. of ascorbic acid/l.	45	0.159
Milk (control)	45	0.159
Milk heated to 80° C. for 1 hr.	46	0.162
Milk heated to 80° C. for 2 hr.	46	0.162

tion of 50 mg. of ascorbic acid did not affect the normal blank value for milk. The addition of 100 mg. of ascorbic acid did increase the value slightly. However, since this is approximately five times the average amount of ascorbic acid found in milk, the normal variations in ascorbic acid would not have a significant effect on the blank values for milk.

Inasmuch as the heating of milk produces various reducing products, trials were conducted to determine the effect of heating. The results presented in table 4 show that the heating of milk at 80° C. for 1 or 2 hr. increased the blank value to a slight extent, but this increase was within the normal variation encountered for unheated milk.

In this study a blank value for milk has been found to vary from 0.150 to 0.170 g. per 100 g. expressed as glucose-galactose equivalent.

SUMMARY AND CONCLUSIONS

The colorimetric determination of sugars by the use of the molybdenum blue reaction as developed by Benham and Despaul (1) has been adapted to follow the enzymatic hydrolysis of lactose in solution and in milk.

The analysis of 14 samples of fresh whole milk containing from 0.1 to 5.0 g. of added glucose and galactose in equal parts per 100 g. of milk gave an average recovery of 98.70 per cent.

A blank value for milk was found to vary from 0.150 to 0.170 g. per 100 g. expressed as glucose-galactose equivalent. The addition of ascorbic acid or the heating of milk to 80° C. for 1 or 2 hr. did not have a significant effect on the blank values.

Values obtained by the colorimetric method for samples of hydrolyzed lactose agreed very closely with those obtained by a modification of the Willstaetter and Schudel method (7).

ACKNOWLEDGMENT

The enzyme preparation used in this study was furnished by the Rohm and Haas Co. of Philadelphia, Pa.

REFERENCES

(1) BENHAM, G. H., AND DESPAUL, J. E. Glucose—a Direct Colorimetric Method for Determining Carbohydrates. Anal. Chem. 20: 933–935. 1948.

(2) BENHAM, G. H., AND PETZING, VIRGINIA E. Direct Colorimetric Method for Carbohydrates —Maltose. Anal. Chem., 21: 991–993. 1949.
(3) HILLIG, F. Colorimetric Determination of Lactic Acid in Milk and Milk Products. J. Assoc. Off. Agr. Chem., 20: 130–140. 1937.
(4) LOWRY, O. H., AND LOPEZ, JEANNE A. The Determination of Inorganic Phosphate in the Presence of Labile Phosphate Esters. J. Biol. Chem., 162: 421–428. 1946.
(5) MANNELLI, G. Colorimetric Determination of Ascorbic Acid. Mikrochemie ver. Mikrochim. Acta, 35: 29–33. 1950.
(6) RAMSDELL, G. A. Determination of Glucose, Galactose and Lactose in their Mixtures. J. Dairy Sci., 28: 671–676. 1945.
(7) ROHM AND HAAS CO., Philadelphia, Pa. Personal communications. 1949.
(8) WILLSTAETTER, R., AND SCHUDEL, G. Bestimmung von Traubenzucker mit Hypojidit. Ber., 51: 780–781. 1918.

THE FURTHER DEVELOPMENT OF MILK REPLACEMENTS FOR DAIRY CALVES[1, 2]

J. B. WILLIAMS AND C. B. KNODT
The Pennsylvania Agricultural Experiment Station, State College, Pa.

Previous work (15) has demonstrated that normal growth can be obtained in dairy calves by the use of limited amounts of saleable whole milk with a milk replacement. Numerous reports (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13) in the literature indicate the possibilities of raising dairy calves on limited amounts of whole milk and dry concentrates.

This report presents additional experiments relative to the improvement of formulas published previously. The principal objectives were to evaluate other plant and animal products and to develop a simpler formula. It was desired to study the comparative value of meat scrap, corn gluten meal, soybean oil meal, blood flour, dried skimmilk and ground raw soybeans, nutri-soy and red dog flour in milk replacements. Previous work at this station (13) indicated a comparison was needed between dried brewers' yeast and distillers' dried solubles.

EXPERIMENTAL PROCEDURE

The male Holstein calves used in the two trials were obtained from Pennsylvania state institutional herds. They were housed in individual solid-wall pens equipped with a water bowl, salt block, hay rack and a concentrate box. To prevent positional effects, the calves were placed at random throughout the artificially lighted and ventilated stable, maintained at a temperature of 65° F. by thermostatically controlled steam heat. Three measures of growth were taken each week, by the same person, at the same time and in the same order. The same person made daily observations on the condition of the feces of each calf. When a case of scours persisted for 24 hr., a 10-g. dose of sulfathalidine was administered orally followed by an additional 5-g. dose at each of the next two successive feedings.

Trial 1. Forty-eight calves were divided into eight comparable groups of six calves each on the basis of body weight, chest circumference and height at withers. Groups I through VII were placed on the experiment not later than the fourth day after birth and were fed the replacement formulas presented in table 1.

Received for publication May 18, 1950.

[1] Authorized for publication on May 10, 1950 as paper no. 1598 in the Journal series of The Pennsylvania Agricultural Experiment Station. This work was supported in part by the Cooperative G.L.F. Exchange, Inc., Ithaca, N. Y. and National Distillers' Products Corp. of N. Y. with the cooperation of the Distillers' Feed Research Council of Cincinnati. The sulfathalidine used in this trial was supplied by Sharp and Dohme, Inc., Glenolden, Pa.

[2] The data contained in this publication are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE 1

Milk replacement formulas—Trial 1

Ingredient	Group						
	I	II	III	IV	V	VI	VII
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Dried skimmilk	50	20	20	10	10	20	5
Dried whey	10	20	20	20	20	20	10
Dist. dr. sol. (Corn)	10	20	20	20	20	20	20
Blood flour	10	.....	.....	10	.....	.....	5
Meat scrap	.....	10	10	10	20	20	.....
Oat flour	5	10	10	10	10	.....	20
Corn gluten meal	.....	20	.....	10	10	.....	.....
Soybean oil meal	.....	.....	20	10	10	20	.....
Ground raw soybeans	.....	.....	.....	.....	.....	.....	40
Dextrose	7.75	.....	.....	.....	.....	.....	.....
Brewers' dr. yeast	4.90	.....	.....	.....	.....	.....	.....
Ground Fenugreek seed	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Irradiated yeast (9F)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Stabilized vitamin A feed[a]	2.20	0.22	0.22	0.22	0.22	0.22	0.22
Minerals[b]	0.042	0.042	0.042	0.042	0.042	0.042	0.042
Dicalcium phosphate	2.5	2.5	2.5	2.5	1.0	1.0	2.5

[a] In mix no. 1 the vitamin A content of the supplement was 220,000 U.S.P. units/lb. In the other mixes the supplement contained 2,220,000 U.S.P. units/lb.

[b] Mineral mixture contained: Ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$) 56.57%
Cupric sulfate ($CuSO_4 \cdot 5H_2O$) 19.73%
Manganese sulfate ($MnSO_4 \cdot 4H_2O$) 21.59%
Cobalt chloride ($CoCl_2 \cdot 6H_2O$) 2.11%

They were fed the mixtures at 100° F. according to the following schedule: First through 4th day—dam's milk; 5th through 7th day—2.5 lb. whole milk, 0.25 lb. milk replacement, 2 lb. water (twice daily); 8th through 10th day—1.0 lb. whole milk, 0.5 lb. milk replacement, 4 lb. water (twice daily); 11th through 49th day—0.7 lb. milk replacement, 5 lb. water (twice daily); 50th to 56th day—0.7 lb. milk replacement, 5 lb. water (once daily).

Group VIII constituted the control group and was placed on the experiment not later than the fourth day after birth. They were fed a total of 372 lb. whole milk (3.4 per cent fat) excluding colostrum according to the following schedule: First day through 4th day—dam's milk; 5th through 14th day—8 lb. milk per day; 15th through 34th day—10 lb. milk per day; 35th day through 41st day—8 lb. milk per day; 42nd day through 49th day—4 lb. milk per day.

All groups of calves were fed a fair grade of timothy hay from birth to 8 wk. and good quality alfalfa from 8 wk. to end of 12 wk. trial, *ad libitum*. Calf starter was fed *ad libitum* until each calf was consuming the maximum of 6 lb. daily and then kept at that level of intake for the duration of the trial. The calf starter was prepared as follows: 406.5 lb. yellow corn meal, 300 lb. wheat bran, 400 lb. crushed oats, 140 lb. linseed oil meal, 280 lb. soybean oil meal, 140 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. dried skimmilk, 100 lb. dried corn distillers' solubles, 0.5 lb. irradiated yeast (9F), 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt and 3 lb. vitamin A feeding oil (2,724,000 USP units of A per pound).

Trial 2. Thirty-six calves were divided into six comparable groups of six calves each. They were fed the mixes in table 2 at a temperature of 100° F.

TABLE 2
Milk replacement formulas—Trial 2

Ingredient	Group					
	I	II	III	IV	V	VI
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Dried skimmilk	50	50	50	50	50	20
Dried whey	10	10	17	17	17	27.338
Dist. dr. sol. (Corn)	10	15	15	15	20	20
Blood flour	10	10	10	.....	.....	.....
Oat flour	5	5	5	5	5	.....
Soybean oil meal (exp. proc.)	.....	.....	.....	10	5	.....
Nutri-Soy	.....	.....	.....	.....	.....	15
Dextrose	7.75	7	.....	.....	.....	.....
Red Dog flour	.....	.....	.....	.....	.....	15
Brewers' dr. yeast	4.90	.....	.....	.....	.....	.....
Irradiated yeast (9F)	0.10	0.10	0.10	0.10	0.10	0.10
Stabilized vitamin A feed[a]	0.22	0.22	0.22	0.22	0.22	0.22
Minerals[b]	0.042	0.042	0.042	0.042	0.042	0.042

[a] The supplement contained 2,220,000 USP uits/lb.
[b] Mineral mixtures same as table 1.

according to the following schedule: Birth through 7th day—colostrum and whole milk; 8th through 14th day—2 lb. whole milk, 0.2 lb. milk replacement, 2 lb. water (twice daily); 15th through 21st day—0.3 lb. milk replacement, 4 lb. water (twice daily); 22nd through 28th day—0.4 lb. milk replacement, 4 lb. water (twice daily); 29th through 42nd day—0.5 lb. milk replacement, 5 lb. water (twice daily); 43rd through 49th day—0.6 lb. milk replacement, 6 lb. water (twice daily); 50th through 56th day—0.6 lb. milk replacement, 6 lb. water (once daily). Since replacement I of trial 2 had been used in previous trials and the growth performance established, it was used as the control and the other mixes were deviations from it. Excellent quality second cutting mixed hay was fed *ad libitum* to 8 wk. and alfalfa from 8 wk. to determination of 12 wk. trial.

The number 1 and 4 calves in each group received the following concentrate in dry mash form: 416.5 lb. yellow corn meal, 300 lb. wheat bran, 400 lb. crimped whole oats, 100 lb. linseed oil meal, 300 lb. soybean oil meal (44 per cent), 150 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. dried skimmilk, 100 lb. dried corn distillers' solubles, 0.5 lb. irradiated yeast (9F), 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt, 3 lb. vitamin A (2,270,000 USP units per pound in dry meal form). The number 2 and 5 calves in each group received the above concentrate in pellet form. The number 3 and 6 calves in each group received the following concentrate in pellet form: 390 lb. yellow corn meal, 100 lb. wheat bran, 100 lb. ground oats, 200 lb. linseed oil meal, 650 lb. soybean oil meal, 100 lb. alfalfa meal, 100 lb. fish meal, 300 lb. dried whey, 20 lb. ground limestone, 20 lb. steamed bone meal, 10 lb. iodized salt, 8 lb. feeding oil (1000 USP units of vitamin A and 400 USP units of vitamin D per gram), 1 lb. anise oil, 1 lb. irradiated yeast.

EXPERIMENTAL RESULTS

Trial 1. Ration VII was lethal to all calves in the group, the calves succumbing at 27, 30, 31, 36, 43 and 58 days, respectively. The last calf was down in the stable for 7 days before being sacrificed for autopsy. Post-mortem revealed enlarged gall bladder, kidney discolorations, distended urinary bladder and excess fluid over the entire body. The condition in all the calves was characterized by muscular weakness and lack of coordination, although pain was not manifested. The calves maintained their appetites until death, although unable to stand up. One calf was lost from group I because of a hip injury, one calf from group IV was suspected actinomycosis, one calf from group V for cause unknown and one calf from group VI because of pneumonia.

All of the calves were easily taught to drink the warm replacement-water mixtures from open pails. Mix VII settled out quickly and mixes II, III, IV, V and VI settled out faster than was desirable. Mix I was very acceptable in water suspension. No serious or prolonged cases of scours occurred.

Growth data in table 3 indicates that calves in groups I and VIII made com-

TABLE 3

Mean daily gains in body weight, withers height and chest circumference, trial 1

Group	Body wt.			Withers ht.			Chest circ.		
	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.
	(*lb.*)	(*lb.*)	(*lb.*)	(*cm.*)	(*cm.*)	(*cm.*)	(*in.*)	(*in.*)	(*in.*)
I	0.45	0.95	1.20	0.13	0.12	0.13	0.07	0.10	0.10
II	0.29	0.71	0.96	0.09	0.10	0.11	0.05	0.07	0.08
III	0.30	0.74	0.98	0.08	0.09	0.11	0.05	0.07	0.07
IV	0.29	0.52	0.79	0.09	0.10	0.10	0.01	0.04	0.06
V	0.26	0.68	0.95	0.11	0.11	0.11	0.01	0.06	0.08
VI	0.36	0.75	0.93	0.08	0.10	0.10	0.05	0.07	0.08
VII	.....	.....	.....	.....	.....	.....	.....	.....	.....
VIII	0.82	0.89	1.23	0.15	0.12	0.14	0.08	0.08	0.10

parable and uniform gains, except that group I calves made less gains the first 4 wk.; however, the appearance and well-being of these two groups of calves were superior to that of other groups. Also, the average consumption of calf starter was significantly less the first 8 wk. for groups I and VIII than for the other groups as presented in table 5.

From these growth data it would seem that corn gluten meal and soybean oil meal are comparable as sources of protein in conjunction with 20 per cent dried skimmilk powder. It also would appear that meat scrap alone is a better protein source than equal amounts of meat scrap and blood flour when dried skimmilk is used at the 10 per cent level.

Trial 2. As in trial 1, palatability was not a problem. All mixes remained in the warm water suspension without difficulty. There were no fatalities among any of the groups, although one calf in group VI failed to make satisfactory gains. The differences in daily gains (table 4) were not significant according to

TABLE 4

Mean daily gains in body weight, withers height and chest circumference, trial 2

Group	Body wt.			Withers ht.			Chest circ.		
	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.
	(*lb.*)	(*lb.*)	(*lb.*)	(*cm.*)	(*cm.*)	(*cm.*)	(*in.*)	(*in.*)	(*in.*)
I	0.48	1.00	1.24	0.12	0.14	0.14	0.05	0.08	0.09
II	0.46	0.98	1.32	0.11	0.13	0.14	0.05	0.07	0.09
III	0.21	0.89	1.10	0.10	0.13	0.14	0.03	0.07	0.08
IV	0.57	0.88	1.06	0.11	0.12	0.13	0.03	0.07	0.07
V	0.32	0.82	1.11	0.08	0.12	0.13	0.03	0.07	0.08
VI	0.39	0.79	0.98	0.09	0.11	0.11	0.00	0.06	0.08

the methods of Snedecor (14).

Table 5 presents the average consumption of calf starter. The difference in

TABLE 5

Average consumption of calf starter at 8 and 12 wk.

Group	First trial		Second trial	
	Av. consumption		Av. consumption	
	8 wk.	12 wk.	8 wk.	12 wk.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
I	45	171	71	190
II	60	187	71	206
III	58	185	60	171
IV	56	175	62	173
V	59	178	56	173
VI	60	184	59	160
VII	.....	.....	.....	.....
VIII	47	161	.....	.....

calf starter consumption up to 8 wk. of age between the group I calves in trial 1 and groups I and II in this trial probably was due to the differences in amount of the milk replacement fed. Each calf in trial 1 was fed 64 lb. of milk replacement and in trial two each calf received 41.2 lb. of milk replacement. Calves in groups I and II consumed a great deal more calf starter to 8 wk. and 12 wk. than did the calves in the other groups, and mean daily gains were higher, although not statistically significant. Further experimentation is planned in respect to the feeding of pellets *versus* mash, and the results of this trial will be reported when additional data are available.

The difference in daily gain between groups I and II and group III is difficult to explain. It may be that a combination of dried whey and dextrose is more beneficial to the infant calf than dried whey alone. Work is in progress at the present time on this phase. The growth data for groups I and II indicate distillers' dried solubles can effectively replace dried brewers' yeast. Growth was rather poor in the calves that received the Nutri-soy-red dog flour diet. Scouring was not a problem in any of the groups, although the group VI calves were rough-coated and generally unthrifty when compared to the calves in groups I and II.

SUMMARY

Ground raw soybeans were not satisfactory when used at a 40 per cent level in the formula studied. All calves in a group of six died between the 29th and 58th day of age. Soybean oil meal and corn gluten meal were of equal value as a source of protein in these milk replacement formulas. Rations containing 50 per cent dried skimmilk gave consistently better results than those containing 20 per cent or less of this ingredient. Dried corn distillers' solubles effectively replaced dried brewers' yeast.

REFERENCES

(1) BENDER, C. B., AND BARTLETT, J. W. A Study of the Factors Affecting the Growth of Dairy Heifers. J. Dairy Sci., 11: 37–49. 1929.

(2) DAVIS, R. N., AND CUNNINGHAM, W. S. Raising Calves on the Minimum Amount of Milk. Ariz. Agr. Expt. Sta. Bull. 111. 1923.

(3) HATHAWAY, I. L., TRIMBERGER, G. W., AND DAVIS, H. P. The Use of Dried Whey and Blood Meal in the Raising of Calves on Limited Amounts of Milk. Nebr. Agr. Expt. Sta. Research Bull. 132. 1943.

(4.) HAYWARD, H. The Rearing of Calves on Milk Substitutes. Pa. Agr. Expt. Sta. Bull. 60. 1902.

(5) INGHAM, L. W., MEADE, D., AND BERRY, M. H. Calf Feeding Investigations: I. Raising Dairy Calves on Nurse Cow, Whole Milk, Remade Skim Milk and Calf Meal. Md. Agr. Expt. Sta. Bull. 319. 1930.

(6) JACOBSON, N. L., AND CANNON, C. Y. Soybean Oil-filled Milks for Feeding Dairy Calves. (Abs.) J. Dairy Sci., 30: 587. 1947.

(7) KRAUSS, W. E., MONROE, C. F., AND HAYDEN, C. C. Dry Feed Systems of Raising Calves. Ohio Agr. Expt. Sta. Bi-mo. Bull. 173: 45. 1935.

(8) MAYNARD, L. A., AND NORRIS, L. C. A System of Rearing Dairy Calves with Limited Use of Milk. J. Dairy Sci., 6: 483–508. 1923.

(9) MORROW, K. S. Dry-feed Systems of Raising Dairy Calves. N. H. Agr. Expt. Sta. Bull. 319. 1940.

(10) NEWMAN, P. E., AND SAVAGE, E. S. Use of Yeast in Calf Meals and Pellets. J. Dairy Sci., 21: 161–167. 1938.

(11) RUPEL, J. W. Raising the Dairy Calf. Wis. Agr. Expt. Sta. Bull. 404. 1929.

(12) SAVAGE, E. S., AND CRAWFORD, C. H. Dry Concentrates as a Partial Substitute for Whole Milk in Calf Rations. N. Y. (Cornell) Agr. Expt. Sta. Bull. 622. 1935.

(13) SCHABINGER, J. R., AND KNODT, C. B. The Value of Distillers' Dried Solubles and Dried Grains with Solubles in the Ration of Dairy Calves. (Abs.) J. Dairy Sci., 30: 585–586. 1947.

(14) SNEDECOR, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press. Ames. 1946.

(15) WILLIAMS, J. B., AND KNODT, C. B. The Value of Milk Replacements in the Rations of Dairy Calves. J. Dairy Sci., 32: 986–992. 1949.

VANILLAS AS ANTIOXIDANTS IN POWDERED ICE CREAM MIXES

HARRY PYENSON[1] AND P. H. TRACY

Department of Food Technology, University of Illinois, Urbana

In a previous study, Pyenson and Tracy (1) has shown that a pure six-fold vanilla concentrate made from Bourbon and Mexican beans had antioxidant properties in powdered cream. Therefore, it was desirable to study other vanillas, both pure and artificial, and vanilla compounds to determine whether they also had antioxidant properties. This study was conducted with powdered ice cream mixes rather than with powdered cream, as vanillas usually are added to powdered ice cream mixes for flavoring. If certain vanillas do act as antioxidants, they then would serve a two-fold purpose in the powdered ice cream mixes. The results obtained on powdered cream mixes probably would be quite similar to those reported here on powdered ice cream mixes.

EXPERIMENTAL PROCEDURE

A 1,100 lb. batch of liquid ice cream mix was made having the composition of 12 per cent butterfat, 11 per cent m.s.n.f., 15 per cent sugar (only one-third of the sugar was added before drying) and 0.2 per cent Dariloid. This mix was made from 35 per cent sweet cream, 34 per cent total solids condensed skimmilk and 9 per cent total solids skimmilk. The mix was pasteurized at 160° F. for 30 min., homogenized on a two-stage machine at 2,500 and 500 lb. pressure per in.^{2}, then cooled to 40° F. and held over-night.

The liquid mix analyzed 13.39 per cent butterfat and 31.92 per cent total solids. Fifteen batches were dried on an experimental spray drier. The kinds and amounts of vanillas or vanilla products added as antioxidant are given in table 1. Each batch of powdered ice cream mix was divided into two parts, one

TABLE 1

Flavoring materials used in powdered ice cream mixes

Batch no.	Amount (%)	Kind of flavoring material	Brand
1	0	.....	—
2	0.1	Conc. Bourbon and Mexican vanilla	A
3	0.1	Conc. Bourbon vanilla	A
4	0.3	Regular vanilla extract (Mexican)	A
5	0.3	Regular vanilla extract (Tahiti 100%)	A
6	0.1	Conc. pure vanilla extract	B
7	0.1	Conc. vanilla extract	C
8	0.2	Powdered pure vanilla	D
9	0.1	Powdered vanilla (Tahiti and Vanillin)	D
10	0.1	Conc. imitation vanilla	A
11	0.01	Methyl vanillin	A
12	0.01	Ethyl vanillin	A
13	0.0025	Vanillic acid	E
14	0.01	Coumarin	E

Received for publication May 24, 1950.

[1] At present Director of Research, Reddi-Whip, Inc., 3938 Lindell Blvd., St. Louis 8, Mo.

part being air-packed and the other part nitrogen-packed. The letter *N* in the batch numbers indicate that the samples were nitrogen-packed.

In drying, the batches were preheated at a temperature of 145° F. and spray-dried at a pressure of 1,000 lb. per in.[2] using a number 69 nozzle and a 2–20 core. The inlet temperature was 310° F. and the outlet temperature was kept as close to 180 to 190° F. as possible. The powder was packed in no. 1 picnic cans, 150 g. of powder to each can. The cans were stored at a temperature of 75 ± 5° F., periodically analyzed for headspace oxygen by the method of Van Slyke and Sendroy (2) and judged for flavor by two or more judges after reconstituting with water in a Stevens mixer to original composition.

The moisture contents of the powdered ice cream mixes, as determined by the Mojonnier method, are recorded in table 2.

TABLE 2

Moisture content of powdered ice cream mixes

Batch no.	Moisture (%)	Batch no.	Moisture (%)
1	0.63	8	0.88
2	0.92	9	1.18
3	1.23	10	1.08
4	0.95	11	1.24
5	0.71	12	0.83
6	0.96	13	1.05
7	0.79	14	0.75

RESULTS

This study was conducted for 1 yr. and the results are summarized in table 3. During this year, seven analyses for oxygen and flavor were made at approximately 1, 2, 3, 4, 6, 9 and 12 mo. of storage. The freshly reconstituted powdered ice cream mixes all were scored 41 on flavor based on the ice cream score card adopted by the American Dairy Science Association in 1941.[2] The flavor scores were determined solely on whether or not samples were oxidized. Some other flavor criticisms noted on the powdered ice cream mixes also will be mentioned. Amounts of vanilla or vanilla compounds used were the quantities thought needed for proper flavoring. Whether the results would have been altered by using greater or lesser amounts of the vanillas or vanilla products is not known.

Some of the other flavor criticisms noted were cooked, strong vanilla, strong artificial, alcoholic and weak. In batches 5 and 5*N*, 0.3 per cent of a 100 per cent Tahiti extract gave a strong vanilla flavor. Batches 1 and 1*N* had a cooked flavor. A strong artificial vanilla flavor was obtained in batches 9, 9*N*, 10 and 10*N*. An alcoholic flavor resulted when 0.01 per cent methyl vanillin was used in batches 11 and 11N. Vanillic acid in the amount of 0.0025 per cent produced a weak flavor. One hundredth per cent coumarin gave a strong flavor to the ic cream mixes in batches 14 and 14N. A weak flavor was noted with 0.1 per cent of a five-fold vanilla extract in batches 7 and 7N and 0.2 per cent powdered vanilla in batches 8 and 8N. Of all the vanillas or vanilla materials tested, the

[2] Excellent, 40 and above; good, 37.5–39.5; fair, 35.5–37.5; poor, 35.5 and below.

most pleasant flavor was produced by the powdered vanilla used in batches 8 and 8N. The harsh flavors produced by methyl vanillin, ethyl vanillin, vanillic acid and couramin might be as objectionable or even more objectionable than oxidized flavor in a commercial product.

All of these vanillas and vanilla compounds were added to the ice cream mixes at the time of preheating just before spray-drying at a temperature of 145° F. The processing or the drying operations did not seem to affect the intensity of the vanilla flavor or vanilla compounds of the reconstituted powdered ice cream mixes.

Table 3 gives a resumé of the changes obtained in oxygen concentration in the headspace gas and the palatability of air-packed and gas-packed powdered ice cream mixes containing vanilla and vanilla compounds as antioxidants. Air-packed control batch no. 1 had a strong oxidized flavor at 37 days and the oxygen in the headspace gas already had started to diminish. After 1 yr. most of the oxygen had been used up and after about 6 mo. the flavor was so oxidized it was given a score of zero. The nitrogen-packed control (1N) also had become oxidized at 37 days storage but the oxidized flavor was not as strong as the air-packed samples throughout the storage period. The vanillas used were much more effective as antioxidants in nitrogen-packed samples than in air-packed. Most air-packed samples containing vanillas were oxidized after only a few months of storage. None of the nitrogen-packed samples containing vanillas were oxidized after 1 yr. of storage at room temperature.

Methyl vanillin, ethyl vanillin, vanillic acid and coumarin had antioxidant properties in powdered ice cream mixes. These compounds were almost as effective in air-packed as in nitrogen-packed samples. Samples containing methyl vanillin (11 and 11N) did not develop an oxidized flavor in either air-packed or nitrogen-packed samples held for 1 yr. Samples containing ethyl vanillin (12 and 12N) showed similar results except that at the sixth month storage period, the nitrogen-packed sample had a slightly oxidized flavor but was not criticized for oxidized flavor at the 9- or 12-mo. periods.

That the vanillas do not mask the oxidized flavors was shown in a previous paper (1). Nevertheless, this possible masking was checked again by adding one of the vanillas to the oxidized control sample. The results again indicated that there was little, if any, masking of the oxidized flavor by the vanilla flavors.

The gas analysis data in table 3 indicate that when vanilla or vanilla compounds were used, there was more oxygen left in the headspace gas than in the headspace gas of the control samples at the end of the storage period. This would indicate that less oxygen was used for oxidation in the powdered ice cream mixes and further proof that the vanillas and vanilla compounds tested have anti-oxygenic properties.

DISCUSSION

All the products studied retarded or prevented the development of an oxidized flavor suggesting the presence of compounds capable of retarding oxygen uptake by the unsaturated fatty acids or phospholipids present in the powder.

TABLE 3

Changes in oxygen concentration in headspace gas and palatability of air-packed and gas-packed powdered ice cream mixes containing flavoring materials

Batch no.		Days of storage at room temperature						
		37	63	95	127	191	263	365
1	% Oxygen	18.46	19.47	18.78	17.28	12.49	3.66	2.48
	Flavor	35*	33*	30*	29*	0*	0*	0*
1N[a]	% Oxygen	2.09	2.52	2.10	2.08	1.05	0.00	0.00
	Flavor	37*	35*	33*	33*	32*	30*	25*
2	% Oxygen	20.00	19.33	18.68	18.08	16.53	13.15	13.52
	Flavor	38*	37.5*	37*	36*	34*	30*	25*
2N	% Oxygen	2.69	2.15	2.79	1.41	1.68	0.00	2.16
	Flavor	41.	40.5	40.	39.5	39.	39.	39.
3	% Oxygen	20.18	20.08	19.75	19.22	17.40	11.53	5.74
	Flavor	40.5	39*	38.5*	37.5*	36*	34*	32*
3N	% Oxygen	3.02	2.76	2.30	1.79	1.74	0.91	1.24
	Flavor	41.	40.5	40	39.5	39	39	39
4	% Oxygen	20.49	19.83	19.84	18.76	17.79	13.39	7.14
	Flavor	40.5	39*	39*	38*	37*	35*	33*
4N	% Oxygen	3.29	2.65	2.72	2.08	1.27	1.91	0.83
	Flavor	41.	40	40	39.5	39	39	39
5	% Oxygen	19.41	19.86	19.95	18.51	17.02	10.42	4.34
	Flavor	40.5	40	39.5	39	39	38	36*
5N	% Oxygen	3.24	3.61	3.07	2.50	1.95	1.01	0.89
	Flavor	41	40	40	39.5	39	39	39
6	% Oxygen	20.00	19.85	20.08	19.93	18.62	15.45	7.81
	Flavor	40.5	40	39.5	39	39	38	37*
6N	% Oxygen	3.45	4.01	3.87	2.91	2.83	2.58	1.55
	Flavor	41	40	40	39.5	39	39	39
7	% Oxygen	20.10	19.71	19.71	19.24	18.13	14.41	7.94
	Flavor	40.5	40	39.5	39*	38*	37*	35*
7N	% Oxygen	3.12	3.42	2.95	2.86	2.68	2.04	0.52
	Flavor	41.	40.	40.	39.5	39	39	39
8	% Oxygen	20.42	20.45	20.15	19.81	19.59	16.76	15.43
	Flavor	40.5	40.	39.5	39	38.5	38.5	37*
8N	% Oxygen	3.06	3.14	3.39	2.75	2.84	2.94	1.18
	Flavor	41.	40	40	39.5	39.5	39.5	39
9	% Oxygen	19.79	20.32	20.00	19.04	18.50	16.54	12.73
	Flavor	40.5	39.5*	39*	39*	38*	38*	37*
9N	% Oxygen	2.74	2.62	2.30	2.33	2.65	1.72	1.82
	Flavor	41	40	40	39.5	39	39	39
10	% Oxygen	20.78	20.17	19.90	20.09	19.25	17.52	15.80
	Flavor	40.5	40.	39.5	39	38.5*	38*	37*
10N	% Oxygen	3.02	2.91	2.74	2.28	1.88	1.76	1.36
	Flavor	41	40	40	39.5	39	39	39
11	% Oxygen	20.28	20.43	20.21	19.88	19.71	17.92	14.40
	Flavor	40.5	40.	39.5	39	38.5	38	38
11N	% Oxygen	3.77	2.93	2.53	3.17	2.28	2.09	1.13
	Flavor	41.	40	40	39.5	39.	38.5	38.5
12	% Oxygen	20.02	20.53	20.11	20.34	19.04	17.68	15.74
	Flavor	40.5	39.5	39.5	39	38.5	38	38
12N	% Oxygen	3.14	2.81	2.81	2.78	2.63	2.25	2.44
	Flavor	41.	40	40	39.5	38*	38.	38.5
13	% Oxygen	20.38	20.57	19.12	19.38	18.05	15.50	8.84
	Flavor	40.5	40.	39.5	39	38*	37*	36*
13N	% Oxygen	3.33	2.57	2.57	2.73	2.98	2.29	1.16
	Flavor	41	40	40	39.5	39	39	39
14	% Oxygen	20.15	20.09	20.11	18.88	18.10	12.14	8.48
	Flavor	40.5	40	39.5	39	38.5	38.	37*
14N	% Oxygen	2.98	2.75	2.67	2.39	1.82	1.35	0.89
	Flavor	41	40	40	39.5	39	39	39

[a] *N* indicates samples were nitrogen-packed, others were air-packed.
* Oxidized.

The explanation for this action is thought to be the structural formation of these compounds.

The structural formulas of methyl vanillin, ethyl vanillin, vanillic acid and coumarin are similar to certain compounds that are known to have antioxygenic properties. At low concentrations numerous phenolic substances have the ability to inhibit the autooxidation of fats. The most effective phenols are those which have some type of oxygen linkage in the ortho and para positions, or both, to the hydroxyl group. Some of the best known antioxidants of this type are hydroquinone, the tocopherols, gum guaiac and nordihydroguaiaretic acid.

Vanillin is the mono-methyl ether of protocatechuic aldehyde, the methoxy group being in the meta position to the aldehyde group. Vanillin is prepared commercially by synthetic methods from eugenole, which yields first iso-eugenole, or from the glucoside coniferin, which yields first coniferyl alcohol. When iso-eugenole and coniferyl alcohol are oxidized, vanillin is formed.

Vanillic acid is the mono-methyl ether of protocatechuic acid with the methoxy group in the meta position to the acid group. It is the acid corresponding to the aldehyde vanillin.

Ethyl vanillin has the same chemical structure as methyl vanillin, except that the ethoxy group is in the meta position instead of the methoxy group. Ethyl vanillin would be the mono-ethyl ether of protocatechuic aldehyde (4 hydroxy 3-ethoxy benzaldehyde).

Coumarin is an odoriferous compound present in tonka beans, the extract of which is used as a substitute for vanilla in some imitation vanilla extracts.

The addition of flavoring compounds having the ability to prevent oxygen uptake by the fatty materials should prove to be a very convenient method of extending the shelf life of a number of foods. It is possible that the extent to which vanilla flavors have been helpful in this respect has not been fully appreciated.

SUMMARY

Studies were made of nine vanillas and four vanilla compounds in powdered ice cream mixes held for 1 yr. at room temperature. The products used represented five different manufacturers of vanillas or vanilla compounds.

Changes in the oxygen concentration of the headspace gas and palatability studies indicated that these vanillas and vanilla compounds have antioxygenic properties in powdered ice cream mixes. The addition of these vanillas or possibly the vanilla compounds would serve a two-fold purpose in powdered ice cream mixes, *i.e.*, as flavoring and as an antioxidant.

REFERENCES

(1) PYENSON, H., AND TRACY, P. H. Manfacture of Powdered Cream for Whipping by Aeration. J. Dairy Sci., 31(7): 539–550. 1948.

(2) VAN SLYKE, D. D., AND SENDROY, J., JR. Manometric Analysis of Gas Mixtures. 1. The Determination by Simple Adsorption of Carbon Dioxide, Oxygen and Nitrogen in Mixtures of these Gases. J. Biol. Chem., 95: 509–529. 1932.

PASTEURIZATION EFFICIENCY OF THE VACREATOR WHEN USED ON ICE CREAM MIX

P. H. TRACY AND RICHARD PEDRICK[1]
Department of Food Technology, University of Illinois, Urbana
AND
H. C. LINGLE
Research Laboratories, Cherry-Burrell Corporation

The Vacreator[2] is a continuous flow type of high-temperature, short-time pasteurizer so constructed as to include three successive stages, each of which operates under a pressure lower than that of the atmosphere (fig. 1). The rapid heating is accomplished by the gravity fall or rain of the liquid through a chamber of expanded steam. This method of heating with steam is the reverse of steam injection. Rapid cooling results from the evaporation of moisture which occurs when the liquid passes to the lower pressure areas existing in the successive stages. A water actuated ejector-type condenser is an integral component of the machine and its high velocity water jet serves to maintain vacua, condense vapors and entrain and eject non-condensable gases.

In normal operation, the incoming and outgoing temperatures of the milk product being processed are maintained at practically the same levels so that the moisture content of the product leaving the Vacreator is essentially that of the product entering the machine. The novel features of the process are the rapid heating of the fluid particles resulting from the controlled addition of more steam than is required to heat the product to a pasteurizing temperature followed by its removal in the second and third chambers, thus providing steam distillation. Temperature control is effected by regulating the pressures maintained within the chambers and not by changing the amount of steam being used. The process should provide an excellent means not only of operating continuously, but also of complete pasteurization without injury to flavor. There also is the possibility of actually improving flavor through the removal of undesirable volatile substances present in the milk product.

The merits of the Vacreator process as applied to cream for buttermaking, milk for cheese making and ice cream mix have been studied extensively by Wilster. In reviewing his own work as well as that of others, Wilster (1) reports that the main advantages of the process are improvement of flavor and high efficiency of pasteurization.

In operating the Vacreator, temperatures can be varied. The usual range, however, is 195 to 205° F. in the first chamber, 160 to 180° F. in the second chamber and 110° F. in the third chamber, with vacuum readings in the three chambers varying from 4 to 9 in. in the first, 15 to 25 in. in the second and 28 in. in the third.

Received for publication May 26, 1950.

[1] Now associated with the Dean Milk Co., Research Laboratories, Rockford, Illinois.

[2] Vacreator—a trademark for vacuum pasteurizers. Registered U. S. Pat. Off. and Canada.

High-temperature, short-time methods of pasteurization for ice cream mix are not commonly used in this country, as public health officials have not as yet established standards for the various time and temperature combinations possible. Commercial and public health interest in the use of the Vacreator for mix manufacture led to the study reported herein.

EXPERIMENTAL PROCEDURE

A no. 3 size Vacreator, having a rated maximum capacity of 3,000 lb. of product per hour, was used (fig. 1). A positive type variable speed stainless steel pump was provided to deliver the product to the Vacreator. A standard two-

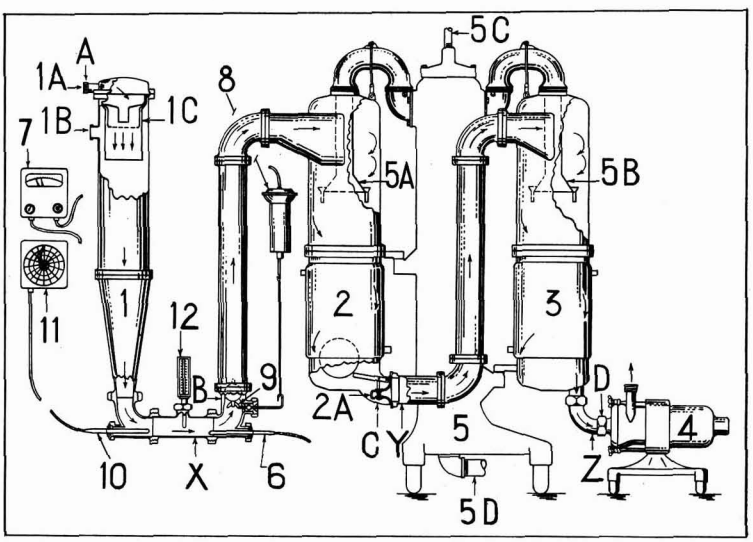


FIG. 1. Diagram of the Vacreator

1. First chamber
1A. Product Inlet
1B. Steam Inlet
1C. Spray Pan
2. Second Chamber
2A. Float Valve
3. Third Chamber
4. Product Discharge Pump
5. Ejector Condenser
5A–5B. Vapor Intake Pipes to Ejector Condenser
5C. Condenser Water Inlet
5D. Condenser Water Outlet Piped to Drain or Water Cooling Tower
6. Bulb of Pasteurizing Temperature Controller
7. Pasteurizing Temperature Controller
8. Regulator between Pasteurizing Temperature Controller and Equilibrium Valve
9. Equilibrium Valve
10. Bulb of Safety Thermal Limit Recorder
11. Safety Thermal Limit Recorder
12. Pasteurizing Temperature Mercury Indicating Thermometer

A–B. First Chamber Effect
B–C. Second Chamber Effect
C–D. Third Chamber Effect

X — Sampling Cock
Y — Sampling Cock
Z — Sampling Cock

stage centrifugal stainless steel pump discharged the product from the third chamber.

The Vacreator was equipped with vacuum and pressure gauges, indicating and recording thermometers and automatic steam control, as well as an automatic pasteurizing temperature controller.

Determination of time required for a liquid to pass through the Vacreator was made using an electric clock calibrated in hundredths of a second. Brine, flowing behind the clear water, upon contacting the first set of electrodes started the clock and stopped it when contact was made with the second set of electrodes.

With pump speeds and steam pressure constant, the rate of mix flowing through the Vacreator will remain constant. However, temperature in the first two effects, where bacterial destruction takes place, can be varied. It was desired to determine the significance of the temperature at these two points in the process, particularly in the first effect. Temperatures in the first effect were varied from 180 to 200° F. The temperature in the second effect was kept at 140° F. by removing the second chamber float valve or at 170° F. with the valve in place.

A mix containing 12 per cent butterfat, 11 per cent milk solids-not-fat and 15 per cent cane sugar was used, unless otherwise specified. The pasteurized mixes were inoculated with 24-hr. cultures of *Micrococcus freudenreichii* M25 just before vacreation took place. The cultures were prepared by growing on tryptone-glucose extract agar at 37° C. The growth was washed from the agar with sterile one-fourth strength Ringer's solution and the washings added to the mix. Samples of the vacreated mix were plated on tryptone-glucose-extract agar and incubated at 37° C. for 48 hr. before counting.

Samples were taken from the first chamber effect by two methods. One method was by gravitational fall into a sterile tube immersed in ice water connected to a cock on the first chamber. The second method consisted of drawing a sample into a continuously evacuated sterile flask. The latter method finally was adopted, as it gave instantaneous cooling of the sample.

As a control measure, a sample of each experimental mix was laboratory pasteurized at 155° F. for 30 min. in a sealed, sterile glass tube.

Experiments also were made to determine to what extent deviations from the normal procedure of operation would affect the pasteurization efficiency of the Vacreator.

RESULTS

As it would be difficult to determine at what point in its passage through the first chamber a mix particle reached the peak temperature, it was decided to measure the time required for a liquid to pass from the intake of the first chamber to the discharge of this chamber. Thus, the data obtained showed the length of time involved in heating to and holding at the indicated temperature of the first chamber. To accomplish this, electrodes were placed under the spray pan where the product first is exposed to live steam. A lead-covered cable was run from the clock through the steam piping into the first chamber to these electrodes. The

stop electrodes were placed immediately in front of the equilibrium valve, which is located at the discharge end of the first chamber. Salt solution was injected 6 in. upstream from the spray pan by means of a syringe. To obtain accurate readings, the resistance between the clock and each electrode was increased to the maximum that would still permit the clock to operate. This insured that only the peak concentration of salt would be timed as it passed each electrode.

This procedure was necessary due to the fact that the temperature of the solution used in these tests increased 80° F. between the two timing electrodes. Tests showed that the conductivity of the salt solution was higher at 190 than at 110° F. Because of this effect of temperature upon conductivity, the most accurate results were obtained by the method described of timing the peak concentrations of salt. Once set, the resistances to the electrodes were not varied, so the effect of varying the steam and product supply could be accurately determined.

When the steam supply used was reduced from 530 lb. per hour to 440, 320 and 230 lb. per hour (table 1), the average time of exposure to the temperature

TABLE 1

Time (seconds) required for water to pass through first chamber of Vacreator

A. Variable steam supply.				
Steam line pressure[a] (*psi*)	38	28	18	10
Steam supply (*lb./hr.*)	530	440	320	230
	0.69	0.78	0.75	0.85
	0.82	0.78	0.75	0.90
	0.75	0.79	0.88	0.94
	0.70	0.78	0.82	0.84
	0.79	0.73	0.81	0.97
	0.77	0.73	0.80	0.90
	0.78	0.83	0.72	0.90
	0.85	0.84	0.84	0.86
	0.69	.....	0.86	0.86
Av.	0.75	0.78	0.80	0.89
B. Variable operating capacity.				
		Steam line pressure (38 *psi*)[a] Steam supply (530 *lb./hr.*)		
Vacreator capacity (*lb./hr.*)	1800	2600	3800	5300
	0.75	0.72	0.70	0.62
	0.64	0.69	0.57	0.61
	0.68	0.72	0.64	0.59
	0.79	0.64	0.61	0.61
	0.80	0.64	0.68	0.63
	0.81	.....	0.69	.....
	0.71	.....	0.67	.....
	0.74	.....	0.61	.....
Av.	0.74	0.68	0.64	0.61

Infeed temperature 110° F.; first chamber temperature 190° F.; second chamber temperature 170° F.; quantity of salt used 115 ml.; capacity of machine 1800 lb./hr.

[a] As delivered through a 0.5 in. diameter-fixed orifice.

of the first chamber was increased from 0.75 sec. to 0.78, 0.80 and 0.89 sec., respectively. When the capacity was increased from 1,800 lb. per hour to 2,600, 3,800

and 5,300 lb. per hour, the time of exposure to the temperature of the first effect was reduced from an average of 0.74 sec. to an average of 0.68, 0.64 and 0.61 seconds, respectively.

Tests also were performed for the purpose of determining the length of time required for complete travel through the Vacreator. In running these tests, the second chamber float valve was removed. This was done in order to determine whether or not, when bacterial destruction effects of the first chamber were measured, the length of time in the second chamber would be sufficient to cause any additional bacterial destruction.

In these tests the stop electrodes were placed in a tee fitting at the discharge elbow leading from the third chamber. Pump capacities of 1,800 and 2,600 lb. per hour and steam pressures of 20 and 40 lb. were used (table 2).

TABLE 2

Minimum time (seconds) required for complete passage through the Vacreator with second chamber float valve removed

Capacity (*lb./hr.*)	2600	1800	1800
Steam line pressure[a] (*psi*)	40	40	20
Steam supply (*lb./hr.*)	550	550	320
	5.69	7.17	7.0
	5.68	6.85	6.9
	5.83	7.07	6.95
	5.33	8.03	7.01
	5.64	6.68	7.07
Av.	5.63	7.16	6.98

[a] As delivered through a 0.5 in. diameter-fixed orifice.

An increase in capacity from 1,800 to 2,600 lb. per hour reduced the time required for complete travel through the Vacreator from an average of 7.16 to 5.63 sec., whereas variation in the steam supply did not significantly alter the elapsed over-all time.

From these results it is evident that when the Vacreator is operated with the second chamber valve removed and the second chamber temperature is held at 140° F. for not more than 5 to 6 sec., a sample taken from the third chamber outlet will reflect only the lethal effect of the first chamber heat treatment. This makes possible the assumption that in tests performed for the purpose of determining the effect of the first chamber heat treatment, samples taken at the discharge end of the Vacreator will be as satisfactory as those taken directly from the first chamber.

A batch of mix after inoculation with *M. freudenreichii* M25 was preheated to 110° F. and passed through the Vacreator, using temperatures in the first chamber varying from 200 to 180° F. at 5 degree increments. The second chamber temperature was kept constant at 170° F. The mix discharge temperature was 110° F. The machine was operated at a capacity of 2,500 lb. per hour. Samples from the second and third chambers were taken by the vacuum method.

The results (table 3) indicate that the lethal effect of the second chamber when operated at 170° F. was not significant until the first chamber temperature

TABLE 3

Relation of first and second chamber temperatures to bacterial destruction in mix

	Bacterial counts per ml. mix.						
First chamber temperature	Natural flora mix	After addition of culture	After first chamber treatment		After second chamber treatment of 170° F.	After third chamber treatment	Lab. past. count 155° F., 30 min.
			Gravity Sample	Vacuum Sample			
(° *F.*)							
200	6,000	760,000	250	500	170	2,900	2,800
200	15,500	630,000	470	300	800	990	1,600
195	20,000	2,200,000	1,100	870	1,570	1,200	3,500
195	800	4,200,000	1,800	3,200	1,200	850	3,900
190	1,700	1,500,000	750	6,200	460	1,070	1,100
190	12,000	3,200,000	1,250	5,200	580	720	2,800
185	19,000	4,300,000	18,000	110,000	720	1,200	2,700
185	1,200	3,600,000	22,000	90,000	1,200	1,500	3,200
180	20,000	4,300,000	127,000	320,000	37,000	20,000	4,000
180	20,000	5,200,000	290,000	530,000	22,000	9,000	5,200

dropped to 190° F. or below. Not until the first chamber temperature was reduced to 180° F. did laboratory pasteurization give results superior to those obtained by vacreation.

To better check the lethal effect of the first chamber, trials were run in which the second chamber float valve was removed and the temperature in this chamber maintained at 140° F. and below. This is not a normal procedure, but it made possible the elimination of any bacterial destruction in the second chamber. The first chamber temperature was maintained at 200° F., then dropped at 5° intervals to 180° F. The mix used first was pasteurized and then inoculated with *freudenreichii*. Samples were taken during each first-chamber temperature condition after the mix had traversed the entire vacreation process. As the second chamber retention time had been found to be approximately 5 sec. with the float valve removed, the time and temperature of this chamber had a negligible effect upon the destruction of the organisms. The removal of the second-chamber float valve had no effect upon the time and temperature maintained in the first chamber. The third chamber at 110° F. had no lethal effect.

This method provided an accurate measurement of the efficiency of the first chamber on which the automatic controls are mounted and in which most of the microorganism destruction takes place. The holding time in the first chamber was exactly that as normally maintained and the error encountered in drawing the sample directly from the first chamber, due to added holding time at a high temperature, was avoided. Sufficient time was allowed between sampling to permit the mix pasteurized at the preceding higher temperature to be completely removed from the system, thus avoiding possible contamination at the subsequent lower temperature pasteurization treatments. The preheating temperatures were standardized at 110° F., and the pump capacity was set at 2,500 lb. per hour in each trial. Results are presented in table 4.

If it is assumed that the heating done in the second chamber is to be considered only as a safety measure and that complete pasteurization must take place

TABLE 4

Pasteurization efficiency of Vacreator with second chamber float valve removed

(Samples taken at the end of the process)

Trial	Natural flora of mix	After addition of culture	Bacteria counts per ml. of mix								
			First chamber temperatures (Second chamber temperature 140° F.)								Lab. past. samples 155° F. 30 min.
			200° F.	195° F.	193° F.	191° F.	190° F.	189° F.	185° F.	180° F.	
1.	700	1,020,000	1,710	760	.....	.....	7,200	.....	219,000	268,000	36,000
2.	6,300	2,200,000	2,600	2,500	.....	.....	11,000	.....	32,000	240,000	7,000
3.	32,000	1,910,000	270	410	.....	.....	7,000	.....	65,000	169,000	30,000
4.	27,000	1,040,000	190	570	.....	.....	23,700	.....	184,000	249,000	1,850
5.	87,000	720,000	320	17,000	.....	.....	112,000	.....	170,000	210,000	2,400
6.	120,000	2,200,000	180	370	.....	.....	16,000	.....	190,000	270,000	2,750
7.	21,000	3,200,000	.....	21,000	34,000	101,000	.....	137,000	.....	.....	3,250
8.	17,000	2,300,000	.....	820	2,600	90,000	.....	89,000	.....	.....	3,250
9.	700	920,000	.....	500	700	3,800	.....	50,000	.....	.....	1,350

Note: The second chamber was maintained throughout these trials at the temperature of 140° F. or below, in order that the bacterial destruction of the first chamber alone could be measured.

TABLE 5

Pasteurization efficiency of Vacreator with second chamber float valve removed
(Samples taken at the first chamber and at the third chamber outlet)

Trial	Natural flora of mix	After addition of culture to mix	Bacteria counts per ml. of mix												Lab. past. samples 155° F. 30 min.
			First chamber temperatures												
			200° F.		198° F.		196° F.		194° F.		192° F.		190° F.		
			First effect	End of process	First effect	End of process	First effect	End of process	First effect	End of process	First effect	End of process	First effect	End of process	
1	300	1,020,000	600	400	630	1,400	740	930	1,250	1,190	7,800	4,900	.....	11,800	1,280
2	270	820,000	500	660	530	690	580	680	760	7,700	1,150	6,200	20,300	13,400	2,100
3	80	1,520,000	660	1,020	400	990	750	1,000	7,200	11,000	12,000	13,800	80,000	73,000	1,600
4	530	810,000	420	430	580	650	570	620	760	4,000	5,000	21,800	49,000	53,000	3,600
5	570	850,000	.....	610	350	530	760	710	1,430	2,900	880	15,000	56,000	111,000	4,600
6	530	720,000	280	290	310	460	510	610	890	930	4,400	6,200	82,000	52,000	16,100
7	270	870,000	260	360	370	390	380	440	630	710	8,900	4,700	144,000	46,000	6,200
8	690	840,000	340	390	240	350	350	260	360	530	7,700	2,300	16,900	39,000	11,100

Note: The second chamber was maintained throughout these trials at the temperature of 140° F. or below, in order that the bacterial destruction of the first chamber alone could be measured.

by the time the mix enters the second chamber, then there is no question but that the first effect temperature must not drop below 190° F. It also is evident that the temperatures as high as 200° F. in the first effect are not necessary to obtain suitable bacterial destruction.

The study was continued using a mix that had been rendered nearly sterile before the addition of the culture. This was accomplished by circulating the mix through the Vacreator at 205° F. It was impossible to obtain a sterile mix due to the presence of Gram-positive, aerobic, spore-forming, rod-shaped organisms. First chamber temperatures were varied from 200 to 190° F. with 2° intervals. Samples were taken both from the first chamber and at the end of the process. The results (table 5) indicate, as in the previous experiment, that temperatures as high as 200° F. are not necessary for proper pasteurization. With one exception, (trial 3) results obtained at 194° F. were superior to those obtained in laboratory pasteurized samples. In general, results obtained at 192° F. were inferior to those obtained on the laboratory pasteurized samples.

Before accepting a new method or process for pasteurizing a liquid dairy product, it is necessary to know what safety precautions must be taken to prevent improper pasteurization from occurring due to accidental or willful misoperation of the process. Better bacterial results were obtained when the mix was passed through the Vacreator at temperatures of 194° F. or higher than when the mix was laboratory pasteurized at 155° F. for 30 min. (tables 3–5). However, the question might be raised as to what would be the result of (a) a reduced infeed temperature, (b) an increase in the infeed pump speed, (c) a sudden reduction in steam line pressures and (d) clogging of the spray pan in the first chamber. Accordingly, experiments using milk were conducted for the purpose of obtaining answers to these questions. Milk was selected as the test liquid for economy reasons and because preliminary studies had shown its suitability for such studies.

Two runs were made at a pasteurizing temperature of 190° F. combined with the abnormally low preheat temperatures of 70 and 49° F. (table 6). Operation at the 70° F. preheating temperature was satisfactory, and bacterial kills were excellent. This was in spite of the fact that the thermometer bulb which operates

TABLE 6

Bacteria counts obtained with low product infeed temperatures, using whole milk

Trial	No. 1	No. 2
Infeed temp.	70° F.	49° F.
Controller past. temp.	190°	190°
Actual past. temp.	186°–190°	182°–196°
Inoculated count	12,400,000	3,500,000
1st chamber count/ml.	450	2,200
2nd chamber count/ml.	250	540
3rd chamber count/ml.	440	460

1st chamber samples were drawn into a continuously evacuated flask. In other tests, nine samples of the inoculated milk pasteurized in the laboratory at 143° F. for 30 min. had counts ranging from 88,000 to 266,000/ml.

The second chamber temperature was 170° F. The capacity was 2,640 lb./hr. and the steam supply was 530 lb./hr.

the recording thermometer and which is located at the entrance of the crossover tube showed fluctuations to as low as 186° F. This indicates that the product was not completely raised to 190° F. until almost out of the crossover tube.

The run at the 49° F. preheat temperature showed 14° F. fluctuations of the pasteurizing temperature, as well as an erratic discharge combined with vacuum fluctuations. A 0.5-in. orifice in the steam line limited the pounds of steam available to 550, and this amount of steam was not sufficient to give a uniform discharge temperature of 190° F. The product fluctuated to as low as 182° F. The safety thermal limit pump stop was not used, as it would have shut off the product flow. Even with these temperature fluctuations, the bacterial destruction remained equivalent to normal operation.

Under some operating conditions, such as a run lasting many hours, precipitated milk proteins or some other solid material might partially clog the holes of the spray pan. Under such conditions, the milk product would not fall as droplets through the live steam as in normal operation, but would run in a stream down the wall of the pasteurizing chamber. This conceivably could reduce the heat transfer and prevent all the product from being raised to a temperature that would give adequate pasteurization.

To study this possibility, 30 gal. of skimmilk were circulated through the Vacreator for 6.5 hr. The steam was superheated 10° F., and for parts of this run a preheat temperature of 125° F. was used so as to condense the product. At the end of the run, the amount of accumulated milk solids or burn-on was not excessive. All the burn-on was around the edge of the pan and not in the zone of the spray holes.

Since other products might cause a heavier burn-on and thus clog the spray pan, a pasteurizing test was made with the funnel to the spray pan closed with cork. The bottom of the pasteurizing chamber was temporarily removed to inspect the flow. Much of the product was seen to run down and drop off the spray guard, while some of it ran down the side of the chamber. No impairment of pasteurization efficiency resulted.

It also was realized that there might be a danger of inadequate pasteurization if the spray pan was accidentally left out when assembling the Vacreator. To exaggerate the conditions of flow that would then occur, a cork with a 0.75-in. diameter hole was placed in the funnel to the spray pan. With the spray pan removed, the product then fell in a solid stream, yet pasteurization again was satisfactory.

Tests were made to determine the pasteurizing efficiency of the Vacreator when operated above the rated maximum capacity of 3,000 lb. per hour. Runs were made at rates of 3,200, 3.800 and 5,300 lb. per hour. All samples taken, with the possible exception of one count (8,360 per ml.) at the highest capacity, indicated satisfactory bacterial reduction. The tests were made at a pasteurizing temperature of 190° F. (table 7).

The infeed pump was overloaded above the 3,800 lb. rate and, thus, could only be run for short periods without being stopped by the overload cut-outs.

TABLE 7

Effect of overloading Vacreator upon bacterial destruction in whole milk

Run no.	1	2	3
Capacity (*lb./hr.*)	3,200	3,800	5,300
Inoculated count/ml.	750,000	2,100,000	3,300,000
First chamber count/ml.[a]	560	370	600
Second chamber count/ml.	420	300	8,360
Third chamber count/ml.	1,080	1,260	770

[a] First-chamber samples were drawn into a continuously evacuated flask. In all 3 runs, the infeed temperature was 190°, the second chamber temperature was 170° and the steam supply was 530 lb./hr.

Vacreator operation at these excessive capacities was satisfactory but was attempted only for short periods. It is reasonable to assume that the operator would correct a highly overloaded situation shortly after it occurred in order to restore proper functioning of the Vacreator. Overloading as much as 50 per cent above the rated maximum capacity apparently did not result in unsatisfactory pasteurization of the milk.

A comparison was made of the results obtained with reduced, normal and excessive steam supply (table 8). In one test the steam line pressure was reduced

TABLE 8

Effect of varying steam quantities on bacterial destruction

Steam supply (*lb./hr.*)	530	320	230	800
Steam line pressure (*psi.*)	40	20	10	45
1st chamber temp. recorded	190°	190°	182–185°	212°
2nd chamber temp.	170°	170°	170°	165°
Capacity (*lb./hr.*)	1,800	1,800	2,000	2,650
Inoculated count/ml.	4,800,000	2,730,000	1,470,000	390,000
1st chamber count/ml.	1,210	880	700	100
2nd chamber count/ml.	930	320	500	.....
3rd chamber count/ml.	1,600	290	1,250	1,600

Steam line orifice removed. The infeed temperature used was 110° F.

to 10 psi., a point where there was insufficient steam to heat the incoming product to the desired 190° F. This low steam supply also decreased the velocity through the first chamber, as shown by time tests. Because of this, the product was held a sufficient length of time to result in good pasteurization below 185° F. This inherent safety feature of a reduced volume of steam resulting in an increased holding time also was demonstrated in the test results obtained using low preheat temperatures (table 6).

SUMMARY

As the amount of steam used in the Vacreator was reduced, the average time required for the test liquid (water) to pass through the first chamber was increased from 0.75 to 0.89 sec. Under set conditions, as the pump capacity was increased from 1,800 to 5,300 lb. per hour the time required for the test liquid to pass through the first chamber was decreased from 0.74 to 0.61 sec. The time required for complete travel through the Vacreator varied from 5.63 to 7.16 sec.,

depending upon the capacity at which the machine was operated. The 170° F. temperature ordinarily used in the second effect was found to be high enough to have considerable lethal effect.

If, from a Public Health angle in the case of mix pasteurization, it is desirable to require the temperature effect of the first chamber treatment to be the equivalent of or better than that obtained with pasteurization at 155° F. for 30 min., then, according to the results obtained, the temperature carried in the first effect should not be less than 194° F.

Dropping the preheating temperature from the standard temperature of 110 to as low as 49° F. did not alter the efficiency of pasteurization. When the infeed pump speed was increased above the rated capacity of 3,000 lb. per hour, there was no change in the efficiency of pasteurization.

When the amount of the steam used in the first chamber was reduced from 500 lb. to approximately 200 lb. per hour, the efficiency of pasteurization remained the same. Partial clogging of the spray jets in the first chamber or removal of the spray pan did not result in a change in the effectiveness of the pasteurization process.

Overloading the Vacreator as much as 50 per cent above the rated maximum capacity did not result in unsatisfactory pasteurization of the mix.

Reducing the steam pressure to below that recommended for proper operation of the Vacreator reduced the velocity of the product passing through the first chamber so that there was sufficient time for proper pasteurization.

When the Vacreator is operated so that a minimum temperature of 194° F. is maintained in the first chamber, results will be obtained that will equal or better the Public Health protection afforded ice cream mix pasteurized at 155° F. for thirty min. in a sealed glass tube.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of E. O. Herreid of the Department of Food Technology, R. V. Hussong of the Kraft Foods Research Laboratory, Glenview, Ill., and Harold Wainess of U. S. Public Health Service, in planning the project.

REFERENCES

(1) WILSTER, G. H. A Compendium of Discussions Relating to Dairy Technology and Dairy Manufacturing. Station Circular of Information, no. 439, Oregon Agr. Expt. Station. 1948.

RELATIVE STORAGE QUALITIES OF FROZEN AND DRIED MILK

P. H. TRACY, JOHN HETRICK[1] AND WALTER S. KRIENKE[2]
Department of Food Technology, University of Illinois, Urbana

The storage of both fat and milk solids-not-fat in frozen form is commonly practiced by the dairy industry (9). The destabilizing effect of the continued storage at freezing temperatures upon the normal dispersion of the milk solids is one of the factors limiting the applications of this method to the storage of cream and condensed milk. Sugar sometimes is added to the milk solids to prevent churning of the fat and destabilization of the milk casein.

In a series of experiments reported by Babcock *et al.* (1–7), it was brought out that homogenized milk remained normal when frozen and stored at a constant temperature. Extremely low (– 40° F.) temperatures were found best. Homogenized milk frozen and stored in the frozen state was found to have the solids more concentrated in the bottom section. Homogenized milk that had been stored frozen was held at usual fluid milk storage temperatures without any more rapid deterioration than would be expected of regular homogenized milk. The addition of sodium citrate to homogenized milk before freezing and storage improved the storage life of the milk, and added ascorbic acid helped preserve the normal flavor of the milk. Homogenized milk was kept as long as 120 hr. before freezing without adversely affecting the keeping quality of the frozen product. These investigators also found that rotating homogenized milk while it was being frozen prevented a segregation of the milk solids but did not improve the keeping quality of the stored milk.

In 1944, Doan and Leeder (8) recommended a procedure for preparation of frozen condensed milk. They proposed a preheating temperature of 180° F. for 15 min., a 3 to 1 concentration of the milk, homogenization of at least 3000 lb. before condensing, limiting the air incorporation to 20 to 30 per cent when freezing with a continuous freezer, holding at a temperature not higher than –10° F. during freezing, storage and dispensing, and reconstitution in 180° F. water. These authors claim the frozen condensed milk can be held for 10 to 12 wk. without effect upon the fat or protein if stored at –15 to –20° F.

EXPERIMENTAL PROCEDURE

Two lots of fresh milk were used for these studies. The first experiment was begun in October, 1944, using milk obtained from a local cheese factory. No attempt was made to select milk of high quality. Upon being received at the University experimental plant, the milk was heated to 85° F. and clarified. It then was heated to 140° F. and homogenized at this temperature using a pressure of 2500 lb. per in.^{2}. The homogenized milk then was pasteurized at 170° F. for 20 min. While it was realized that the high temperature used for pasteurizing

Received for publication May 26, 1950.

[1] Now Research director, Dean Milk Co., Rockford, Ill.

[2] Now with the Dept. of Dairy Husbandry and Dairy Manufactures, University of Florida.

the milk would lower the flavor score of the fluid milk as well as the concentrated milk, it had been established in previous experiments by numerous investigators that if dried whole milk is not made from milk preheated to temperatures as high as 170° F. oxidized flavors are likely to develop in a short time after storage. It also was realized that the high heat treatment would retard or prevent the development of an oxidized flavor in the frozen milk (2).

A portion of the pasteurized milk was cooled to 40° F., filled into quart paper containers (American Can type) and frozen in the hardening room at $-10 \pm 5°$ F. The balance of the milk was cooled to 145° F. and condensed to a 11.5° Baume. A portion of the condensed milk was cooled to 40° F. and put into quart paper containers and frozen in an ice cream hardening room. Another batch of this condensed milk was frozen to a slush (27 to 28° F.) in a Creamery Package continuous ice cream freezer, packaged directly into quart paper milk containers and stored at the low temperature. To another portion of the condensed milk, 3 per cent by weight of dextrose was added; it was packaged and placed in the ice cream hardening room. The remainder of both the sweetened and unsweetened condensed milk was dried, using an experimental spray drier. A no. 72 nozzle and 1000 lb. spray pressure with a 140° F. spray temperature were used.

The dried milks were packed in no. 1 cans with a packing density of 0.5 g. per milliliter at 110° F. Half of the cans were air-packed and the remainder were nitrogen-packed. The nitrogen-packed samples were evacuated twice, one gassing following the other immediately. Half of the air- and nitrogen-packed samples of powder from each batch were stored in the hardening room with the frozen fluid and condensed samples. The other samples of powder were stored at room temperature. The room temperature was thermostatically controlled at 72° F. during the time the building was heated.

At intervals during storage, the powdered samples were gas-analyzed and all samples were reconstituted to the original milk composition before use. The frozen products were partially defrosted by submerging the quart paper containers in a water bath having a temperature of 110° F., which is just below the melting point of the paraffin coating the container. To the block of frozen condensed milk was added the amount of water (160° F.) required to restore the milk solids to their normal concentration. The mixture was agitated gently at intervals until thawed. Pre-thawing was necessary to remove the frozen block without having paraffin attached to it. So tenaciously was the paraffin attached that "peeling" of the carton resulted in transfer of a considerable portion of the paraffin from the carton to the frozen block and subsequent incorporation in the defrosted product. Shrinkage during storage of the frozen, condensed milk resulted in a similar removal of paraffin by the frozen block in the samples that had been pre-frozen in the ice cream freezer. Still-frozen samples did not shrink.

The reconstituted and thawed samples were examined for fat separation, curdy and flaky appearance and ascorbic acid content and were judged for flavor by at least three experienced milk judges using the value of 25 as a perfect score, 23 to 25 no criticism and anything under 12 as unsalable.

The second experiment was started in December, 1944, using University of

Illinois herd milk. The following procedure was followed in processing the milk: (a) clarified at 90° F.; (b) pasteurized at 170° F. for 20 min. (after clarification); (c) cooled to 145° F. in the vat and a portion homogenized at 2500 lb. pressure; (d) the remainder was condensed and then homogenized at 2500 lb. pressure at a temperature of 135° F.; (e) to a portion of the condensed milk was added 1.5 per cent dextrose; (f) the spray pressure used for drying the condensed milks was 500 lb.; and (g) the powder had a packing density of 0.484 g. per milliliter. The samples were stored and observations were made in a manner similar to those listed in the first experiment except that the condensed milk to which dextrose was added was divided into two lots. One lot was placed directly into paper containers before being placed in the hardening room and the second lot first was passed through a continuous freezer and reduced to a temperature of 27 to 28° F. before being placed in the paper containers.

The analytical data on the experimental samples when freshly prepared are given in tables 1 and 2 for experiments number 1 and 2, respectively.

TABLE 1

Analytical data on the relative keeping quality of frozen milk, frozen condensed milk, and spray-dried whole milk powder

(*Experiment 1*)

Product	B.F.	T.S.	Acidity	Vit. C	Bact. count	Coli count	Copper	Iron	Initial oxygen	Moisture
	(%)	(%)	(%)	(*mg./l.*)			(*ppm*)	(*ppm*)		(%)
Standardized milk	3.5	12.05	0.155	15.4	14,200	12	0.10	0.51	.....	
Pasteurized milk	.....	.....	.....	13.3	600	0	.....	.....	.....	
Condensed milk	12.65	43.22	0.55	9.8	760	0	0.40	2.77	.....	
Condensed milk plus 3% dextrose	12.33	45.17	.....	.....	850	8	.....	.....	.....	
Powdered whole milk	28.88	98.0	0.14*	.....	600*	.....	1.43	7.65	0.95	2.0
Powdered milk (Dextrose added)	27.53	97.2	0.135*	.....	1000*	.....	1.40	7.40	0.94	2.8

Powder sample identification
Experiment 1

Cond. no.	Product dried	Sample no.	Type of pack	Symbol used	Storage temp.	Symbol used
(1)	Condensed milk	100	Air	A	Room	R
		101	Nitrogen	N	Room	R
		102	Air	A	−10° ± 5° F.	
		103	Nitrogen	N	−10° ± 5° F.	
(2)	Condensed milk and 3% Dextrose	104	Air	A	Room	R
		105	Nitrogen	N	Room	R
		106	Air	A	−10° ± 5° F.	
		107	Nitrogen	N	−10° ± 5° F.	

* After reconstitution.

TABLE 2

Analytical data on the relative keeping quality of frozen, frozen condensed and spray-dried whole milk powder
(Experiment 2)

	B.F.	T.S.	Acidity	Vit. C	Copper	Iron	Initial oxygen	Moisture
	(%)	(%)	(%)	(*mg./l.*)	(*ppm*)	(*ppm*)		(%)
Standardized milk	3.5	12.6	0.16	16.4	.....	.....	.....	.....
Pasteurized milk	.....	.....	.....	11.7	.....	.....	.....	.....
Condensed milk	11.5	41.97	0.155*	10.6*	.....	.....	.....	.....
Powdered milk	27.39	98.5	0.15*	8.6*	1.50	3.3	.....	1.50
Powdered milk (dextrose added)	26.52	98.0	0.15	8.0	1.42	2.8	.....	2.0

Powder sample identification
Experiment 2

Cond. no.	Product dried	Sample no.	Type of pack	Symbol used	Storage temp.	Symbol used
(3)	Condensed milk	136	Air	A	Room	R
		137	Nitrogen	N	Room	R
		138	Air	A	Hardening room	HR
		139	Nitrogen	N	Hardening room	HR
(4)	Condensed milk and 1.5% dextrose	140	Air	A	Room	R
		141	Nitrogen	N	Room	R
		142	Air	A	−10° ± 5° F	
		143	Nitrogen	N	−10° ± 5° F	

* After reconstitution.

At intervals during the storage period, thawing observations, ascorbic acid determinations and flavor scores were made on the thawed products; analyses of the headspace gas of nitrogen-packed powder were made and ascorbic acid values and flavor scores were determined on the reconstituted powdered milk, both air- and nitrogen-packed. In the first experiment, sixteen periodic observations were made over a storage period of 523 days (approximately 17 mo.). In the second experiment, the milk samples were stored 365 days and twelve periodic observations were made.

EXPERIMENTAL RESULTS

The data on the thawing observations of the frozen products, the oxygen content of the powdered milk and the flavor scores and ascorbic acid values of both types of products are given in tables 3 and 4. At the beginning of the storage period, the score of the reconstituted condensed milk was higher than that of pasteurized homogenized fluid milk and that of the reconstituted powdered milk was higher than the flavor score of the reconstituted condensed milk. These samples had much less cooked flavor as a result of the condensing and drying opera-

TABLE 3

The flavor, ascorbic acid, thawing observations and oxygen content of fluid, concentrated and powdered whole milk on storage (Experiment 1)

Sample	How Frozen	Analysis	Storage time (*days*)[b]														
			Initial	23	48	79	105	140	175	198	227	269	307	338	364	425	464	523
Pasteurized[a] and homogenized	HR	Flavor score	19	19	19	19	19	19	18.5	18.5	18.5	18.0	19.0	19.0	18.0	18.5	19.0	19.0
		Flavor criticism[c]	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
		Vit. C (*mg./l.*)	10.3	.....	9.6	9.7	8.0	7.2	7.4	8.2	9.4	8.9	8.9	8.9	9.0	7.6	8.0	6.5
		Thaw Obs.															Sl.Cd.	Sl.Cd.
Condensed 3.5–1	HR	Flavor score	21.0	22.0	21.5	21	21.0	21	20.0	19.0	19.0	19	19.5	20	19.0	19.0	21.0	20.5
		Flavor criticism	.....	.....	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF
		Vit. C (*mg./l.*)	9.8	.....	9.6	8.1	6.6	4.8	7.1	6.7	7.5	4.5	4.0	8.8	8.7	8.0	8.3	6.3
		Thaw Obs.																
Condensed 3.5–1	F.	Flavor score	21.0	22.0	21.5	21.0	21.0	21	20.0	19.5	19.5	19.5	19.5	19.5	19.5	19.0	20¾	19.0
		Flavor criticism	.....	.....	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF
		Vit. A (*mg./l.*)	9.8	.....	9.6	8.3	4.7	5.3	6.2	6.0	8.9	3.9	3.9	8.6	7.5	7.6	8.5	7.0
		Thaw Obs.													Sl.Cd.	Sl.Cd.	Sl.Cd.	Cd. F.S.
Condensed 3.5–1 and 3% dextrose added	F.	Flavor score	21.5	22.0	21.5	21	21.0	21.0	20.0	20.0	20	20	19.5	20	19.5	19.0	20¾	19.5
		Flavor criticism	.....	.....	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF	LF
		Vit. C (*mg./l.*)	.....	.....	9.1	8.3	5.2	5.5	6.4	6.1	7.5	4.0	3.8	7.9	7.8	7.3	8.3	6.6
		Thaw Obs.														Sl.Cd.	Sl.Cd.	Cd. F.S.

[a] Raw milk scored 22 in flavor and contained 15.4 mg./l. vitamin C.

[b] Concentrated milk samples (stored at $-10° \pm 5°$ F.).

[c] Key to criticisms for tables 3 and 4.

Symbol used	Flavor criticism	Symbol used	Flavor criticism
C	Cooked	Sy	Salty
M	Metallic	Sl.Ox.	Slightly oxidized
LF.	Lacks Freshness	Sl.S	Slightly stale
Ox.	Oxidized	Sl.Cd.	Slightly curdy
		F.S.	Slight fat separation
		Cd.	Curdy
		Sl.M	Slightly metallic

TABLE 3 (*continued*)

Powdered whole milk samples					Storage time[b] (*days*)														
Sample no.	Made from cond. no.	Type pack	Where stored	Analysis	Initial	23	48	79	105	140	175	198	227	269	307	338	364	425	464	523
100	1	A	R	Flavor score	22.0	22.0	19.5	19	17.0	16.5	16.0	15.0	15.0	10.	10.0	0	0	.....	.....	.....
				Flavor criticism	.....	.....	Sl.Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	.....	.....	.....
				Vit. C. (*mg./l.*)	.....	.....	5.9	3.1	4.0	3.6	4.4	4.4	3.6	3.2	3.6	3.3	3.5	.....	.....	.....
101	1	N	R	Flavor score	22.0	22.0	20.0	19.5	18.5	18.5	18.0	18.0	18.0	17.0	16.0	13.0	10.0	.....	8.0	15
				Flavor criticism	.....	.....	.....	.....	.....	.....	.....	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	.....	Ox.	Ox.
				Vit. C. (*mg./l.*)	.....	.....	7.5	5.6	6.0	6.7	6.4	7.5	7.8	6.8	7.1	6.5	6.5	6.7	6.5	6.1
				% O_2	.95	1.92	1.70	2.22	1.46	1.31	1.33	1.33	1.21	.84	.96	1.09	2.11	.68	.93	.86
102	1	A	HR	Flavor score	22	22	21.5	21.5	20.0	20.0	17.0	19.0	14.0	17.0	16.5	14.0	.....	.....	.....	.....
				Flavor criticism	.....	.....	LF	.....	.....	.....	Sl.M.	Sl.M.	.....	M	M	M	.....	.....	.....	.....
				Vit. C. (*mg./l.*)	.....	.....	8.0	7.5	7.0	5.2	5.9	8.4	7.8	6.8	7.8	7.4	.....	.....	.....	.....
103	1	N	HR	Flavor score	22.0	22.0	22.0	22.0	21.0	20.5	20.0	20.0	19.5	19.5	18.0	17.5	18.0	17.5	17.5	12.0
				Flavor criticism	.....	.....	LF	.....	.....	F	.....	LF	.....	.....	M	M	M	M	M	M
				Vit. C. (*mg./l.*)	.....	.....	8.0	7.7	7.0	5.7	6.9	8.4	8.3	7.4	7.8	7.4	8.0	7.1	7.5	6.5
				% O_2	.95	1.69	1.93	1.63	2.12	1.94	2.15	2.18	1.98	1.96	1.92	.95	2.03	2.15	1.94	2.18
104	2	A	R	Flavor score	22.0	22.0	20.5	20.0	19.0	17.5	17.0	16.5	14.0	15.0	13.0	3.0	0	0	10	10
				Flavor criticism	.....	.....	Sl.S	.....	.....	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.
				Vit. C. (*mg./l.*)	.....	.....	6.4	5.1	4.0	4.1	4.4	4.9	4.7	4.2	7.6	3.7	4.0	2.7	3.5	1.7
				% O_2									.....	.....	.....	.....	17.36	.....	.....	.....
105	2	N	R	Flavor score	22.0	22.0	20.5	20.5	18.5	19.0	19.0	18.5	17.5	17.0	17.0	14.5	13.5	16.0	8.0	17.0
				Flavor criticism	.....	.....	Sl.S	Sl.Ox.	.....	.....	.....	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.M	Ox.
				Vit. C. (*mg./l.*)	.....	.....	7.0	5.1	6.0	6.2	5.9	7.1	6.8	6.8	7.1	7.0	7.5	5.8	6.5	6.1
				% O_2	.94	1.57	1.93	1.90	1.55	1.35	1.29	.99	.84	.53	2.23	1.34	.90	1.20	1.20	.80
106	2	A	HR	Flavor score	22.0	22.0	21.5	21.0	20.5	20.5	20.0	17.0	17.0	13.0	16.0	14.0	15.0	14.0	.....	14.0
				Flavor criticism	.....	.....	.....	.....	.....	.....	M	M	M	M	M	M	M	M	.....	M
				Vit. C. (*mg./l.*)	.....	.....	7.0	7.1	7.0	3.6	6.9	8.0	6.2	6.8	7.5	7.4	7.5	6.7	.....	6.5
107	2	N	HR	Flavor score	22.0	22.0	21.5	21.0	20.0	21.0	19.0	18.5	19.5	19.0	17.5	17.0	17.0	17.0	18.0	16.0
				Flavor criticism	.....	.....	.....	.....	.....	.....	.....	M	.....	M	M	M	M	M	M	M
				Vit. C. (*mg./l.*)	.....	.....	7.5	7.1	7.0	5.7	7.4	8.0	7.8	7.4	7.5	7.4	7.5	6.2	7.5	6.5
				% O_2	.94	1.45	1.58	1.21	1.56	1.51	1.73	1.64	1.19	1.46	1.26	1.45	1.48	1.78	1.67	1.50

TABLE 4

The flavor, ascorbic acid, thawing observations and oxygen content of fluid,[a] concentrated and powdered whole milk on storage. (Experiment 2)

Sample	How Frozen	Analysis	Storage time[b] (*days*)										
			Initial	22	48	92	119	148	176	210	248	280	306	365
Pasteurized and homogenized	HR	Flavor score	.....	19.5	20.0	19.0	18.5	18.0	18.0	18.0	19.5	19.0	19.0	19.5
		Flavor criticism	.....	C	C	C	C	C	C	C	C	C	C	C
		Vit. C. (*mg./l.*)	11.7	9.2	9.0	8.1	8.5	8.2	9.2	9.0	9.6	9.9	10.0	7.6
		Thaw Obs.												
Condensed 3.5–1	HR	Flavor score	.....	21.5	21.0	20.0	19.5	19.0	19.0	19.0	19.5	19.5	19.5	20
		Flavor criticism	.....	.....	.....	.....	.....	.....	C	C	C	C	C	C
		Vit. C. (*mg./l.*)	10.6	8.7	8.3	7.2	7.6	5.9	8.4	5.1	5.9	8.6	8.3	7.1
		Thaw Obs.												
Condensed 3.5–1	F	Flavor score	.....	21.5	21.0	20	19.5	19.5	19.5	19.5	19.5	20.0	20.0	19.75
		Flavor criticism	.....	.....	.....	.....	.....	C	C	C	C	C	C	C
		Vit. C. (*mg./l.*)	10.6	9.4	7.5	7.1	7.3	5.6	8.2	5.4	6.6	9.9	7.6	7.6
		Thaw Obs.												
Condensed 3.5–1	HR	Flavor score	.....	21.5	21.0	18.5	20.0	20.0	20.0	19.0	19.5	19.5	19.5	20.5
		Flavor criticism	.....	Sl.C	C	C	C	C	C	C	C	C	C	C
		Vit. C. (*mg./l.*)	.....	8.1	7.6	8.0	7.8	6.8	9.2	4.6	5.8	10.7	8.0	7.0
		Thaw Obs.												
Milk condensed 3.5–1 and 1.5% dextrose added	F	Flavor score	.....	21.5	21.0	18.5	20.0	20.0	20.0	19.0	19.5	19.5	19.5	20.25
		Flavor criticism	.....	C	C	C	C	C	C	C	C	C	C	C
		Vit. C. (*mg./l.*)	.....	9.3	7.8	7.7	7.0	6.1	8.4	5.1	6.6	10.2	7.4	8.2
		Thaw Obs.												

[a] Raw milk scored 19 in flavor and contained 16.4 mg./l. vitamin C.
[b] Concentrated Milk Samples (stored at $-10° \pm 5°$ F.)
[c] See table 3.

TABLE 4 (*continued*)

Powdered whole milk samples					Storage time (*days*)										
Sample no.	Cond. no.	Type pack	Where stored	Analysis	Initial	22	48	92	119	148	176	210	248	280	306	365
136	3	A	R	Flavor score	.....	19.0	19.0	18.5	17.5	15.5	15.0	15.0	.....	9.0	.....	.....
				Flavor criticism	.....	Sl.Ox.	.....	.....	Ox.	Ox.	Ox.	Ox.	.....	Ox.	.....	.....
				Vit. C(*mg./l.*)	8.6	7.1	6.0	5.1	5.5	5.5	5.0	3.7	.....	5.1	.....	.....
137	3	N	R	Flavor score	.....	19.5	19.5	19.0	19.0	17.0	16.5	16.5	15.0	12.0	15.0	.....
				Flavor criticism	.....	Sl.Ox	.....	.....	.....	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.	.....
				Vit. C(*mg./l.*)	8.6	7.7	6.5	7.1	7.0	7.7	6.7	7.4	7.5	7.4	7.5	.....
				% O_2	.....	1.81	1.79	1.44	1.65	1.45	1.09	.72	.85	.59	1.04	.....
138	3	A	HR	Flavor score	.....	20.5	21.0	21.0	19.5	18.5	17.0	17.0	15.0	17.5	13.5	16.5
				Flavor criticism	.....	C	.....	.....	.....	.....	M	M	M	M	M	M
				Vit. C(*mg./l.*)	8.6	6.6	7.5	7.6	8.0	8.2	7.6	8.0	8.2	7.9	8.0	6.7
139	3	N	HR	Flavor score	.....	20.0	20.5	20.0	20.0	20.0	20.0	20.0	19.0	18.5	18.5	17.5
				Flavor criticism	.....	C	.....	C	.....	.....	.....	.....	Sl.M	M	S	S
				Vit. C(*mg./l.*)	8.6	7.7	8.0	8.1	8.0	8.2	7.6	8.0	8.6	7.9	8.0	7.1
				% O_2	.....	1.44	1.52	1.59	1.47	1.60	1.20	1.11	1.50	1.74	1.98	1.69
140	4	A	R	Flavor score	.....	20.0	19.0	19.0	18.0	16.5	17.5	17.5	.....	.....	.....	.....
				Flavor criticism	.....	Sl.Ox.	.....	.....	Ox.	Ox.	Ox.	Ox.	.....	.....	.....	.....
				Vit. C(*mg./l.*)	8.0	7.1	5.0	5.6	6.0	5.9	5.0	4.8	.....	.....	.....	.....
141	4	N	R	Flavor score	.....	20.0	19.5	18.5	18.5	18.0	18.0	18.0	16.0	15.0	15.0	.....
				Flavor criticism	.....	Sl.Ox.	.....	.....	.....	.....	.....	.....	M	Ox.	Ox.	.....
				Vit. C(*mg./l.*)	8.0	7.1	6.5	7.1	7.5	7.7	7.6	8.0	7.5	8.4	8.5	.....
				% O_2	.....	1.26	1.36	1.14	.87	1.14	.78	.45	.85	.00	.63	.....
142	4	A	R	Flavor score	.....	20.0	21.0	21.0	20.5	19.0	18.0	18.0	16.0	16.5	.....	.....
				Flavor criticism	.....	C	.....	.....	.....	.....	M	M	M	M	.....	.....
				Vit. C(*mg./l.*)	8.0	7.7	6.5	7.6	8.0	8.2	7.6	8.0	8.2	8.4	.....	.....
143	4	N	HR	Flavor score	.....	19.5	20.5	18.0	20.0	19.5	18.5	19.0	18.0	18.0	18	17.0
				Flavor criticism	.....	C	.....	.....	.....	.....	M	M	M	M	S	S
				Vit. C(*mg./l.*)	8.0	8.2	6.5	7.6	8.0	8.2	7.6	8.0	8.2	8.8	9.0	7.6
				% O_2	.....	1.03	1.16	1.28	1.17	1.30	1.20	1.13	1.31	1.27	1.42	1.24

tions, indicating that at least a portion of the substances responsible for cooked flavor are volatile.

Fluid or condensed milks can be kept frozen at low temperatures for considerable lengths of time without showing any curdy, oily or flaky appearance upon defrosting (tables 3 and 4). In the second experiment (table 4), at the end of a year of storage, the fluid and condensed milk still defrosted satisfactorily. In the first experiment (table 3) there was normal thawing and reconstitution up to 364 days of storage when the condensed milk frozen in the freezer had a slightly curdy appearance on reconstitution. The next sample to show a curdy appearance was the sweetened condensed milk frozen in the freezer, which was curdy and showed fat separation when examined on the 425th day. This condition in these two samples became progressively worse for the duration of the study. The pasteurized fluid milk showed a slight curdy appearance at the end of 1 yr. The condensed milk frozen in the quiescent state in the ice cream hardening room had a satisfactory appearance even after 523 days of storage. However, the frozen condensed milk was particularly sensitive to heat-shocking. Samples brought out to room temperature and then returned to the hardening room showed a destabilized condition of the milk proteins in a few days. Similar effects were produced when the frozen milk was transferred from the sub-zero temperature to one slightly above 0° F.

The greatest reduction in ascorbic acid values came as a result of the heating that occurred during processing. The amount of ascorbic acid retained in the thawed frozen products was only slightly less than it was when the products were first stored. The concentration of milk solids, the addition of dextrose or the method of freezing did not seem to influence to any extent the retention of ascorbic acid on storage. Although initially powdered whole milk will show less ascorbic acid content, the retention of ascorbic acid in the nitrogen-packed product compared favorably with that of the frozen products. In the milk powder samples, greater ascorbic acid loss occurred in the air-packed than in the nitrogen-packed samples and more loss of ascorbic acid occurred in those stored at room temperature than in those stored at $-10° \pm 5°$ F.

The frozen fluid milk and frozen concentrated milks possessed better flavor keeping qualities than the gas-packed whole milk powder made from the same lot of milk and stored at the same temperature. As the storage period advanced, the powder stored in the hardening room usually became progressively metallic or oxidized while the frozen milk and concentrated milk lost their fresh milk flavor but remained highly palatable. The flavor of the frozen products remained satisfactory throughout the storage period of 523 days in the case of experiment 1 and for 1 yr. in the case of experiment 2, while the gas-packed whole milk powder stored at the same temperature became unsatisfactory after a storage period of 307 days for experiment 1 and approximately 280 days for experiment 2. The air-packed powder stored at $-10 \pm 5°$ F. possessed a metallic flavor after 175 days storage in experiment 1 and 176 days of storage in experiment 2. When powder was stored at room temperature, an oxidized flavor was observed after 198 days of storage in the nitrogen-packed samples of experiment 1 and 176 days

in the second experiment. At room temperature the air-packed samples became unsatisfactory at 105 days and 119 days for the two experiments, respectively.

After thawing, the frozen milks in some instances were held at 40° F. for 72 hr. yet they did not change in flavor.

The concentration of the milk (approximately 3.5 to 1), the addition of dextrose prior to freezing or drying, the method of freezing (slow freezing at $-10 \pm 5°$ F. *vs.* freezing initially to a slush in a continuous ice cream freezer) did not prove to be important factors in flavor changes, ascorbic acid retention or stability of the milk solids on thawing of the frozen products.

CONCLUSIONS

Fluid milk or milk concentrated approximately 3.5 to 1 can be satisfactorily stored at a uniformly low temperature ($-10 \pm 5°$ F.) for at least 1 yr. Milk concentrated approximately 3.5 to 1 or fluid milk can be stored in frozen state for 1 yr. with less flavor change than the same milk stored at the same temperature in dried form (gassed or ungassed).

Condensing before freezing or the addition of dextrose to the milk did not prove to be important factors in storing milk in a frozen state either from the standpoint of flavor changes or changes in the physical state of the milk proteins.

REFERENCES

(1) BABCOCK, C. J., ROERIG, R. N., STABILE, J. N., DUNLAP, W. A., AND RANDALL, R. Frozen Homogenized Milk. I. Effects of Freezing and Storage Temperatures on the Physical Characteristics of Homogenized Milk. J. Dairy Sci., 29: 699–706. 1946.

(2) BABCOCK, C. J., ROERIG, R. N., STABILE, J. N., DUNLAP, W. A., AND RANDALL, R. Frozen Homogenized Milk. II. Effect of Freezing and Storage Temperatures on the Chemical and Bacteriological Properties of Homogenized Milk. J. Dairy Sci., 30: 49–54. 1947.

(3) BABCOCK, C. J., STABILE, J. N., RANDALL, R., AND WINDHAM, E. S. Frozen Homogenized Milk. III. Stability of Milk Solids Distribution in Frozen Homogenized Milk. J. Dairy Sci., 30: 733–736. 1947.

(4) BABCOCK, C. J., STABILE, J. N., RANDALL, R., AND WINDHAM, E. S. Frozen Homogenized Milk. IV. Keeping Quality of Frozen Homogenized Milk after Thawing. J. Dairy Sci., 31: 805–810. 1948.

(5) BABCOCK, C. J., STABILE, J. N., WINDHAM, E. S., EVANS, L. B., AND RANDALL, R. Frozen Homogenized Milk. V. Effect of Age before Freezing on the Keeping Quality of Frozen Homogenized Milk. J. Dairy Sci., 31: 811–815. 1948.

(6) BABCOCK, C. J., STABILE, J. N., WINDHAM, E. S., AND RANDALL, R. Frozen Homogenized Milk. VI. The Use of Stabilizers in Frozen Homogenized Milk. J. Dairy Sci., 32: 175–182. 1949.

(7) BABCOCK, C. J., WINDHAM, E., AND RANDALL, R. VII. Effect of Agitation during Freezing on Keeping Quality of Frozen Homogenized Milk. J. Dairy Sci., 32: 812–816. 1949.

(8) DOAN, F. J., AND LEEDER, J. G. Milk Can Be Frozen for Sale to Consumers. Food Ind., 16(7): 532–534, 579. 1944.

(9) TURNBOW, G. D., TRACY, P. H., AND RAFFETTO, L. A. *The Ice Cream Industry.* 2nd ed., John Wiley and Sons. pp. 53–54. 1947.

MOTILITY OF BOVINE SPERMATOZOA AND CONTROL OF BACTERIA AT 5 AND 25° C. IN EXTENDERS CONTAINING SULFANILAMIDE, PENICILLIN, STREPTOMYCIN AND POLYMYXIN

R. H. FOOTE AND R. W. BRATTON

Laboratory of Animal Breeding and Artificial Insemination
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

In artificial breeding the general practice is to preserve bovine spermatozoa in a buffered yolk medium by cooling to approximately 5° C. and holding the extended semen at that temperature until used. This procedure is based on considerable experimental evidence which has been reviewed by Anderson (1) showing that spermatozoa survive longer at 5° C. than at higher temperatures. Considerable expense is involved in packaging and storing semen so as to maintain a temperature of 5° C. until the semen is used for insemination. Therefore, any method of preserving the spermatozoa which is cheaper than refrigeration is of practical importance. Foote and Salisbury (5, 6) have shown that the motility of spermatozoa stored at 20° C. in a citrate-phosphate buffer is prolonged by the addition of a number of antibacterial agents. Since egg yolk is an excellent medium for bacterial growth, one of the problems of preserving spermatozoa in egg yolk at "room" temperature appears to be that of controlling bacterial growth at this temperature. Dimitropoulos *et al.* (2) and Hennaux *et al.* (7, 8) have reported that antibacterial agents were beneficial in preserving the motility of spermatozoa extended with citrate-yolk and incubated at 37° C. The present paper is a report of comparisons of spermatozoan motility and bacterial growth in extended semen when it is stored at 5° C. and at 25° C. in extenders containing various antibacterial agents.

EXPERIMENTAL PROCEDURE

The basic medium employed for extending the semen consisted of egg yolk mixed with an equal amount of buffer containing 3.6 g. sodium citrate dihydrate per 100 ml. of water redistilled in glass. Based on experiments previously reported by Foote and Bratton (4), five different extenders were prepared by adding the following amounts of antibacterial agents per milliliter of basic extender: (a) 3 mg. sulfanilamide, (b) 500 Oxford Units of crystalline sodium penicillin G, (c) 500 γ (units) of streptomycin base ($CaCl_2$ complex), (d) 500 γ equivalents of polymyxin B sulfate[1] and (e) a combination of a, b, c and d. In addition, the basic extender was included as a control. Thus, with six extenders and two storage temperatures, 5 and 25° C., a total of 12 treatments were involved. To accomplish a simultaneous comparison of all 12 treatments, each semen sample was divided into six equal portions, and each portion extended with one of the six extenders to give approximately 15×10^6 motile spermatozoa per milliliter of

Received for publication May 27, 1950.

[1] "Aerosporin" brand polymyxin B was kindly supplied by D. S. Searle of the Burroughs Wellcome and Co., Inc., Tuckahoe, New York. The amount used was equivalent to 500 γ of pure standard.

TABLE 1

The counts of bacteria in semen before storage and after 24 hr. of storage at 5 and 25° C. in citrate-yolk extender with and without sulfanilamide and antibiotics

Sample	Semen as collected	After extension of semen	After 24 hr. of storage in extenders containing:											
			No antibacterial agent		Sulfanilamide		Penicillin		Streptomycin		Polymyxin		All antibacterial agents	
			5° C.	25° C.	5° C.	25° C.	5° C.	25° C.	5° C.	25° C.	5° C.	25° C.	5° C.	25° C.
			(*1,000's of bacteria/ml.*)											
1	33.0	0.6	2.8	120	2.4	28.0	0.0	0.1	0.0	86.0	1.4	2.0	0.0	0.0
2	61.0	1.1	1.7	26,000	1.2	1.0	0.2	1.0	0.1	1.0	0.7	1.0	0.0	1.0
3	71.0	0.7	1.4	110,000	0.9	24.0	0.7	30,000.0	0.5	2,000.0	1.1	30,000.0	0.3	0.0
4	0.7	0.01	0.5	31	0.5	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
5	0.4	< 0.01	0.6	21	0.3	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	130.0	1.0	3.2	57,000	3.3	3.0	0.0	1.0	0.2	0.0	2.9	1.0	0.0	0.0
7[a]	160.0	1.7	9.4	49,000	8.9	27.0	0.8	64.0	0.4	69.0	2.4	22.0	0.3	0.0
8	59.0	1.4	7.7	51,000	6.7	11.0	0.0	0.0	0.1	0.0	3.2	1.0	0.0	0.0
9[a]	13.0	0.2	0.5	410,000	0.5	6.6	0.2	800.0	0.0	0.0	0.5	0.0	0.0	0.0
10[a]	110.0	1.5	5.6	800,000	7.1	460.0	2.3	100,000.0	7.6	30,000.0	0.0	0.0	0.0	0.0
11[a]	140.0	1.6	3.0	27,000	0.1	0.0	2.7	1,800.0	0.0	1.0	0.0	12.0	0.0	0.0
12[a]	28.0	0.3	1.9	81,000	1.5	2.4	0.0	25,000.0	0.0	4.3	0.2	30,000.0	0.0	0.0
13	9.0	0.08	0.9	110	0.9	4.0	0.1	40.0	0.2	2.0	0.3	1.0	0.0	0.0
14[a]	31.0	0.3	3.4	790,000	3.3	5.8	3.0	30,000.0	3.8	50,000.0	0.0	0.0	0.0	0.0
15	100.0	1.0	20.0	84,000	12.0	18.0	0.2	0.0	0.6	370.0	10.0	1,000.0	0.0	0.0
16	31.0	0.3	7.3	240,000	1.7	1.5	0.2	0.2	0.1	0.0	0.3	510.0	0.0	0.0
17	8.0	0.08	1.0	4	0.9	0.0	0.0	1.0	0.1	0.0	1.2	0.0	0.0	0.0
18	500.0	11.0	39.0	45,000	57.0	35.0	2.1	5.0	0.6	2.0	21.0	10.0	0.1	0.0
Av.	83.0	1.3	6.1	150,000	6.1	35.0	0.7	10,000.0	0.8	4,600.0	2.5	3,400.0	0.04	0.06

[a] Bacterial flora included pseudomonas type organisms.

extended semen, the average extension rate being about 1 to 70. From each of these extended portions, duplicate sub-samples were taken so that the six extenders could be stored at 5 and 25° C. The sub-samples were examined microscopically for the per cent of motile spermatozoa and the rate of progressive movement after 2, 24 and 72 hr. of storage. This extension and storage procedure was replicated with 18 samples of high quality semen obtained from 18 bulls in the active stud of the New York Artificial Breeders' Cooperative, Inc.

The approximate number of living bacteria present in each semen sample immediately following collection and in all samples of extended semen after 24 hr. of storage was determined by the plate count method as employed in this laboratory (4). The 24-hr. period of storage was chosen as the most desirable time to estimate the numbers of bacteria present because most bovine semen is used for insemination at about this time.

The statistical significance of the differences between the average per cent of motile spermatozoa in the different extenders stored at the two temperatures was tested by analysis of variance (9).

RESULTS

The number of bacteria present in the freshly collected semen, in the unstored extended semen and in the extended semen stored for 24 hr. is shown in table 1. Most of the freshly collected semen samples had a bacterial count of less than 120,000 per milliliter. After the semen was mixed with nearly sterile extender so as to contain approximately 15 million motile spermatozoa per milliliter, the bacterial counts, with the exception of semen sample 18, were reduced to less than 2,000 per milliliter, with sample 5 having a count of less than 10 per milliliter. After 24 hr. of storage at 5° C., bacterial growth was not excessive in any of the extended semen samples. Usually, when used separately, each of the antibiotics and sulfanilamide partially inhibited bacterial growth. The combination of antibiotics with sulfanilamide usually inhibited bacterial growth completely.

At 25° C. bacteria multiplied rapidly when no antibacterial agent was present. Sulfanilamide consistently reduced bacterial growth. Penicillin, streptomycin and polymyxin were highly bactericidal when the predominating types of bacteria were sensitive to the particular drug present. In samples 10 and 14, *Pseudemonas pyocyaneus* (usually sensitive to streptomycin) thrived at 25° C. in the presence of streptomycin, but was inhibited completely by polymyxin. Again the combination of antibiotics with sulfanilamide was highly bactericidal.

Microscopic examinations within 2 hr. after the semen was extended indicated that the percentages of motile spermatozoa were similar for all treatments. However, after 24 and 72 hr. of storage, large differences existed which are evident in table 2. At 5° C. the per cent of motile spermatozoa was similar in all extenders throughout the 72-hr. storage period. At 25° C. after 24 and 72 hr. of storage, the combination of sulfanilamide plus antibiotics was more effective ($P < 0.05$) in preserving the motility of the spermatozoa than was penicillin, streptomycin or polymyxin. While penicillin, streptomycin and polymyxin did not differ from each other, each was superior to no antibiotic at this temperature. At 25°

TABLE 2

The per cent of motile spermatozoa in extenders containing different antibacterial agents and stored at 5 and 25° C. (Av. of 18 ejaculates)

Length of storage	Temperature	Extenders containing:					
		No antibacterial agent	Sulfanilamide	Penicillin	Streptomycin	Polymyxin	All antibacterial agents
(*hr.*)	(° *C.*)	(*Percentage of motile spermatozoa*)					
24	5	63	62	63	62	63	62
	25	41	54	49	43	46	58
72	5	56	55	56	56	57	53
	25	8	34	17	17	23	39

C. spermatozoan motility generally was poorer than at 5° C. but at the 24-hr. storage interval the difference between the per cent of motile spermatozoa at these two temperatures in extenders containing sulfanilamide plus antibiotics was not significant statistically ($P > 0.05$).

DISCUSSION

The results of these experiments indicate that bovine semen in extenders containing sulfanilamide, penicillin, streptomycin and polymyxin can be stored for at least 24 hr. at 25° C. with much of the deleterious effect of high temperatures eliminated by the inhibition of bacterial growth. Sulfanilamide alone was nearly as effective in maintaining the motility of the spermatozoa as was the combination of sulfanilamide and antibiotics, but bacterial growth was more completely inhibited by the combination. Frequently, the antibiotics used alone were more bactericidal than sulfanilamide used alone, but the percentage of motile spermatozoa was higher when sulfanilamide was present.

Putrefaction of the egg yolk consistently occurred when bacterial growth was high. The percentage of samples exhibiting a putrid odor after 72 hr. of storage at 25° C. was 72, 6, 11, 22, 17 and 0, respectively, for the extenders containing no antibiotics, sulfanilamide, penicillin, streptomycin, polymyxin and the combination of sulfanilamide and antibiotics. The products of egg yolk putrefaction may have been spermicidal. The pH of all samples after 72 hr. of storage at 5° C. was approximately 6.70, while at 25° C. it was 6.50. This small difference would appear to eliminate H-ion concentration as an important factor in reducing spermatozoan motility at the higher temperature.

Fertility data obtained by Drake (3) indicate that semen processed and stored without refrigeration may be used for insemination in cool weather (April) but is not satisfactory for use in warm weather (July). The poorer results in July may have been caused by the direct deleterious effects of high temperatures on the spermatozoa or the citrate-yolk extender, since the bacteriological data presented in this paper indicate that bacterial growth is effectively inhibited at 25° C. by a combination of sulfanilamide and antibiotics. As a consequence of this control of bacterial growth in extenders stored unrefrigerated, the problem of prolonging the motility and fertility of spermatozoa in unrefrigerated extenders now appears to be one of achieving sufficient control of spermatozoan metabolism.

SUMMARY

The feasibility of storing bovine semen at 25° C. for use in artificial insemination to eliminate the expense of refrigerating the semen at 5° C. was investigated. Sulfanilamide, penicillin, streptomycin, polymyxin and a combination of these were added to 3.6 per cent citrate-yolk extender. The citrate-yolk extender containing no sulfanilamide or antibiotics served as the control. Eighteen semen samples were stored in each of the six extenders at 5° C. and at 25° C. The per cent of motile spermatozoa after 24 hr. of storage was lower when the semen was stored at 25° C. than when it was stored at 5° C. except in the extender containing the combination of antibacterial agents. In nearly all samples, this combination of sulfanilamide and antibiotics completely inhibited bacterial growth at both temperatures. This combination of antibacterial agents gives promise of making possible the development of an extender for bovine semen which will not require refrigeration.

REFERENCES

(1) ANDERSON, J. The Semen of Animals and its Use for Artificial Insemination. Imp. Bureau Animal Breeding and Genetics, Tech. Comm., Edinburgh. 1945.

(2) DIMITROPOULOS, E., HENNAUX, L., AND CORDIEZ, E. L'action des sulfamides sur la vitalité du sperme de taureau. Compt. rend. soc. biol., **141**: 1283–1286. 1947.

(3) DRAKE, M. Personal communication. 1949.

(4) FOOTE, R. H., AND BRATTON, R. W. Motility of Spermatozoa and Control of Bacteria in Bovine Semen Extenders Containing Sulfanilamide, Polymyxin and Aureomycin. J. Dairy Sci., **33**: 539–543. 1950.

(5) FOOTE, R. H., AND SALISBURY, G. W. The Effect of Pyridium, Penicillin, Furacin, and Phenoxethol upon the Livability of Spermatozoa and upon the Control of Bacteria in Diluted Bull Semen. J. Dairy Sci., **31**: 763–768. 1948.

(6) FOOTE, R. H., AND SALISBURY, G. W. The Effect of Sulfonamides upon the Livability of Spermatozoa and upon the Control of Bacteria in Diluted Bull Semen. J. Dairy Sci., **31**: 769–778. 1948.

(7) HENNAUX, L., DIMITROPOULOS, E., AND CORDIEZ, E. L'action de la streptomycine sur la vitalité du sperme de taureau. Compt. rend. soc. biol., **141**: 1272–1274. 1947.

(8) HENNAUX, L., DIMITROPOULOS, E., AND CORDIEZ, E. L'action de la penicilline sur la vitalité du sperme de taureau. Compt. rend. soc. biol., **142**: 408–410. 1948.

(9) SNEDECOR, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press, Ames. 1946.

RELATIONSHIP OF HYALURONIDASE CONCENTRATION TO FERTILITY OF DAIRY BULL SEMEN[1]

JAMES E. JOHNSTON[2] AND JOHN P. MIXNER
New Jersey Agricultural Experiment Station, Sussex

The possibility that the concentration of the enzyme hyaluronidase in mammalian semen is a critical factor in fertility has led the authors to investigate this relationship in dairy bulls.

In fertility studies in rabbits, Rowlands (8) found that by adding seminal plasma from killed spermatozoa suspensions containing hyaluronidase to dilute spermatozoa suspensions the median effective spermatozoa concentration for fertility was reduced to one-sixth of that of the controls. In similar experiments, however, Chang (1) indicated that the increased fertilizing capacity obtained by adding seminal plasma to dilute suspensions of spermatozoa was not due to hyaluronidase but to some other seminal plasma factor. Seminal plasma in which the hyaluronidase had been inactivated by heat was similarly effective, whereas added bull testes hyaluronidase had no effect.

Kurzrok *et al.* (5) reported that in six cases of human female infertility where the female was apparently not at fault and where the male seminal hyaluronidase concentration was low, the application of bull testes hyaluronidase to the uterine cervix with subsequent coitus resulted in pregnancies. Later, Kurzrok (4) reported that 33 out of 102 similar clinical patients conceived following the application of bull testes hyaluronidase to the uterine cervix. Entirely negative results were obtained by Seigler (10) in a series of 48 cases of human female infertility where hyaluronidase was applied and where the female presumably was not at fault. Semen samples from the male partner were not assayed for hyaluronidase in these cases.

A zero order correlation coefficient of -0.32 (significant at the 5 per cent level) between hyaluronidase titer and fertility of dairy bull semen was obtained by Sallman and Birkeland (9). The hyaluronidase assays in this case were made within 20 hr. of the time of ejaculation. In considering the negative correlation which they obtained, these authors suggested that the removal of hyaluronidase from semen might improve the fertility of the semen. In this general connection it is interesting to note that Johnston and Mixner (3) found a first order partial correlation (limiting effect of sperm concentration) of -0.30 (significant at the 5 per cent level) between percentage of live spermatozoa in dairy bull semen and hyaluronidase concentration.

METHODS

One hundred and eighty-seven semen samples were obtained from 24 Guernsey and Holstein bulls at the bull stud of the Dairy Research Farm, New Jersey Agri-

Received for publication May 31, 1950.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, Department of Dairy Industry.

[2] Present address: Dairy Department, Louisiana State University, Baton Rouge.

cultural Experiment Station, Sussex, from November, 1947, to July, 1949. Semen samples were diluted for use at a rate not exceeding 1 to 100 and usually much less. Inseminations were made by the technicians of the Sussex County Co-operative Breeding Association, Inc. Fertility estimates were based on the percentage of non-returns to service, after a 60- to 90-day period following service, to first and second service cows.

Seminal hyaluronidase was assayed by the turbidimetric method, as outlined by Leonard *et al.* (6) and modified by Mixner and Johnston (7). Hyaluronidase potencies are expressed as milligrams equivalent to a standard preparation of bull testes hyaluronidase[3] (30 TRU per mg.). The Klett-Summerson photoelectric colorimeter with red no. 66 filter was used to measure turbidity. Two hyaluronidase assays were made upon the seminal plasma of each semen sample, the first assay being made within 1 hr. after the collection of the semen and the second assay being made after 24 hr. of incubation at 37° C. under toluene. These assay periods were chosen after a consideration of the scheme of development of hyaluronidase in bull semen (Johnston and Mixner, 2). Both of these measures of hyaluronidase concentration in semen were correlated with the fertility data.

The data on the semen samples were classified into three groups for purposes of calculation according to the number of first- and second-service cows bred to each sample. The groups are as follows: (a) 10–19 cows bred per sample, including 82 samples from 21 bulls for a total of 1,086 breedings, (b) 29 or more cows bred per sample, including 105 samples from 18 bulls for a total of 3,568 breedings, and (c) summation groups a and b, comprising 187 samples from 24 bulls to which 10 or more cows were bred per sample for a total of 4,654 breedings.

RESULTS AND DISCUSSION

The mean values, standard deviations and ranges for initial and 24-hr. hyaluronidase levels and for fertility are given in table 1 for each of the semen sample groups.

TABLE 1

Means, standard deviations and ranges for fertility, initial and 24-hr. hyaluronidase levels for each semen sample group

Character	Mean and standard error	Standard deviation	Range
(a) 10–19 cow bred/sample			
Initial hyaluronidase (mg./ml.)	45.3 ± 2.1	18.7	14–104
24-hr. hyaluronidase (mg./ml.)	119.0 ± 4.6	41.4	47–208
Fertility (% 60- to 90-day non-returns)	65.8 ± 1.5	13.9	37–92
(b) 20 + cows bred/sample			
Initial hyaluronidase (mg./ml.)	41.0 ± 1.7	17.4	14–98
24-hr. hyaluronidase (mg./ml.)	110.3 ± 4.1	41.4	44–246
Fertility (% 60- to 90-day non-returns)	66.4 ± 1.1	11.2	36–83
(c) 10 + cows bred/sample (groups *a* and *b*)			
Initial hyaluronidase (mg./ml.)	42.9 ± 1.3	18.1	14–104
24-hr. hyaluronidase (mg./ml.)	114.1 ± 3.0	41.6	44–246
Fertility (% 60- to 90-day non-returns)	66.1 ± 0.9	12.4	36–92

[3] The bull testes hyaluronidase was furnished through the courtesy of Schering Corp., Bloomfield, N. J.

Analysis of variance of the data revealed that there were highly significant differences among bulls with respect to initial and 24-hr. hyaluronidase levels and, also, with respect to the fertility estimation on each of the semen sample groups.

It is interesting to note (see table 1) the marked increase in hyaluronidase concentration of the seminal plasma which occurs between the time of the initial assay and the 24-hr. assay. This increase is of the order of 166 per cent and apparently represents the release of hyaluronidase by dying and dead spermatozoa into the seminal plasma (Johnston and Mixner, 2).

To determine whether any relationships existed between either of the measures of hyaluronidase concentration and fertility, zero order coefficients of correlation were calculated for each semen sample group of data on a "total," "between bull" and "within bull" basis (table 2). None of these correlations achieve statistical significance.

TABLE 2

Zero order coefficients of correlation between initial and 24-hr. hyaluronidase levels and fertility estimates

Semen sample group	No. semen samples	Initial hyaluronidase			24-hr. hyaluronidase		
		Total	Between bull	Within bull	Total	Between bull	Within bull
10-19 cows bred	82	+0.16	+0.42	−0.12	+0.01	+0.32	−0.22
20+ cows bred	105	−0.17	−0.24	−0.12	−0.08	−0.01	−0.12
10+ cows bred	187	0.00	+0.08	−0.07	−0.03	+0.10	−0.11

Highly significant relationships have been shown to exist between hyaluronidase levels and the two factors, spermatozoa concentration and percentage of live spermatozoa (Johnston *et al.*, 3). Also, a significant relationship exists between these latter two factors and fertility (Stone *et al.*, 11). However, since none of the coefficients of correlation between seminal plasma hyaluronidase levels and fertility achieved statistical significance, further statistical manipulation of the data did not seem justified.

The bulls used in this study generally would be considered highly fertile. However, bulls occasionally have been eliminated from the breeding stud because of lowered fertility. There has been no indication in any case of a change in hyaluronidase levels associated with this lowered fertility.

SUMMARY AND CONCLUSIONS

The possibility that hyaluronidase is a critical factor in the fertility of dairy bull semen was investigated. One hundred and eighty-seven semen samples were collected from 24 dairy bulls. Hyaluronidase assays on seminal plasma were made initially and after 24 hr. of incubation at 37° C. under toluene on all samples. Semen samples were classified for statistical analysis into three groups according to the number of first- and second-service cows bred per sample: (a) 10 to 19 cows bred; (b) 20 or more cows bred; and (c) total of *a* and *b*. Coefficients of correlation were obtained between fertility estimates and hyaluronidase con-

centrations in each group on a "total," "between bull" and "within bull" basis. None of these coefficients of correlation attained statistical significance. From these results it seems doubtful if any significant relationship exists between the hyaluronidase and fertility levels of semen from bulls of relatively high fertility when the semen is diluted at a rate of 1 to 100 or less.

REFERENCES

(1) CHANG, M. C. Effects of Testis Hyaluronidase and Seminal Fluids on the Fertilizing Capacity of Rabbit Spermatozoa. Proc. Soc. Exptl. Biol. Med., **66**: 51–54. 1947.

(2) JOHNSTON, J. E., AND MIXNER, J. P. Development of Hyaluronidase in Bull Semen. J. Animal Sci., **7**: 440–446. 1948.

(3) JOHNSTON, J. E., STONE, E. J., AND MIXNER, J. P. Hyaluronidase Relationships in Dairy Bull Semen. J. Dairy Sci., **32**: 574–579. 1949.

(4) KURZROK, R. The Clinical Evaluation of Hyaluronidase in Human Infertility. Am. J. Clin. Path., **18**: 491–498. 1948.

(5) KURZROK, R., LEONARD, S. L., AND CONRAD, H. Role of Hyaluronidase in Human Infertility. Am. J. Med., **5**: 491–506. 1946.

(6) LEONARD, S. L., PERLMAN, P. L., AND KURZROK. A Turbidimetric Method for Determining Hyaluronidase in Semen and Tissue Extracts. Endocrinol., **39**: 261–269. 1946.

(7) MIXNER, J. P., AND JOHNSTON, J. E. The Adaptation of a Standard Curve to the Turbidimetric Method of Assay of Hyaluronidase in Bull Semen. J. Dairy Sci., **32**: 570–573. 1949.

(8) ROWLANDS, I. W. Capacity of Hyaluronidase to Increase the Fertilizing Power of Sperm. Nature, **154**: 332–333. 1944.

(9) SALLMAN, B., AND BIRKELAND, J. M. Interrelationships of Spermatozoa Count, Hyaluronidase Titer and Fertilization. Am. J. Physiol., **182**: 271–279. 1948.

(10) SIEGLER, S. L. Clinical Evaluation of Hyaluronidase in Infertility. Paper presented at Third Annual Convention of Amer. Society for Study of Sterility. 1947.

(11) STONE, E. J., JOHNSTON, J. E., AND MIXNER, J. P. Live Spermatozoa Relationships and Fertility of Dairy Bull Semen. J. Dairy Sci., **33**: 442–448. 1950.

COMPARATIVE FERTILITY OF DILUTED BULL SEMEN TREATED WITH CALCIUM CHLORIDE COMPLEX STREPTOMYCIN OR DIHYDRO STREPTOMYCIN SULFATE[1, 2]

H. L. EASTERBROOKS, P. HELLER,[3] W. N. PLASTRIDGE AND E. L. JUNGHERR

Storrs (Connecticut) Agricultural Experiment Station, Department of Animal Diseases, University of Connecticut, Storrs

In 1949 Easterbrooks *et al.* (1) reported that the addition of 100 units (μg.) of streptomycin sulfate per milliliter of diluted bull semen increased fertility significantly on the basis of a split sample study when used in the routine operation of the Connecticut Artificial Breeding Association. Mixner (3), reporting simultaneously showed neither significant increase nor decrease when streptomycin calcium chloride complex and penicillin were both added at rates of 1,000 units per milliliter. One source of variation between these studies was that different compounds of streptomycin were used.

The purpose of the present investigation was to test dihydro (DH) streptomycin sulfate and streptomycin calcium chloride complex (CCC), for evidence of incompatibility with semen diluent buffers, as well as for their comparative effectiveness in increasing fertility rates.

EXPERIMENTAL

The problem was considered from two aspects. In the first phase of the study graduated concentrations of the two compounds varying from 100 to 1,000 units per milliliter in final dilution were added to phosphate and citrate buffers of various concentrations above, below and including those used routinely by artificial breeding organizations. After standing a few moments, a visible precipitate, calcium phosphate,[4] appeared in all tubes containing phosphate buffers to which streptomycin CCC had been added in excess of 100 units per milliliter. Because of the precipitation of these major components, the use of streptomycin CCC in phosphate containing diluents is considered contraindicated by the writers. No visible precipitation occurred at any level of either compound in any concentration of the citrate buffers.

Laboratory tests with the two compounds in citrate buffers revealed no measurable variation in respect to bactericidal activity or effect upon sperm livability.

The second phase of the work consisted of subjecting 14 ejaculates used by the Association over a period of 7 consecutive weekends in October and November, 1949, to a split sample study involving the two drugs at levels of 500 units per milliliter of diluted semen. This level was chosen in contrast to that previously

Received for publication June 3, 1950.

[1] Streptomycin used in this investigation was supplied by Merck and Co., Inc., Rahway, N. J.

[2] Supported in part by funds received under project NE1 of the Research and Marketing Act of 1946.

[3] Manager, Connecticut Artificial Breeding Association.

[4] The authors respectfully acknowledge the help of E. Lippincott who conducted the qualitative chemical analysis of the precipitate.

reported (1) because unpublished data indicate a level between 300 and 900 units to be optimum for increasing fertility; also 500 units per milliliter will destroy *Vibrio fetus* organisms which might be present in semen from infected bulls (4). The diluent used was composed of a 2.9 per cent sodium citrate and 0.6 per cent sulfanilamide in sterile distilled water buffer plus an equal volume of egg yolk. Comparisons were based on 60- to 90-day non returns (N.R.) to first service percentages. Five hundred eighty cows were inseminated with diluted semen containing DH streptomycin and 586 with diluted semen containing streptomycin CCC. First services only were used in compiling the data. The N.R. per cent for the DH streptomycin-treated semen was 71.6 as compared to 68.4 for the semen containing streptomycin CCC. The actual difference was 3.2 N.R. per cent and by a weighted analysis 2.3 N.R. per cent. These figures should be considered as precedent to those published by Easterbrooks *et al.* (2) in an abstract at an earlier date. No significance could be found associated with these data by statistical treatment; however, the data do suggest that DH streptomycin may be the drug of choice for addition to citrate containing diluents.

SUMMARY

Five hundred and eighty cows were inseminated with diluted semen containing 500 units of dihydro streptomycin sulfate per milliliter and 586 cows with split portions of the same semen containing 500 units of streptomycin calcium chloride complex. No statistical significance was associated with the 3.2 per cent non-returns increase for the dihydro streptomycin sulfate-treated group.

Streptomycin calcium chloride complex was found to be incompatible with phosphate buffers.

REFERENCES

(1) Easterbrooks, H. L., Heller, P., Plastridge, W. N., Junghere, E. L., and Elliott, F. I. Fertility Studies with Streptomycin in Bovine Semen. (Abs.) J. Animal Sci., 8: 639–640. 1949.

(2) Easterbrooks, H. L., Heller, P., Plastridge, W. N., Junghere, E. L., and Elliott, F. I. A Comparison of Two Streptomycin Compounds Used in Diluted Bull Semen. (Abs.) J. Dairy Sci., 33: 394–395. 1950.

(3) Mixner, J. P. The Effect of a Combination of Penicillin and Streptomycin in Semen Dilutors on the Fertility of Dairy Bulls. (Abs.) J. Animal Sci., 8: 642. 1949.

(4) Plastridge, W. N., and Easterbrooks, H. L. Unpublished data. 1949.

VOLUME XXXIII NOVEMBER, 1950 NUMBER 11

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BUTTER

O. F. HUNZIKER, SECTION EDITOR

776. Moderne dansk smørfremstillingsteknik. (Modern Danish butter manufacturing technique). H. HEDEMANN, Odense. Maelkeritidende, **63,** 25: 543–547. June 23, 1950.

The various conditions under which Danish butter has been produced have contributed toward the standard which must be maintained. The conditions which the Danish butter experts consider important for producing their ideal butter are considered. Through the cooperation of Danish milk producers, creamery operators, Danish merchants and English buyers, a certain type of butter has been developed. This type of butter must have a clean, pleasant aroma, a smooth-textured, waxy consistency, a good spreadability, pleasing color and salt content satisfactory to the majority of customers. The moisture and fat contents must comply with the law. After 14 d. in storage at 13° C. the butter must not be leaky, show mold or have deteriorated. The aroma of Danish butter is considered to be its most outstanding characteristic; it results partly from products produced during ripening by bacteria and partly from the feed of the cows. Some of the older creamery operators have suggested that feed may have given a certain spicy aromatic quality to the butter, a flavor which they cannot seem to duplicate now when scarcely any weeds or wild flowers grow in grass and clover fields. Possibly, weeds and wild flowers can influence, in a minor degree, the tendency toward a slightly higher aroma development during cream ripening. Milk from cows allowed to graze along roadsides had a slightly better aroma production than milk from cows on good pasture lands which were free from most weeds and wild flowers. During the past 25 yr., there has been marked progress in equipment for handling milk and cream for butter-making. It is unlikely that any roll-type churns are in use in Danish creameries today. The rollerless churn constructed of either stainless steel or wood has replaced the older types, but much still depends upon the man who operates the churn, regardless of how automatic it is. Reducing the tendency to oxidation in Danish butter still is a problem. In Sweden and Finland a special salt (A.I.V.) is used for the purpose of acid reduction, but this seems inadvisable for Danish butter. Any neutralizing agent, however sparingly used, might have an unfavorable influence upon the famed fresh aromatic flavor of Denmark's butter. When some butter appears to be more resistant to oxidation than some other it is not that the pH is too high but that the oxygen tension is lower than it ought to be.

The Danish butter industry has made marked progress in packaging butter in consumer-size packages of as fine quality as in the present wooden casks. The most efficient techniques for every phase of butter manufacture and marketing are being used to perpetuate the high reputation of Danish butter. G. H. Wilster

777. Continuous butter working apparatus. C. E. NORTH. U. S. Patent 2,521,398. 3 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, **638,** 1: 217. 1950.

Freshly churned cream, with the butter in granular form is introduced into 1 end of this device, which consists of a horizontal cylinder, in which rotates a shaft holding 4 spiral-shaped blades adjacent to the cylinder wall. The butter is fed in at such a rate that it continuously falls from the rotating blades and moves forward because of the slight spiral shape of the blades. Wash water is sprayed on the top of the cylinder and withdrawn with the butter through a drain at the end of the cylinder. R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

778. Ostens Gaeringsvarme (Fermentation temperature of cheese). Anonymous. Danish State Experimental Creamery, Hillerød. Maelkeritidende, **63,** 24: 524. June 16, 1950.

In order to measure the amount of heat produced in cheese during ripening, 2 identical wooden cabinets were built and placed in a room where the temperature was controlled automatically. Each cabinet was provided with a contact thermometer, a relay and a built-in lamp for supplying the necessary heat. The cheese was placed in 1 of these cabinets. The amount of heat produced in the cheese then could be determined by noting the difference between the amount of electricity consumed in the cabinet holding the cheese and in the empty cabinet. The cabinets were at 12° C., about 2° C. higher than in the room in which the cabinets were placed. A slight difference in the amount of electricity consumed in each cabinet, even when both were empty, made it necessary to measure the electricity used in each cabinet before and after the test, for periods of 3–5 d. Periods of 5–13 d. were allowed for the measurement of electricity consumed during the test. Since cheese containing mold had been shown to produce more heat than cheese without mold, Roquefort cheese was chosen for some of the experiments. The greatest amount of heat was produced during the period of greatest mold growth, about 8 d. after pricking.

Six cheeses with a combined weight of 20 kg. were used. The results showed a variation from 2.5–5.0 cal./kg. of cheese for each 24-hr. period. The marked variation in the heat could have been due to the method of measuring. Danish, Swiss and Gouda cheese, used for a similar experiment, were 5–6 wk. old when tested. The amount of heat produced in this cheese was not enough to be significant, since it was only about 0.5 cal./kg. of cheese for each 24-hr. period.

G. H. Wilster

779. Process of making cheese. C. Tone (assignor to Armour and Co.). U. S. Patent 2,520,183. 4 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, **637,** 5: 1389. 1950.

Milled curd is filled directly into containers lined with an air-impervious wrapper and sealed. The cheese is kept in the container until cured.

R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

780. Method and apparatus for gassing the contents of cans. W. M. Tomkins (assignor to Continental Can Co.). U. S. Patent 2,518,100. 12 claims. Aug. 8, 1950. Official Gaz. U. S. Pat. Office, **637,** 2: 505. 1950.

Powdered or ground food materials such as milk, eggs, coffee, etc., packed in open-end cans, are gassed with nitrogen or other gas, prior to sealing the cans. A bell-shaped baffle is lowered into the center of the packed can until it is about 1 in. from the bottom. The gas is admitted into the bell and as it flows down through the powder and up on the outside it displaces the air. The bell is withdrawn and the can sealed.

R. Whitaker

781. Method of heating food products in sealed containers LaV. E. Clifforn, G. T. Peterson and J. M. Boyd (assignors to Continental Can Co.). U. S. Patent 2,517,542. 9 claims. Aug. 8. 1950. Official Gaz. U. S. Pat. Office, **637,** 2: 364. 1950.

Liquid food products, such as evaporated milk, are sterilized rapidly by rotating the cans end over end about an axis located outside the cans and at such speed that the air bubble in the cans moves about half way up the sidewall of the can from the end and then returns to the end during 1 complete rotation of the can. This specific movement of the air bubble provides turbulence of the contents, resulting in rapid heating and cooling. R. Whitaker

782. Milk chocolate. B. K. Hallquist and L. O. J. Campbell (assignors to Svenska Mjolkprodukter Aktiebolag). U. S. Patent 2,519,833. 6 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, **637,** 4: 1181. 1950.

Spray-dried milk powder having an average grain porosity of not over 10% by volume is used with cocoa, fat and sugar to make milk chocolate.

R. Whitaker

783. Milk proteins and lactose from dried skimmilk. S. R. Hoover and E. L. Kokes, Eastern Regional Research Lab., Philadelphia 18, Pa. Ind. Eng. Chem., **42,** 9: 1910–1912. 1950.

The recovery of protein and lactose from dried skimmilk by a countercurrent extraction process with water acidified with HCl was demonstrated. The dried skimmilk was leached in 4 stages with 5 times its weight of 0.25% NaCl at pH 4.1 to give a soluble extract of 14% lactose, whey salts

and added salt, riboflavin and minor constituents. The extracted solid consisted of the milk casein and heat-coagulable whey protein and contained 86% protein, 2% ash and 0–3% lactose. Lactose could be recovered by the usual processes of evaporation and crystallization. B. H. Webb

784. Process of concentrating milk. J. F. KOWALEWSKI and G. SPERTI (assignor to Institutum Div. Thomas Foundation). U. S. Patent 2,520,939. 5 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, **638,** 1: 99. 1950.

Pasteurized homogenized milk is concentrated by freezing to a slush and separating the ice from the concentrated milk solids. R. Whitaker

785. Shortening. G. C. NORTH, A. J. ALTON, and W. C. BROWN (assignors to Beatrice Creamery Co.). U. S. Patent 2,520,954. 1 claim. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, **638,** 1: 103. 1950.

A powdered shortening in which the fat particles are surrounded by a blend of non-fat milk solids and egg solids is described. R. Whitaker

786. Casein manufacturing process. P. F. SHARP (assignor to Golden State Co.). U. S. Patent 2,519,606. 5 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, **637,** 4: 1125. 1950.

Skimmilk is coagulated with a coagulant and at the same time an inert gas is injected in such a manner as to cause the coagulated casein to form a foam. The foam is floated off the whey and dried after washing with a water spray.

R. Whitaker

787. Process for preparing casein. J. A. REYMIERS (assignor to Amino Acids, Inc.). U. S. Patent 2,518,493. 2 claims. Aug. 15, 1950. Official Gaz. U. S. Pat. Office, **637,** 3: 737. 1950.

A weak acid is added to skimmilk with continuous agitation at a temperature of between 2 and 16° C. The fine, flocculent pptd. casein is separated from the whey, washed, dried and used as a food supplement. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

788. Bactericidal efficiency of quaternary ammonium compounds. C. T. BUTTERFIELD, E. WATTIE and C. W. CHAMBERS, Pub. Health Service, Cincinnati, O. Pub. Health Reports, **65,** 33: 1039–1056. Aug. 18, 1950

Bactericidal efficiency of 11 quaternary ammonium compounds used as active agents of 40 commercial sanitizers was determined by the method presented. Tests also were made for residual active agents. The nature of the water in which the compound was dissolved definitely influenced its germicidal efficiency. Interference induced by different waters occurred almost instantaneously and did not increase with time. This interference was reduced in some cases after removing dissolved gasses by boiling or aeration. Presence of even small amounts of soap or other detergents usually reduced action markedly. The higher the temperature of the solution used, within the usual range of 12–46° C., the more effective the toxic action. Changes in pH of the solution affect its activity but the direction of the change varied with the compound. A decrease in pH increased potency of some compounds and reduced that of others. Very unreliable results were obtained with test procedures available for measuring active bactericidal content of residuals. Because of this, it is essential to make bacterial examinations with the product under conditions in which it is to be used.

D. D. Deane

789. Kan Syrningsvanskeligheder nu Effektivt Afhjaelpes? (Can starter failures be effectively prevented?) A. J. OVERBY. Maelkeritidende, **63,** 24: 526. June 16, 1950.

To some extent it has been possible to prevent starter failures due to bacteriophage by making a fog in the creamery rooms, using a 5% solution of hypochlorite disinfectant. However, since bacteriophage may be present in milk received daily at the creameries this method is only a partial control. To change from 1 source of starter culture to another has been of benefit. Two creameries which had experienced regular starter failures were able to prevent failure when a starter from the Utterslev creamery was used. Some bacteriophages were isolated by the dairy laboratory from a starter culture which had become inactive and these were found to possess specific characteristics. Some of the bacteriophages attacked the organisms in only 1 of the starters to which it was added. Some strains of bacteriophage were active against 2 of the starters, while others were active against 3 or 4 starter cultures. Commercial cultures, A, B, and C were affected by 6 of the 11 bacteriophages, while culture D was affected by 10 of these. The starter from the Utterslev creamery was not affected by any of the isolated bacteriophages. The starter from the Utterslev creamery had been used with good results for 21 yr. This starter was highly aromatic, with 20% of the isolated bacteria being betacocci. Of the streptococci present, 65% were good acid producers. This percentage was higher

than had commonly been found in starters and this might be the reason for this starter being particularly resistant to the action of the bacteriophage. G. H. Wilster

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

790. Browning of ascorbic acid in pure solutions. M. P. LAMDEN and R. S. HARRIS, M.I.T., Cambridge, Mass. Food Research, **15,** 1: 70–89. Jan.–Feb., 1950.

Ascorbic acid heated in the presence of citric acid in pure solution underwent deepening of color. Other common organic acids cause a similar action. Increase in color and destruction of ascorbic acid did not depend on the presence of oxygen, but color was a function of the initial concentration of ascorbic acid. Furfural was obtained in the heating of ascorbic acid and citric acid at the boiling point but was not noted with dehydro ascorbic acid. These and other findings are discussed in their relationship to the browning reaction, especially as it pertains to citrus products. F. J. Doan

791. Process for recovery of lactalbumin. G. J. STREZYNSKI (assignor to the DeLaval Separator Co.). U. S. Patent 2,520,615. 19 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, **637,** 5: 1500. 1950.

Whey, having a pH of between 4 and 7 is heated to a temp. of about 165–190° F. to ppt. the albumin. After cooling to not lower than 130° it is fed into a centrifuge from which the albumin is continuously discharged from the periphery and the whey and lactose from the inner part of the bowl. R. Whitaker

792. Babcock test mixer. G. F. MASSEY. U. S. Patent 2,520,556. 5 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, **637,** 5: 1486. 1950.

A mechanical agitator mixes the contents of Babcock fat test bottles. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

793. Butterfat samples as affected by weigh can design. V. SCHWARZKOPF, Lathrop Paulson Co., Chicago, Ill. Sou. Dairy Prod. J., **48,** 1: 44, 46, 90–91. July, 1950.

Samples of milk taken further from the strainer in rectangular weigh cans when the milk is not properly agitated, are richer. Ten to 30 sec. are required for agitation by air or mechanically when it is necessary. Sufficient mixing of the milk may be obtained from its own velocity alone in narrow weigh cans of medium length with not less than 1.5 in. pitch/ft. toward the outlet valve, with 3/16 in. perforated strainer set high and kept free from baffles or louvers, and having a minimum depth of about 8 in. at the front. The use of a blender to convert the small streams from the strainer into a heavy body of milk is recommended. F. W. Bennett

794. Using an in-the-line milk filter as a sediment tester. Anonymous. Milk Dealer, **39,** 11: 42–43. Aug., 1950; Sou. Dairy Prod. J., **48,** 3: 129–130. Sept., 1950.

A standard in-the-line milk filter equipped with sediment disc to take an accurate last minute sediment test on all milk handled in the plant is being used successfully at the Brook Hill Certified Milk Farms at Genessee Depot, Wis. The filter is made up of a series of stainless steel horizontal plates with cotton media, the suspension of which is controlled in the milk flow by stainless steel spiders. All milk must flow upward through this series of parallel pads which is placed between the dump tank and the cooler. Because of the upward flow of milk, the tops of the discs are clean, indicating that no foreign matter passes through to the finished product. The bottom, or upstream side of the pad, however, indicates whether any foreign matter has been introduced into the milk during either the milking or plant handling. C. J. Babcock

795. Plastic coating protects dairy equipment surfaces against corrosion. Anonymous. Milk Dealer, **39,** 11: 51, 58. Aug., 1950.

Corrosite is a plastic which combines chemically with the metal surface it covers and, because it is non-porous, results in an anti-corrosive, acid-resistant surface that does not crack or peel. It hardens rather than deteriorates with age. Its use as a coating for pasteurizing, bottle-washing and bottle-filling equipment for milk at the Walker-Gordon Lab. Co., at Plainsboro, N. J., has demonstrated the practicability of plastics for the dairy industry in protecting metals exposed to daily washings, caustics, detergents and lactic acid. The successful use of vinyl film ("corrosite") on cement feed troughs on dairy farms also is reported. C. J. Babcock

796. Size refrigerant lines for low cost. Anonymous. Power, **94,** 9: 91–93. Sept., 1950.

Cost of suction and discharge piping usually is a small part of the total outlay for a refrigeration plant, but undersizing piping can increase annual operating costs from 5–30% or more depending

on the pressure drop of the system. Piping of proper size will cause only a moderate pressure drop and will cost little more than undersized piping.

Excessive pressure drop in the suction line causes superheating of the suction vapor and causes the compressor to operate at a lower suction pressure. Low suction pressure causes poor operating economy and reduces the compressor capacity.

Undersized discharge lines from the compressor to the condenser causes a decrease in capacity and an increase in power input. Refrigerant pipe resistance depends upon the compressor capacity, vapor velocity, pipe length, number of bends and the pipe size. A table presents safe velocities for suction line and discharge line for ammonia, Freon-12, methyl chlorine and carbon dioxide. H. L. Mitten, Jr.

797. 19 ways operators ruin refrigeration equipment. G. HOLMAN. Operating Eng., **3,** 7: 36–37. July, 1950.

A list of the 19 most common mistakes is presented with a brief explanation and suggestion as to prevention. H. L. Mitten, Jr.

798. Hot tips on cold plants. H. WELCH. Operating Eng., **3,** 8: 36–37. Aug., 1950.

When welding flange joints and fittings, remove valve bonnet to vent line to atmosphere. Oil vapors in receivers and shell and tube condensers are dangerous when warm. Ammonia and oil vapors are explosive when mixed so that 17–27% of the total is ammonia.

Check valves should be installed on all compressor discharge lines directly above the unit. The angle-type is better than the horizontal because it operates noiselessly and cannot be jacked open. Pipelines should be free to expand and contract with temperature changes. They should also be accessible at all points for inspections.

Corroded bolts on flange joints should not be tightened while liquid lines are under full pressure. Corroded flange bolts may be removed from line under low pressure without shutting down, provided a C-clamp is placed on the flange and the bolts are cut and replaced one at a time.

Inspect and test controls and gauges regularly. The operator should be acquainted thoroughly with the local codes governing type of plant in his charge. Each member of the operating crew must be instructed in the use of safety equipment for emergencies. H. L. Mitten, Jr.

799. How to apply mineral wool heat insulation. P. W. SWAIN, Power, 330 W. 42nd St., New York, 18. Power, **94,** 9: 86–90. Sept., 1950.

Application of mineral wool insulation is presented pictorially with brief, to-the-point descriptions. The applications presented include between-masonry walls, between-metal sheets, wire-impaled blankets, types of supports, nail fastening, expansion joints, cement on brick wall, covers for tanks, and pipe and fitting covers.

H. L. Mitten, Jr.

800. Grout—and here's how it's used. J. J. O'CONNOR, Operating Engineer, Albany, N. Y. Operating Eng., **3,** 7: 32–33. July, 1950.

Grouting has an advantage over shimming or wedging for machine bases because it takes up any uneveness in both concrete foundation and machine base so that the machine will rest firmly on the whole foundation rather than on a few points.

In setting a machine, pour the foundation and set the anchor bolts. See that foundation is clean and wet before machine is set in place and grout is poured. The best grout mix for most jobs is 1 volume cement to 2 volumes of sand. Water should be held to 6 gal./sack of cement. Shrinkage of grout increases and strength decreases as amount of mixing water increases. Before pouring, let grout set 2 hr. after it is mixed, then remix without adding water and pour immediately. This procedure will reduce settling. Use 1–1.5 in. of grout under bed plate edge. Pour through grout holes in base plate or under plate between base and forms. Special bases may require air venting. H. L. Mitten, Jr.

801. Being practical about oil viscosity. Anonymous. Power, **94,** 9: 117. Sept., 1950.

In sleeve bearings the journal load is supported by a continuous oil film. Maintenance of the film under varying speeds, loads and temperatures is the deciding factor in lubricant selection. With ball or roller bearings the oil film is not continuous because of the high unit pressure between the rolling elements and the races.

Most industrial designs provide large safety margins for oil viscosities so that machines can meet the practical variations in operating conditions. A chart presents recommended oil viscosities for various loads and operating speeds.

H. L. Mitten, Jr.

802. Relief valve. R. HINRICHES (assignor to Tri-Clover Machine Co.). U. S. Patent 2,521,166. 3 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, **638,** 1: 157. 1950.

A spring-loaded pressure relief valve fitted into a standard sanitary cross fitting is described.

R. Whitaker

803. How good maintenance worked in a dairy. G. GRIFFEL. Operating Eng., **3,** 8: 20–22. Aug., 1950.

Standby spares are kept for pumps, motors and drives. Spares for the main pieces of processing equipment cannot be justified because of cost.

The heart of the maintenance program is the report which provides for a request for maintenance by the production department and a report on parts, labor and comments by the maintenance department. These reports serve as guides for equipment replacements, budgets and maintenance scheduling.

Maintenance men can best be trained on the job. They should be permitted to go over each new piece of equipment with the manufacturer's representative. The shop should contain a supply of replacement parts, a lathe, drill press, power hacksaw, grinder, welding equipment and other portable tools. Special test rigs may be built and installed in the shop.

Chief engineer's job is to schedule maintenance, train men, requisition new equipment and sell value of maintenance to management.

H. L. Mitten, Jr.

Also see abs. no. 777, 781, 814, 815.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

804. Selection and inplant training of production men in the industry. C. E. KREY, Sou. Dairies Inc., Washington, D. C. Ice Cream Trade J., **46,** 8: 56, 58. Aug., 1950.

A college student committee was set up to supervise training of a number of promising young men in college and those graduated. Each member is responsible for visiting certain allotted schools during the year and interviewing all dairy students interested in summer jobs or graduates seeking employment. Accepted students are placed on a 13-wk. summer schedule. A college graduate, new to the company, is put in a 60-wk. schedule designed to include every phase of the production work and prepare him as a supervisor of some department. With this system management is confident that alert capable and responsible men are supporting present production superintendents. W. H. Martin

805. Trends and influencing factors in consumption of dairy products. L. SPENCER, Cornell Univ., Ithaca, N. Y. Sou. Dairy Prod. J., **48,** 3: 36–38, 40. Sept., 1950.

We now are eating more fruits and vegetables other than potatoes, more eggs, more dairy products, more fats and oils, much less bread and cereals and much less potatoes per capita than were consumed 40 yr. ago. In 1949 we consumed 9% more of all dairy products except butter than the average for 1935–39. There was a 38% decrease in the consumption of butter.

The increase in the consumption of oleo accounts for less than half the loss in the consumption of butter. The reduced consumption of bread and other foods on which to spread butter or oleo and the increased consumption of vegetables on which salad oil or dressings are used also are important factors in reducing butter consumption.

The quantities of dairy products the markets will absorb will be affected mostly by (a) trend and characteristics of population, (b) consumer incomes in relation to cost of living, (c) relative retail prices of foods, (d) developments in production and distribution and (e) consumer acceptance.

On Sept. 15, 1949, the average retail prices of dairy products had increased 85.3% and the average prices of all foods had increased 104.2% as compared with the 1935–39 average.

F. W. Bennett

806. Examining ice cream distribution costs. E. R. HUBBARD, Hubbard, Dilley and Hamilton, New York, N. Y. Ice Cream Trade J., **46,** 8: 34, 35, 86–88. Aug., 1950.

The magnitude of distribution costs, as well as their alarming increase—far in excess of the increased cost of labor involved—has prompted a study designed to increase efficiency of marketing and reduce distribution costs. A thorough knowledge of present distribution costs, obtained by an adequate accounting procedure, will evaluate costs relative to the various distribution functions performed. Wastes in internal distribution should be eliminated and distribution cost data from other manufacturers should be compared. When a logical system of distribution cost control is installed and operating, success is determined only by management studying facts it reveals and taking action where required.

W. H. Martin

HERD MANAGEMENT

H. H. HERMAN, SECTION EDITOR

807. Milking machine. A. E. ANDERSON. U. S. Patent 2,518,589. 10 claims. Aug. 15, 1950. Official Gaz. U. S. Pat. Office, **637,** 3:761. 1950.

The novel feature of this milker is a manifold below the udder, so arranged that rearward counterpoise is provided when the front teat cups are

pulling and a forward counterpoise when the rear cups are pulling. R. Whitaker

808. Overhead carriage and hoist for milk cans. B. V. CULP. U. S. Patent 2,520,238. 2 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, **637,** 5: 1403. 1950.

A motor-driven hoist mounted on an overhead rail facilitates placing cans of milk in a cooling tank, etc. R. Whitaker

809. Gutter side wall cleaner for dairy barns. C. A. GILBERT. U. S. Patent 2,519,645. 3 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, **637,** 4: 1134. 1950.

The side walls of barn gutters are scraped clean by 2 beveled scraper blades and held against the side walls by a coil spring in a tube separating the 2 blades. A handle attached to the tube facilitates movement of the scraper along the gutter. R. Whitaker

810. Animal confining means. E. S. DIEHL. U. S. Patent 2,520,385. 7 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, **637,** 5: 1440. 1950.

A stanchion for cows has a device for adjusting the size to fit the animal. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

811. Weight changes in packaged ice cream at cabinet temperatures. J. A. MEISER, JR., Mich. State College, East Lansing. Sou. Dairy Prod. J., **48,** 3: 26–27, 52, 54, 56. Sept., 1950.

Ice cream packaged in untreated pt. paper containers lost 16–29 g. during 12 wk. of storage at cabinet temperatures. Losses in weight in the individual packages were at a practically constant rate. Containers constructed of the heaviest paper and possessing the minimum surface area resisted desiccation of the ice cream to the greatest degree.

Containers coated with paraffin permitted losses in weight of only 0.3–6.3 g./pt. in 12 wk. Containers paraffined on both the inner and outer surfaces offered the greatest protection. Coating the containers with glassine or vinylite also retarded moisture loss. F. W. Bennett

812. High serum solids content in quality ice cream. A. J. GELPI, JR. and F. I. DOWDEN, La. State Univ., Baton Rouge. Sou. Dairy Prod. J., **47,** 6: 42–44, 47–48. June, 1950.

In the experiment reported, mono- and diglycerides with high grade gelatin retarded crystallization of lactose in mixes of high serum solids content, improved the whipping ability, produced a smoother and richer tasting finished product, decreased shrinkage and enabled the ice cream to withstand heat shock to a remarkable degree. The possibility of manufacturing a highly satisfactory ice cream containing 14.5–16% serum solids stabilized with 0.2% gelatin and 0.2–0.25% monostearate or other mono- or diglycerides was demonstrated. Ice cream from such mixes may be drawn at higher overrun and still meet legal requirements. F. W. Bennett

813. Method of making ice cream layer cake. G. A. ZABRISKIE and F. ZABRISKIE (assignors to Airline Foods Corp.). U. S. Patent 2,517,756. 7 claims. Aug. 8, 1950. Official Gaz. U. S. Pat. Office, **637,** 2: 418. 1950.

Several thin rectangular wafers or crackers are held in notches on the sides of the carton, so as to make a series of compartments or spaces of equal size. Soft ice cream is filled into the spaces, the carton closed and placed at a low temperature to harden. R. Whitaker

814. Mixing and scraping machine, especially adapted for use as ice cream freezer. P. CARPIGIANI. U. S. Patent 2,519,543. 3 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, **637,** 4: 1108. 1950.

A cylindrical container is caused to rotate by a shaft extending downward through the container from an overhead drive. A second rotating shaft between the drive shaft and container wall causes whipping and ice removal by a blade which is so formed that all inside surfaces of the container are scraped. R. Whitaker

815. Apparatus for filling containers with ice cream, with cutter means and container controlled circuit breaking means for stopping the apparatus. R. M. HESSERT. U. S. Patent 2,517,107. 4 claims. Aug. 1, 1950. Official Gaz. U. S. Pat. Office, **637,** 1: 145. 1950.

This device, installed in an ice cream cabinet and driven by an exterior motor, mechanically packs pt. or qt. containers from bulk containers. The bulk container is inverted on a platform, a rotating blade cuts off small pieces which drop down into a screw conveyor which packs the pieces into the retail packages as they are sold. R. Whitaker

816. Detachable cover and service bar for frozen foods containers. W. S. FREDENHAGEN and M. S. SCHMIDT. U. S. Patent 2,518,134. 4 claims.

Aug. 8, 1950. Official Gaz. U. S. Pat. Office, **637,** 2: 513. 1950.

This device is designed to convert a conventional ice cream cabinet into a display cabinet for self service stores or into a soda bar. It is placed on top of the cabinet after removing the sleeve covers. Wells for dispensing syrups, nuts, flavors, etc. provided, as well as sliding doors and a counter. R. Whitaker

817. Method of making ice cream sandwiches and to ice cream sandwiches and wrappers therefor. L. D. OVERLAND.. U. S. Patent 2,521,403. 11 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, **638,** 1: 218. 1950.

Two edible wafers are held spaced and parallel by a paper wrapper. Ice cream from the freezer is filled into the space and the completed sandwich hardened. R. Whitaker

818. Precut ice cream cake and method of making same. F. ADAMS. U. S. Patent 2,520,522. 11 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, **637,** 5: 1477. 1950.

Pie-shaped pieces of ice cream are frosted on the sides and top and fitted together to form a complete circular unit. R. Whitaker

819. Ice cream dispensing package. J. S. MILLER. U. S. Patent 2,519,271. 4 claims. Aug. 15, 1950. Official Gaz. U. S. Pat. Office, **637,** 3: 942. 1950.

Ice cream is pushed out of the top of a cylindrical container by a second cylinder which telescopes into the bottom of the top container. R. Whitaker

820. Costing ice cream mix. A. SEARLES, JR., Cornell's Dairy Prod., Endicott, N. Y. Ice Cream Trade J., **46,** 8: 44, 45, 89. Aug., 1950.

After selling surplus milk on the market for 60–70% of cost, Cornell's Dairy decided to convert it to ice cream mix. The making of mix does not increase property tax, insurance, band cost or even depreciation of equipment, and general overhead is minimized if large scale production is not necessary. A form is completed for each batch made, listing types and amounts of ingredients used, labor costs and any overhead. Another form completed monthly, lists batches of mix made, value of mix, total labor charges and mix on hand; this acts as a check on the daily mix reports. The difference between gross sales and costs of production is only one profit; the hidden profit is the difference between mix sales, on a butterfat basis and the price the surplus butterfat would have brought if mix wasn't being manufactured. W. H. Martin

821. Gas station sites with ice cream stores. Anonymous. Ice cream Trade J., **46,** 8: 30, 31, 95. Aug., 1950.

The Friendly Ice Cream Corp. has leased a retail outlet built and owned by the Atlantic Refining Co. An attractive colonial-type building is situated next to the gasoline stations. This arrangement by which gas stations and ice cream stores cooperate to the mutual benefit of both is expected to increase. W. H. Martin

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

822. Effect of thyroxine on oxygen consumption and heart rate following bile duct ligation and partial hepatectomy. B. GRAD and C. P. LEBLOND, McGill Univ., Montreal, Can. Am. J. Physiol., **162,** 17–23. July, 1950.

In studies on male albino rats, these authors present confirmaiton of previous studies in which it has been maintained that the liver excretes and inactivates excess amounts of thyroid hormone in the body. V. Hurst

823. Influence of environmental temperatures and thyroid status on sexual development in male mouse. M. MAQSOOD and E. P. REINEKE, Mich. State Coll., East Lansing. Am. J. Physiol., **162:** 24–30. July, 1950.

Groups of young male mice were maintained at either 24 or 30° C. They were fed varying levels of thyroprotein or thiouracil and at the end of 3 or 4 wk. the animals were sacrificed and the testes and seminal vesicles weighed and sectioned.

Thiouracil fed alone depressed the weight of the testicles and seminal vesicles at both 24 and 30° C. Thyroprotein fed in dosages causing mild hyperthyroidism increased both testicular and seminal vesicle weight at 24 and 30° C. The optimal stimulating dosage of thyroprotein at 24° was 10 times the optimal stimulating dosage at 30°. Severe hyperthyroidism caused decreased testicular and seminal vesicle weights at both 24 and 30°. The dosage range of thyroprotein which increased testicular size at 30° was considerably more restricted than the range of dosages causing stimulation at 24°. Histologically, mild hyperthyroidism stimulated spermatogenesis and caused epithelial proliferation of the mucosa of the seminal vesicles, whereas hypothyroidism produced the opposite effects. V. Hurst

what is Directed Flow?

In Mojonnier Vats circulation of hot water, steam, or cold water is *directed* by small channels, seam-welded to the entire side and bottom surfaces of the inner shell. These channels force all the heat exchange medium against every square inch of surface at high velocity. By-passing and sluggish flow can not occur because the path of the medium is *fixed* and *directed*. Complete contact and high velocity result in exceptionally high heat transfer rates.

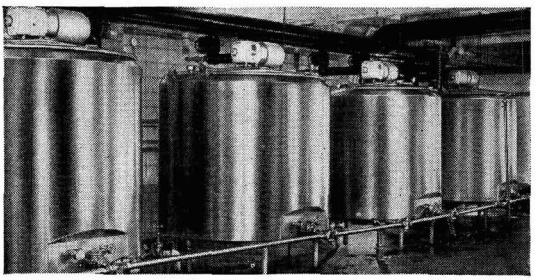
Mojonnier Vats also feature:

1 Specially Designed Agitators
pitched so as not to incorporate air yet gently, thoroughly agitating product to insure fast heat transfer rates.

2 Sturdy Construction
to withstand high pressures.

3 Sanitary Power Units

MOJONNIER BROS. CO.
4601 W. OHIO ST., CHICAGO 44, ILL.



Four 1,000 gallon Mojonnier Zone-Control Vats processing buttermilk. Borden Dairy Co., Detroit.

For better vat value, specify:

Mojonnier ZONE-CONTROL VATS

Globe, Cerelose and Puritose are registered trade-marks of Corn Products Refining Co., New York, N. Y.

Contributes to the quality of your products...

ICE CREAM manufacturers have found Puritose high conversion Corn Syrup performs better ...is of consistently uniform high quality.

In the manufacture of ices and sherbets Cerelose has won much favor... producing smoother products with a minimum of ice crystals.

Full technical service, no obligation

CORN PRODUCTS REFINING CO., 17 BATTERY PLACE, NEW YORK

Copies of the 20-Year Index covering Volumes I to XX, inclusive are available at the following prices:

MEMBERS	
Cloth Bound	$2.35
Paper Bound	2.00
NON-MEMBERS	
Cloth Bound	$5.50
Paper Bound	5.00