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THE KEEPING QUALITY, SOLUBILITY, AND DENSITY OF POWDERED WHOLE MILK IN RELATION TO SOME VARIATIONS IN THE MANUFACTURING PROCESS. II. SOLUBILITY AND DENSITY[1]

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In judging the quality of whole milk powder, first consideration should be given to palatability. Of almost equal importance for proper consumer acceptance is the solubility of the powder and the ease with which it may be reconstituted.

REVIEW OF LITERATURE

Solubility. There are many reports which show that the temperature-time relationship of preheating of the milk has a direct relationship to the solubility of the resulting powder. However, it should be borne in mind that solubility is relative and depends upon the solubility test used.

Crossley and Johnson (4) concluded that the solubility of milk powder was least impaired when the preheating temperature did not exceed 159° F. for 20 seconds. For temperatures of between 150 and 163° F. for 20 seconds, followed by a 3 to 5 minute holding period at a slightly lower temperature, the mean solubilities found were nearly a constant value only just below that obtained at 159° F. Even at 167° F. the reduction in solubility was not of commercial significance. Hollender and Tracy (6) compared the solubility indices of powders made from whole milk preheated at 150, 170 and 190° F. for 30 minutes and found that the least soluble powders were those made from the milk preheated at 190° F. However, Crossley (3), by using the short-time preheating method, found little loss in solubility in powder made from milk preheated at 190° F. as compared to that in powder made from milk preheated at 165° F., and the very small loss of solubility appeared to be more than offset by the increased keeping quality with respect to flavor.

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Wright (20), in studying milks of concentrations varying from 20 to 50 per cent total solids and preheated at 194, 203 and 212° F., found that for a given temperature, the higher the concentration of the milk the more readily was the protein rendered insoluble. He reported that when milk powder containing 2.5 per cent moisture was exposed to temperature-time relationships ranging from 212° F. for 10 hours to 282° F. for 40 seconds, 50 per cent of the casein was rendered insoluble. In another experiment, Howat and Wright (7) heated spray-dried whole milk powder for 6 hours at 221 to 230° F. Sixty per cent of the protein was insoluble when the powder was reconstituted at 68° F. Crossley and Johnson (4) found that prolonged exposure of milk powder to hot air currents, even at 194° F. or less (in bag dust collectors), resulted in significantly lowered solubility.

In 1931, Lampitt and Bushill (10) reported that the decrease in solubility of powders of comparable moisture content (less than 6 per cent moisture) was almost negligible when stored at room temperature and very much slower than at 86° F. Lea and Smith (12) concluded that changes in solubility due to storage at ordinary temperatures for a number of years would be exceedingly slight with gas-packed powders at reasonably low moisture contents. Lea *et al.* (11) found that a moisture content of 2.2 per cent had no appreciable effect on solubility.

Hollender and Tracy (6) stated that when powders were stored at temperatures below 68° F., variations in the moisture content between 2.32 and 5.39 per cent had no significant effect on the solubility of the various powders studied. They observed that those samples which discolored during storage became less soluble, that 5 per cent moisture content is about the critical point and upper limit for samples to be stored at room temperature without discoloration, and that at temperatures of 68° F. or above discoloration takes place regardless of the moisture content. They concluded that conditions favorable for brown discoloration and decrease in solubility of milk powders were preheating the milk at 190° F. for 30 minutes, a moisture content of 5 per cent or higher and storing the powder at temperatures of 68° F. or higher.

Wilster *et al.* (19) listed small particle size, small, uniform-sized fat globules and care not to overheat the powder in the drying chamber as favorable factors for good solubility of whole milk powder.

Density and particle size. Webb and Hufnagel (18) found that the density of milk powder could be increased by increasing the degree of preconcentration. Mook (15) described a patented method in which the milk was preheated at 160 to 170° F., condensed to 35 to 40 per cent total solids, and then superheated at not less than 170° F. and preferably at 180 to 210° F. until slight coagulation occurred. The milk powder from this milk was reported to be of greater density and to reconstitute to a fluid with four to eight times the viscosity of reconstituted milk from powders produced by the usual procedures.

Miyawaki (14) found that milk powder made from milk sprayed without preheat treatment had more and larger air cells than the powder made from

preheated milk. Coulter and Jenness (2) reported that the removal of foam from the condensed milk prior to spraying reduced the amount of entrapped air in the powder particles and that, on the other hand, whipping air into the precondensed milk resulted in greater numbers of air cells and air cell clusters in the powder particles. Studying the effect of preheat treatment of milk upon the density of the milk powder, Stamberg and Bailey (16) found that the densest powder was made from milk receiving the most severe heat treatment. The preheat treatments studied were: 150 and 200° F., and 150 and 200° F., followed by superheating. The holding time during preheating was constant for all samples, but it was not definitely recorded.

Hunziker (8) stated, "It has long been observed that an increase in the concentration of the fresh milk is accompanied by an increase in the particle size of the resulting spray powder." According to Hetrick and Tracy (5) and Hollender and Tracy (6), large-size powder particles were obtained by using a low homogenization pressure, low spray pressure and low spray temperature.

EXPERIMENTAL PROCEDURE

The manufacturing and storage procedures used were given in an earlier publication (13).

Solubility test. The solubility indices of the powders were determined by the method of Cone and Ashworth (1) at intervals of 24 hours and 1, 2 and 6 months following manufacture.

It was noted that powder made from the 40 per cent total solids concentrate had a higher solubility index than the powder made from the 20 per cent total solids concentrate. A portion of the original unconcentrated milk for powders no. 108 and 109 was homogenized and subjected to the solubility test. The homogenized concentrated milk from which powders 94 to 109, inclusive, were made also was subjected to the solubility test and the results compared to the solubility index of the fresh powder. In addition, the homogenized concentrates from which powders no. 108 and 109 were made were examined microscopically to determine if any differences in homogenizing efficiency existed due to differences of concentration of the milk. The per cent of fat globules greater than 5 μ in diameter in given fields under the oil immersion objective of the microscope was estimated by Cone and Ashworth (1) by use of a previously calibrated Whipple eyepiece.

Density and particle size. The apparent density was determined on the powders stored at 45° F. for 6 to 7 months by using the following procedure: Light mineral oil (no. 1) manufactured by the Standard Oil Company was employed as the displacing medium. Eight per cent Babcock milk test bottles were found to serve excellently as improvised pycnometers (17). The Babcock test bottles were carefully calibrated at 25° C. with freshly-boiled distilled water at three different levels between the 6.5 and the 8.0 per cent marks on the necks of the bottles. The level of the bottom of the meniscus was estimated to 0.1 of the 0.1 per cent divisions. The density of the oil was determined by use of Hubbard-Carmick specific gravity bottles and by use of the above-mentioned calibrated

Babcock test bottles. In both cases the average value obtained was 0.8407 with a standard deviation of $\pm$ 0.00001. However, the figure 0.841 was used in the density determinations of the powder since the reading of the meniscus of the oil level to 0.1 of a 0.1 per cent division was an estimation to 0.002 ml. From 7 to 11 g. of the powder were introduced into each calibrated Babcock test bottle by use of a 2-inch glass funnel from which the stem had been removed. The apex of the funnel was placed within the flared opening of the test bottle and the powder in the funnel was vibrated into the bottle by use of a "vibro" glass marking tool. Mineral oil then was added so as to fill the bulb of the test bottle about three-fourths full. The powder and oil were mixed thoroughly by vigorous shaking and then were subjected to 20 inches of vacuum for 20 minutes to remove interstitial and adsorbed air. The above conditions for the removal of interstitial and adsorbed air were found to give constant results, whereas a lesser period of time resulted in the appearance of free air bubbles and a period of 30 minutes did not change the results obtained. Upon removal from the vacuum, the oil level was raised with additional oil to approximately the 7 per cent mark. The samples then were tempered in a 25 $\pm$ 0.1° C. water bath. At the end of 30 minutes the levels of the bottom of the menisci were recorded, the bottles were wiped dry and weighed, and the densities calculated.

The solubility effect of the mineral oil on the constituents of the milk powder was checked by determining the specific gravity of mineral oil filtered from completed milk powder density determinations. The oil had been in contact with the whole milk powder 2.25 hours at the time filtration was begun; filtration required an additional 2.75 hours. Triplicate determinations on the specific gravity of the oil gave 0.8409, 0.8409 and 0.8410 as the specific gravity.

Centrifugation also was tried as a means of removing the interstitial and adsorbed air. Although the results obtained were approximately the same as when vacuumization was used, the appearance of very fine particles in the oil meniscus precluded accurate reading of the oil level in the stem of the test bottles.

The sizes of the dry milk particles were determined by examining under the oil immersion objective a mineral oil suspension of the powder placed between a glass cover slip and a glass slide. The particle sizes were estimated by use of a previously calibrated Whipple eyepiece with a grid division of 2.5 μ. The number of particles measured per batch varied from 1000 to 5900 particles.

RESULTS AND DISCUSSIONS

Solubility. All solubility indices given in table 1 are the averages of three determinations. The degree of preconcentration has a very distinct effect on the solubility of milk powder. In every comparison of paired milk powders, the powder made from the 40 per cent total solids concentrate had a higher initial solubility and maintained a higher solubility during storage than did the powder made from 20 per cent total solids concentrate. Powders made from either 20 or 40 per cent total solids concentrate showed little if any decrease in solubility when stored at 45° F. for 6 months. However, when stored at 100° F. for 6

months, powders made from 20 per cent total solids concentrates showed a distinct lowering of solubility indices. This decrease was least for the powders made from milk preheated at 160° F. for 30 minutes.

The powders made from the 40 per cent total solids concentrate, except those made from milk preheated at 180° F. for 10 minutes, retained to a remarkable degree their initial solubility when stored at 100° F. for 6 months. The powder made from milk preheated at 180° F. for 10 minutes decreased rapidly in solubility. It is suggested that the denaturing of the casein initiated by the high preheat treatment lowers the solubility of milk powders during storage as is indicated also in the data presented by Hollender and Tracy (6). They found that powders made from milk preheated at 150 and 170° F. for 30 minutes did not de-

TABLE 1

Solubility indices of whole milk powders

Preheat treatment	Milk conc., average	No. of samples	Powder, ave. % moisture	Average solubility index after storage for			
				1 day	1 mo.	2 mo.	6 mo.
	(% *T.S.*)						
			Storage at 45° F.				
160° F.	20.2	6	2.7	97.4	97.3	97.4	97.3
30 min.	38.1	6	2.2	98.8	98.5	98.5	98.5
170° F.	21.9	4	2.2	98.1	98.5	98.4	98.3
10 min.	40.7	4	2.0	99.3	99.3	99.3	99.3
170° F.	21.0	6	2.3	97.8	98.5	98.4	98.3
30 min.	40.9	6	2.1	99.2	99.4	99.4	99.4
180° F.	22.8	6	2.3	98.4	98.9	99.0	99.0
10 min.	40.3	6	2.0	99.1	99.4	99.4	99.3
			Storage at 100° F.				
160° F.	20.2	6	2.7	97.4	97.2	97.3	96.9
30 min.	38.1	6	2.2	98.8	98.7	98.8	98.5
170° F.	21.9	4	2.2	98.1	97.5	97.6	96.7
10 min.	40.7	4	2.0	99.3	99.3	99.3	99.1
170° F.	21.0	6	2.3	97.8	98.1	98.1	96.9
30 min.	40.9	6	2.1	99.2	99.4	99.4	99.1
180° F.	22.8	6	2.3	98.4	98.1	98.2	96.9
10 min.	40.3	6	2.0	99.1	99.2	99.1	97.4

crease in solubility as did powders made from milk preheated at 190° F. for 30 minutes when stored at room temperature for 67 days.

The preheat treatment of the milk, as used in this experiment, appears to affect the initial solubility of the milk powder only to a very slight degree. The initial solubility of the milk powder made from milk preheated at 160° F. for 30 minutes was slightly lower than that of milk powder preheated at the other temperatures. The initial solubilities of the powders made from milk preheated at 170 and 180° F. for 10 minutes and at 170° F. for 30 minutes were about equal when the powders were made from the 40 per cent concentrate. These results are not exactly comparable to those reported by others (6), who showed that the powder made from milk preheated at 190° F. for 30 minutes was less soluble than the powder made from milk preheated at 150 and 170° F.

TABLE 2

Comparison of the solubility index of homogenized whole milk, homogenized concentrated milk and the powder made from the concentrated milk

Powder no.	Milk and conc. milk	% of fat globules over 5μ in diam.	Solubility index	
			Milk	Powder
	(% *T.S.*)			
	12.8[a]	10	97.8	
108	22.6	5	98.9	97.6
109	38.6	less than 1	99.3	97.9

[a] Original milk from which concentrates for powders no. 108 and 109 were made.

for 30 minutes. These differences probably are due to the shorter period of exposure and the lower maximum temperature employed and possibly due to the differences in method employed to determine solubility.

Efficiency of homogenization. It appeared from microscopic examination that the more highly concentrated milk is homogenized more efficiently. The per cent of fat globules 5 μ or over in size progressively decreased as the preconcentration increased. The solubility test (1) when applied to these milks indicated a direct relationship between high solubility indices and efficient homogenization. Under the conditions of the solubility test employed, it is impossible to have a solubility index of 100 per cent. Thus, the unconcentrated, unhomogenized sample shows the lowest solubility index because at least a part of the cream layer is retained as insoluble material (table 2).

In table 3 is shown a further comparison of the solubility indices of concen-

TABLE 3

Comparison of the solubility of homogenized concentrated milk and the powder made from it

Powder no.[a]	Preheat treatment	Milk conc.[b]	Initial solubility index	
			Milk conc.[b]	Powder
		(% *T. S.*)		
106	160° F.	23.3	98.6	97.9
107	30 min.	40.1	99.5	99.3
98	170° F.	22.8	98.9	98.7
99		38.9	99.4	98.9
100		23.4	98.3	97.5
101	10 min.	42.0	99.1	99.2
104		22.7	99.1	98.5
105		39.4	99.3	99.4
94	180° F.	20.6	98.4	99.2
95		41.6	99.3	99.1
96		21.4	99.1	98.1
97		39.9	99.2	99.1
102		39.0	99.1	99.4
103	10 min.	26.5	99.2	99.3
108		22.6	98.9	97.6
109		38.6	99.3	97.9

[a] The powders are paired. The even-numbered powders and the following odd-numbered powders are made from the same milk.

[b] Homogenized.

trated milks and the corresponding powders made from them. The solubility indices obtained with the concentrated fluid milk samples no. 94 to 109, inclusive, range from 98.3 to 99.5. Generally, the less concentrated samples gave lower values than did the more concentrated samples. The effect of concentration of the milk on the solubility of the powder was even greater than when the test was applied only to the concentrated milk. More efficient homogenization of the more viscous, heavier concentrate may have resulted in the differences of the solubility indices of the milks and the greater differences observed in the powders made from the 20 and 40 per cent total solids concentrate.

Color and solubility. The powders made in this experiment had moisture contents which ranged from 1.5 to 3.1 per cent. None of the powders was discolored noticeably after storage for 180 days at 100° F. Krienke and Tracy (9) showed that brown discoloration of powder takes place at all storage temperatures, the discoloration increasing with increased moisture in the powder. Discoloration was accompanied by decreasing solubility. Hollender and Tracy (6) stated that it was evident that there was a critical point in the neighborhood of 5 per cent moisture content which represented the upper limit of moisture for samples stored at room temperature without deterioration in flavor and color. However, they found discoloration of milk powders would occur at 68° F. or higher, regardless of moisture content. They stored samples of milk powder with moisture contents varying from 2.32 to 5.39 per cent at 98.6° F. All of their samples discolored within 67 days. The lower average moisture content of the present samples may have prevented noticeable discoloration.

Density and particle size. The apparent density of the powdered milk is important for it affects packing volume and ease of handling as well as ease of reconstitution. The effects on packing volume and ease of handling or packaging are well known in the commercial field.

Webb and Hufnagel (18) found that increasing preconcentration of the milk resulted in powders with increased densities. In agreement with their findings, it was found in this work that with a given preheat treatment there was a direct correlation between density and preconcentration of the milk (table 4). This direct correlation of density with degree of precondensing was noted in every instance except for powder no. 94. In the production of this powder, trouble was experienced with continuous clogging of the air outlet of the spray nozzle. It is believed that this prevented normal atomization of the milk, resulting in a heavier, coarser powder.

Increasing the degree of preconcentration from 20 to 40 per cent total solids resulted in whole milk powder not only of greater density but with a higher percentage of the larger-size powder particles (table 5). Wilster *et al.* (19) stated that one of the factors favorable to reconstitutability of dry milk was small particle size. The powders with the highest solubility indices, as reported in this paper, had the highest percentage of the large size particles. However, it must be noted that in nearly all of the powders examined, 95 per cent of the particles were less than 10 μ in diameter (table 5). All of the

TABLE 4

Whole milk powder densities

Powder no.	Preheat treat.	Preconc.	Powder		Densities at 25° C.	Av. density	
			% fat[a]	% T. S.		sample	group
		(% *T.S.*)					
70		21.9			1.161, 1.162, 1.162	1.162	
72		18.0			1.180, 1.178, 1.181	1.180	
76	160° F.	19.9	26.3	97.7	1.176, 1.175, 1.170	1.174	
106		23.3	28.4	97.5	1.152, 1.153	1.153	1.167
	for						
71		38.9			1.214, spilled	1.214	
73	30 min.	38.6			1.216, 1.213, 1.211, 1.212, 1.215	1.213	
77		39.3	26.3	97.8	1.206, 1.209, 1.208	1.208	
107		40.1	28.4	97.6	1.189, 1.188	1.188	1.206
88		19.6	26.5	97.9	1.162, 1.165, 1.163	1.163	
92		20.5	26.3	97.9	1.163, 1.160, 1.162	1.162	
100	170° F.	23.4	29.4	97.6	1.135, 1.136	1.136	
110		23.9	28.3	97.6	1.155, 1.158, 1.157	1.157	1.154
	for						
89		42.2	26.6	98.2	1.198, 1.197, 1.201	1.199	
93	10 min.	41.3	26.5	98.5	1.198, 1.199	1.199	
101		42.0	29.5	97.9	1.185, 1.188, 1.186	1.186	
111		37.4	28.3	97.5	1.193, 1.193, 1.192	1.193	1.194
74		20.8			1.178, 1.178, 1.177	1.178	
78		22.0	26.4	97.6	1.169, 1.170, 1.170	1.170	
80		21.0	26.1	97.7	1.164, 1.165, 1.165	1.165	
82	170° F.	19.3	26.1	97.8	1.159, 1.160	1.160	
84		20.3	26.1	97.9	1.158, 1.158	1.158	
104		22.7	27.9	97.5	1.157, 1.155	1.156	1.165
	for						
75		44.4			1.195, 1.192, 1.193	1.193	
79	30 min.	40.7	26.5	97.9	1.204, spilled	1.204	
81		40.3	26.2	98.2	1.206, 1.209	1.208	
83		40.3	26.2	98.1	1.199, 1.195	1.197	
85		40.3	26.1	98.0	1.202, 1.201, 1.202	1.202	
105		39.4	27.9	97.7	1.192, 1.194	1.193	1.198
86		22.0	28.1	97.8	1.164, 1.159	1.162	
90		22.1	26.7	97.4	1.154, 1.157	1.156	
90[b]		22.1	26.7	97.4	1.152, 1.155	1.154	
94[c]		20.6	28.4	98.1	1.196, 1.194, 1.197, 1.193, 1,195, 1,196	1.195	
98		22.8	28.3	97.9	1.139, 1.140, 1.138, 1.138, 1.141, 1.141	1.140	
103	180° F.	26.5	28.3	97.2	1.162, 1.164	1.163	
108		22.5	29.0	97.6	1,151, 1.151	1.151	1.154
	for						
87		41.6	28.2	98.4	1.189, 1.190	1.190	
91	10 min.	41.9	27.8	98.2	1.196, 1.197	1.197	
95		41.6	27.5	98.0	1.182, 1.182, 1.179, 1.182	1.181	
99		38.9	28.3	98.0	1.187, 1.189	1.188	
102		39.0	28.4	97.4	1.189, 1.189	1.189	
109		38.6	29.0	97.8	1.189, 1.188	1.188	1.189

[a] Calculated per cent fat based on per cent fat in the fresh milk.

[b] Adsorbed and interstitial air removed by centrifugation.

[c] During the production of this powder, the air outlet of the atomizing nozzle was apparently out of adjustment and became clogged continuously, preventing normal atomization. This result, therefore, is not included in the average.

powder produced for this experiment may be classified as of small particle size, for Hunziker (8) indicates the size of milk powder particles produced commercially by the spray process ranges from 10 to 100 μ, with 80 per cent of the particles being in the relatively coarse group.

In the solubility tests, it was noted that the powders from the 20 per cent concentrate always were highly charged. Even the slightest handling of this lighter powder markedly increased the static charge and made the handling of this powder difficult. Consequently, the dense powder may play a favorable role in the reconstitutability of whole milk powder, because its lesser tendency

TABLE 5

Size distribution of powder particles

Powder no.	Preheat treat.	Preconc.	% of particles—Diameters in microns						
			0–5	5–10	10–15	15–20	20–25	25–30	30–45
		(% *T.S.*)							
72		18.0	83.0	15.2	1.7	0.1			
70	160° F.	21.9	79.1	16.3	3.6	0.7	0.24		
73	30 min.	38.7	68.4	26.2	4.1	0.8	0.53		
77		39.3	61.4	29.7	6.0	1.8	0.50	0.50	
88		19.6	88.0	11.5	0.5				
110	170° F.	23.9	82.6	16.5	0.5	0.1			
111	10 min.	37.4	61.9	31.3	5.5	1.2	0.10		
101		42.0	67.1	25.4	4.5	2.1	0.33	0.47	0.21
74		20.8	81.7	16.5	1.5	0.4	0.03		
80	170° F.	21.0	80.6	18.1	1.1	0.2	0.09		
85	30 min.	44.3	70.4	23.4	4.6	1.1	0.39	0.16	0.02
75		44.4	65.9	27.2	5.1	1.0	0.47	0.16	0.10
94		20.6	74.9	21.6	3.1	0.3	0.04		
98	180° F.	22.8	83.4	14.9	1.5	0.2	0.08		
109	10 min.	38.6	73.9	22.5	2.9	0.5	0.13		
95		41.6	72.2	21.5	3.7	0.9	0.75	0.25	0.57

to build up a high electrostatic charge appears to reduce the wettability of the powder, and the dense powder naturally has less occluded air.

SUMMARY AND CONCLUSIONS

(1) Precondensing milk to a level of 40 per cent total solids resulted in a spray-dried powder of higher solubility than when the milk was precondensed to a level of 20 per cent total solids.

(2) The powders made from the milk of all preheat treatment levels and concentrated to the 40 per cent level reconstituted quickly and without a visible film of specks on the glassware. For all practical purposes, they were 100 per cent soluble at the end of 6 months of storage at 45° F.

(3) The solubility of the powders made from milk preheated at the lower temperatures and precondensed to 40 per cent total solids did not decrease appreciably when stored for 6 months at 100° F. A preheat treatment at 180° F. for 10 minutes appears to induce heat denaturation of the protein, which is con-

tinued when the powder is stored at 100° F., resulting in continued loss of solubility during storage.

(4) A method for quickly and accurately determining the density of whole milk powder is presented.

(5) The density of milk powder increases with increasing preconcentration of the milk. The powders made from milk concentrated to 40 per cent total solids were easier to reconstitute, did not take up as much space, and did not as readily develop a high electrostatic charge as did the powders made from the 20 per cent total solids concentrate.

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EFFECT OF THYROXINE ON OXYGEN CONSUMPTION OF BOVINE SPERMATOZOA AND SEMEN[1]

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Previous studies have shown that the rate of oxygen consumption of spermatozoa may be related to fertility (3, 9). Other studies have shown that thyroxine increases the oxygen consumption of some tissues *in vitro* (1, 2). Since certain oxidative mechanisms may play a role in spermatozoan physiology similar to that in other respiring cells (4, 5), the purpose of this experiment was to determine the effect of thyroxine on oxygen consumption and, ultimately, to study the effect of thyroxine on semen fertility. The results obtained with a measured amount of thyroxine on the oxygen consumption of bovine spermatozoa and semen are reported in this paper.

METHODS

Bovine semen was collected by use of the artificial vagina under conditions as aseptic as possible. Experiments with washed and unwashed spermatozoa were carried out. In experiments with washed spermatozoa, the semen was cooled slowly to 4.5° C. immediately after collection and diluted with a phosphate buffer.[2] The diluted semen was centrifuged and the diluted seminal fluid removed. Phosphate buffer then was added in amounts to make up the original diluted volume. Experiments on unwashed semen were made on samples collected as described above and diluted with a diluent composed of one part fresh egg yolk to ten parts phosphate buffer. The rate of semen dilution varied for different samples. Since preliminary experiments indicated that thyroxine exerts its effect on respiration rate only after it has been in contact with the spermatozoa for several hours, the determinations in these experiments were made 10 to 30 hours after addition of thyroxine to the semen or spermatozoa. Only a limited number of the centrifuged and washed semen samples maintained respiratory activity at a level high enough for a reliable determination after 10 to 30 hours of storage. Preliminary work (7) indicated that from 0.3 to 1.0 γ of DL-thyroxine in 10 ml. of diluted semen brought about an average increase in oxygen consumption. Therefore, 0.7 γ of DL-thyroxine per 10 ml. diluted semen or washed spermatozoa was used in all of the determinations.

The DL-thyroxine was prepared by isolation from iodocasein which was thyroidally active.[3] A weighed amount of this thyroxine was added to distilled water, completely dissolved by adding 0.1 N NaOH drop by drop and this solu-

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2 Made up of 20 g. $Na_2HPO_4 \cdot 12\ H_2O$ plus 2 g. KH_2PO_4 in 1,000 ml. boiled distilled water.

3 Supplied by Dr. E. P. Reineke of Michigan State College, East Lansing

tion made up to a volume giving 100 γ thyroxine per ml. For use in treating semen, this thyroxine solution was diluted further with a phosphate buffer so that 1 ml. of solution contained 5 γ. A portion of each semen sample was treated with thyroxine and another portion used as a control. The pH was adjusted so that it was the same in treated and control portions.

Oxygen consumption was determined with a Barcroft-Warburg respirometer at 37° C. The manometers were shaken at a rate of 110 oscillations per minute. Care was taken to mix the semen thoroughly before dividing it into portions. To determine the variability of results due to sampling and operation of the respirometer, the oxygen consumption of two untreated portions of a semen sample was determined. The standard deviation of the difference between two like samples for ten determinations was found to be 1.24 mm.^{3} oxygen. The concentration of spermatozoa in the original semen was determined with a hemocytometer.

To determine the influence of bacterial contamination on results, oxygen consumption determinations were made on thyroxine-treated and untreated portions of semen in which the spermatozoa had been killed by adding a measured quantity of water to the raw semen before dilution or by warming and cooling of the semen. Also, oxygen consumption determinations were made on treated and control semen to which 800 units of pencillin per ml. had been added. Dilution was one part semen to four parts buffer.

EXPERIMENTAL

The effect of thyroxine in a concentration of 7 γ per cent on the oxygen consumption of bovine spermatozoa and semen is shown for individual semen samples in table 1. On a basis of the oxygen consumed per 100 million spermatozoa per hour, the results show that the average was 8.48 mm.^{3} for control semen and 9.20 mm.^{3} for thyroxine-treated semen. These values were obtained with semen stored from 10 to 30 hours at 4.5° C. and with varying concentration of spermatozoa. On the same basis, control semen consumed an average of 8.12 mm.^{3} oxygen when the concentration of spermatozoa in the original semen was below 800,000 per mm.^{3}, whereas these same semen samples when treated with thyroxine consumed 8.00 mm.^{3}. Apparently, no average change in the amount of oxygen consumed was brought about with the addition of thyroxine. Control semen with a spermatozoan concentration of more than 800,000 per mm.^{3} and less than 1,400,000 per mm.^{3} in the original semen consumed 8.66 mm.^{3} oxygen, whereas these same semen samples treated with thyroxine consumed 9.64 mm.^{3} oxygen, an average increase of 11.2 per cent.

The results obtained were correlated with spermatozoan concentration of the original semen. In general, as the spermatozoan concentration of the original semen increased from 800,000 to 1,400,000 per mm.^{3}, there was a progressively greater increase in oxygen consumption resulting from the presence of DL-thyroxine. With semen having a spermatozoan concentration of less than 800,000 per mm.^{3}, there was little or no change in the oxygen consumption due to the presence of thyroxine. The correlation coefficient representing the degree of this relationship was found to be + 0.60. This is a statistically highly sig-

nificant correlation for the number of determinations involved. This relationship apparently was not due to spermatozoa number *per se*, because the actual number of spermatozoa present in each determination was not related to increasing oxygen consumption resulting from thyroxine treatment. The correlation coefficient representing the degree of the latter relationship was + 0.19. Therefore, it is believed that differences in changes of oxygen consumption due to thyroxine are due to variations of some character of semen that is associated

TABLE 1

Effect of 7 γ DL-thyroxine per 100 ml. of diluted semen on oxygen consumption

Sperm concentration in original semen	No. of sperm per 2 ml. diluted sample	Oxygen consumption		% of control
		Control	Treated	
(*Thousands/mm.*³)	(*Millions*)	(*mm.*³)	(*mm.*³)	
	Washed, diluted semen			
700	280	26.56	23.54	88.6
800	266	12.16	14.06	115.6
800	266	24.60	27.68	112.5
900	450	33.47	35.76	106.8
1,000	600	32.06	36.09	112.6
1,160	1,160	29.41	31.22	106.1
	Unwashed, diluted semen			
300	120	11.44	11.08	96.9
350	170	14.36	13.84	96.4
450	360	23.34	26.06	111.7
550	220	23.99	23.57	98.3
688	688	36.74	33.94	92.4
700	350	24.28	22.73	93.6
725	482	37.61	42.39	112.7
800	400	75.81	72.63	95.8
820	410	28.58	28.39	99.3
900	360	16.68	21.37	128.0
980	290	21.36	22.86	107.0
1,000	1,000	52.84	59.79	113.2
1,000	100	20.27	22.00	108.5
1,100	220	27.08	32.48	119.9
1,100	440	46.46	48.30	103.9
1,000	440	27.69	34.47	124.5
1,130	900	65.68	75.62	115.1
1,200	480	45.61	50.45	110.6
1,220	490	29.53	34.27	116.1
1,220	1,220	43.25	51.11	118.2
1,375	680	111.60	136.40	122.2

with spermatozoan concentration of the original sample and not to sperm numbers *per se*.

Although not enough evidence has been obtained on semen from individual bulls to draw definite conclusions, it appears that semen from some bulls usually increases in its ability to consume oxygen when thyroxine is present, and semen from these bulls usually has a high spermatozoan concentration. Other bulls seem to produce semen that is depressed or not influenced in its ability to consume oxygen when thyroxine is added and usually is low in spermatozoan concentration. In cells such as bovine spermatozoa that vary considerably in their

physiology, as is indicated by their varying ability to effect fertilization, it is not surprising that the reaction to experimental conditions varies. Varying effects on the oxygen consumption of different tissues due to the influence of thyroxine have been reported by other workers (2, 8).

Bacterial contamination cannot be avoided entirely in semen collection, and its influence on total oxygen consumption may affect results. Therefore, the oxygen consumption of semen samples with the spermatozoa killed was determined for 12 samples. One portion of the sample containing dead spermatozoa was treated with thyroxine and another untreated portion served as a control. The average oxygen consumption of these 12 samples with dead spermatozoa was 3.26 mm.^{3} per hour, with a standard deviation from the mean of 1.97 mm.^{3}. Actual variation was from an amount of oxygen which could not be detected to 6 mm.^{3} per hour. For these same semen samples with thyroxine added, the average oxygen consumption was 3.68 mm.^{3} per hour. With semen to which penicillin had been added immediately after collection and dilution, there appeared to be a similar response to thyroxine, except that the percentage increase in oxygen consumption with thyroxine was less than that to which no penicillin had been added. Increases of from 2 to 12 per cent in the oxygen uptake were obtained with 8 semen samples. The decreased response may be due to the interference by penicillin with oxidative mechanisms of the spermatozoa as well as that of bacteria.

The results obtained in these experiments indicate that total oxygen consumption of spermatozoa is influenced by contaminating substances. However, since washed spermatozoa, as well as unwashed and penicillin-treated semen, showed a similar pattern of response to added thyroxine, it is assumed that spermatozoan metabolism was affected by the treatment.

Wide variability between the oxygen consumption of individual semen samples is influenced by individuality of the semen sample, length of time the semen was stored, the rate of dilution (6) and probably by unavoidable differences in handling individual semen samples.

SUMMARY

Bovine spermatozoa and semen, diluted with a phosphate buffer, were treated with 7 γ per cent DL-thyroxine.

Oxygen consumption generally was increased with the addition of thyroxine in semen samples with an original spermatozoan concentration of from 800,000 to 1,400,000 per mm.^{3}

Semen samples with a spermatozoan concentration of less than 800,000 per mm.^{3} in the original semen were not influenced on the average by the presence of thyroxine in the concentration used.

The ability of semen to respond with increased respiration rate to added thyroxine appears to be related to some factor that is associated with original spermatozoan concentration.

The influence of bacterial and other contamination on results has been discussed. Its exact influence remains unevaluated.

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RETENTION OF ASCORBIC ACID, CHANGES IN OXIDATION-REDUCTION POTENTIAL, AND THE PREVENTION OF AN OXIDIZED FLAVOR DURING FREEZING PRESERVATION OF MILK

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Outstanding among beverage milk problems is the prevention of the flavor described as oxidized, tallowy or cappy, that tends to develop in milk that is produced under sanitary conditions and has a low bacterial content.

Preservation of milk by freezing is a means of studying the oxidized flavor problem which has been little utilized. In the frozen state, milk is more stable than in the unfrozen state, and its changing characteristics can be studied over a longer period. Babcock *et al.* (1), using samples packaged in paper at a large dairy, found the milk of acceptable beverage quality after storage at –32.8° C. for 115 days. At higher temperatures frozen milk is not as stable and, in the author's experience, always eventually becomes oxidized in flavor. The onset of this defect is a limiting factor in the preservation of milk by freezing and, therefore, is of economic importance.

There are at least four forms of ascorbic acid—two that are levorotatory and are called ascorbic acid and dehydroascorbic acid and two that are dextrorotatory. The latter two are not biologically active. Of the former, each of which is equally biologically active, ascorbic acid is the only form of vitamin C present in milk in the mammary gland (6). The oxidized flavor ordinarily is detected in milk containing dehydroascorbic acid, the first oxidation product of ascorbic acid, as well as ascorbic acid. Krukovsky and Guthrie (7) concluded that ascorbic acid oxidation is an essential link in the chain of the reactions resulting in the development of the tallowy (oxidized) flavor in milk, and that apparently the oxidation of the lipid fraction of milk is coupled to that of ascorbic acid when a certain equilibrium between ascorbic acid and dehydroascorbic acid has been established.

Greenbank (4) believes that the development of an oxidized flavor in milk is related to a change in the oxidation-reduction potential, and that variations in milks can be explained on the basis of differences in their poising action.

Dahle and Palmer (3) have expressed the situation as follows: "A condition in the milk which is favorable to the production of the oxidized flavor is apparently favorable to the destruction of vitamin C." They found that the ascorbic acid content gradually decreased during the holding period in all cases, regardless of the development of off-flavor. The reduction in vitamin C was nearly as great in the normal milk as in the milk that developed the off-flavor.

EXPERIMENTAL METHODS

The titration of the milk for reduced ascorbic acid was carried out according

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to the procedure published by Sharp (9). The solution of sodium 2,6-dichlorophenolindophenol was prepared and standardized as described by Stewart and Sharp (11).

In obtaining the milk, no attempt was made to select milk from individual cows, based on their tendency to produce milk that readily developed an oxidized flavor. Morning milk was utilized and was processed in the course of the forenoon. Copper contamination was avoided by employing hand milking and by having the milk delivered in the can into which it was poured from the milk pails.

To delay and prevent the onset of an oxidized flavor, Sharp *et al.* (10) deaerated milk. A convenient laboratory apparatus for deaeration of milk is the one devised by Mottern and Von Loesecke for deaeration of citrus juices (8). This was utilized in the present work.

In deaerating the milk, 12–l. balloon flasks were used and the vacuum was 29 inches. The warm deaerated milk was poured directly into clean well-tinned cans of about 150 ml. capacity, the cans sealed and their contents frozen. Three cold-air storage spaces were utilized: one had a temperature of –10° C., another, –16° C., and the third, –27° C. The temperature of the air in these spaces varied several degrees Fahrenheit. The ascorbic acid was dissolved in a small volume of distilled water and added to the milk.

Unhomogenized milk was found less suitable for these experiments than homogenized milk, since it is less resistant to the onset of the oxidized flavor (13) and, on thawing, shows inferior distribution of insoluble solids. Homogenization was effected at 2,500 lb. pressure per square inch immediately after heating the milk at 62° C. for 30 minutes. The frozen milks were thawed by immersing the cans in warm water.

Oxidation-reduction (E_h) measurements were made with a battery-operated pH meter, using a calomel half-cell. One end of an agar-KCl glass bridge rested in a saturated potassium chloride solution and the other in the sample of milk in a glass beaker. Also partially submerged in the potassium chloride solution was the calomel half-cell and, in the milk was a gold foil electrode fused to a gold wire; these were connected to the jacks of the meter. Each day the electrodes were used, they first were cleaned in the flame of an alcohol lamp, this flame being more suitable than the less pure flame of a Bunsen burner. Each E_h value in the following data is the average of three readings obtained with three electrodes. With but few exceptions, results with the three electrodes were in excellent agreement.

The E_h values of a set of thawed milk samples were obtained either late in the forenoon and again in the afternoon or in the course of the same afternoon but an hour or two apart. In case of differences between the two readings on the same sample, an average value usually was regarded as most representative. The older the samples, the greater this difference was likely to be.

The hand-drawn, uncooled morning milk was processed as follows: *C* (the control)—At the end of the holding period of 30 minutes at 62° C., the milk was homogenized at 2,500 lb. pressure per square inch, cooled, canned and

frozen. *CD*—Similar to *C* except that it was deaerated as already described above its boiling point (50° C.) just before it was canned. *P 20*—Same as the control, except that a solution of ascorbic acid was added to the cooled milk at the rate of 20 mg. per l. of milk. *P 20 D*—Same as *P 20* except that it was deaerated above its boiling point after the addition of the vitamin. *P 40*—Same as *P 20* except that 40 mg. of ascorbic acid per l. of milk was added instead of 20. *P 40 D*—Same as *P 40* except that the warm milk was deaerated after the addition of the vitamin. *20 P*—Same as *P 20* except that the ascorbic acid was added to the raw milk. *20 PD*—Same as *20 P* except for deaeration just before canning. *40 P*—Same as *P 40* except that the vitamin was added to the raw milk. *40 PD*—Same as *40 P* except that the vitamin fortified milk was deaerated before it was canned.

EXPERIMENTAL RESULTS

Figure 1 presents the results of an experiment designed to show the retention at –27° C. of ascorbic acid in milk prepared under various conditions and the

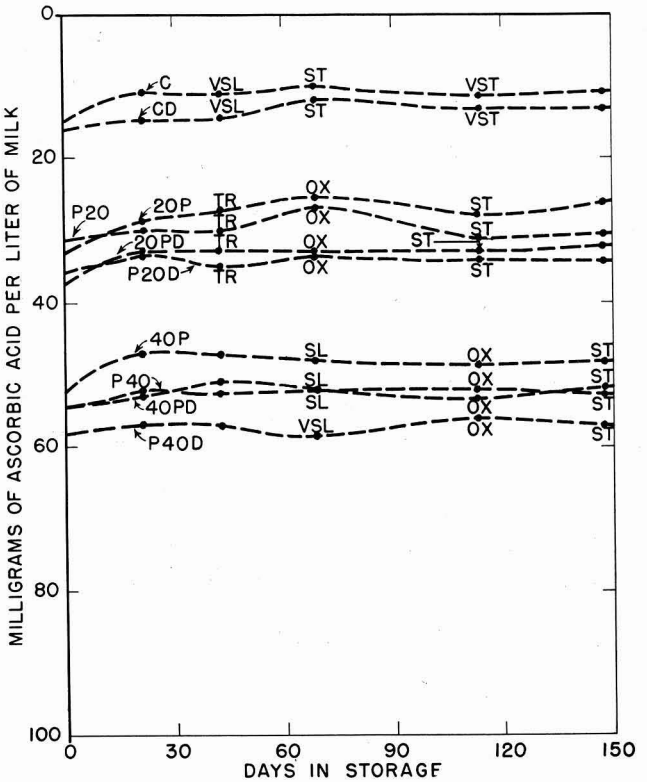

FIG. 1. Retention of ascorbic acid in milk stored at –27° C. and the effect of deaeration and of fortifying the milk with ascorbic acid upon the rate of development of an oxidized flavor. Oxidized flavor intensity is indicated on the curves as follows: tr = trace, v sl = very slight, sl = slight, ox = oxidized, st = strongly oxidized, and v st = very strongly oxidized.

rate of development of an oxidized flavor. To hasten the rate of freezing, these cans of warm milk were immersed in alcohol at –16° C. to which dry ice was added to obtain agitation and to prevent a rise in temperature. Samples were examined after they had been in storage 21, 42, 68, 112 and 147 days. As soon as each fresh milk had been prepared, a small portion was placed in a room maintained at 2 to 4° C. After 9 days, only staleness was detectable in these fluid samples. They were not oxidized.

The ascorbic acid content of the fresh samples was determined 3 to 4 hours after they were placed in the cold room. There was substantially more ascorbic acid in the deaerated than in the undeaerated fresh milks, and this difference was essentially the same in all the thawed samples that were examined during the storage period. Little significance is attached to the apparent advantage of adding ascorbic acid after pasteurization rather than before. Additional evidence on this point is needed.

A straight line drawn from the beginning of each curve to its end would be

TABLE 1

Reduced ascorbic acid content and E_h of the samples of experiment 2 after 4 days at 2 to 4° C.

Sample no.	Reduced ascorbic acid	Oxidation-reduction potential
	(*mg./l.*)	(*millivolts*)
C	0.0	284
CD	0.0	281
P 20	0.0	289
P 20 D	2.4	278
P 40	4.2	280
P 40 D	13.8	256
P 60	16.2	252
P 60 D	31.8	237

nearly horizontal, especially those lines representing portions of the milk that were fortified with ascorbic acid after pasteurization. Although the concentration of ascorbic acid decreased only slightly, these samples all developed a strong oxidized flavor but at different rates. On a percentage basis, ascorbic acid retention was least in the control and greatest in the deaerated milk that was fortified after pasteurization.

The body of all the thawed samples was good. There was no apparent flakiness or fat separation.

Figures 2, 3, and 4 illustrate the relationship in thawed milk between retention of ascorbic acid, the oxidation-reduction potential and the onset of the oxidized flavor. The morning milk was hand-drawn and, except as indicated, was processed and labeled as in the preceding experiment. All canned samples were in a hardening room before noon for freezing and storage at –16° C. Four days after samples of the freshly prepared milks were placed in the 2 to 4° C. room there was a trace of an oxidized flavor in the control, and the deaerated samples were not as flat in flavor as the non-deaerated ones. Their ascorbic acid content and E_h values are given in table 1.

The reduction in E_h in the freshly prepared samples, as additional units of ascorbic acid were added, is shown in figure 2. The average of several measurements on different milks, in which increments of 25 mg. of ascorbic acid per l. of milk instead of 20 were added, is as follows: first, 25 mg./l., 38 millivolts; second, 25 mg./l., 20 millivolts; third, 25 mg./l., 11 millivolts; and fourth, 25 mg./l., 7 millivolts.

After 23 days in storage, a set of samples was thawed by placing the cans in warm water. Each can was shaken, opened and a portion poured into a small

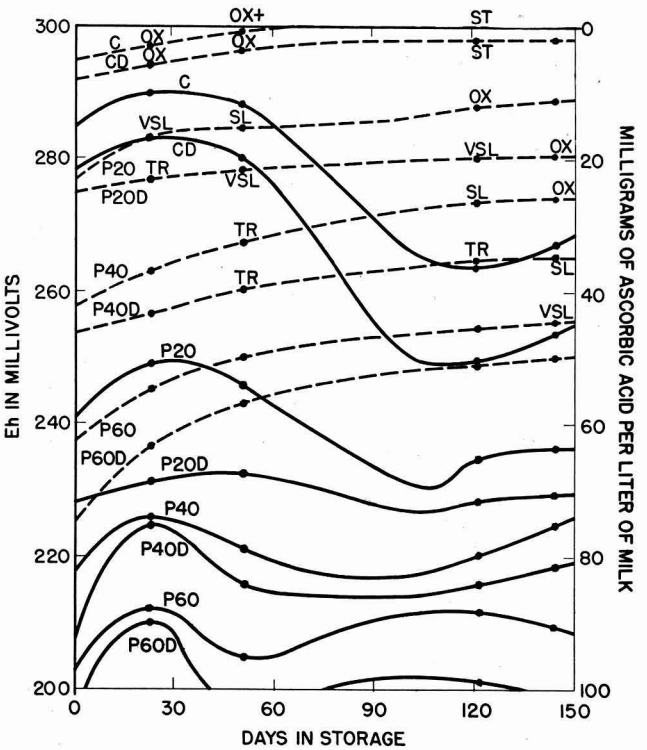

FIG. 2. Retention of ascorbic acid, changes in E_h, and the development of an oxidized flavor in milk stored at −16° C. Broken lines represent mg./l. of ascorbic acid, solid lines the E_h of the samples. (Flavor designations as in fig. 1.)

beaker, after which the opened cans were covered and stored at 2 to 4° C. The beaker samples were warmed to 30° C. and their ascorbic acid content and oxidation-reduction (E_h) values determined. The titrations showed losses in the reducing agent (ascorbic acid), and the E_h measurements disclosed increases in oxidation-reduction potential. Examinations of samples thawed at later dates indicated a continuing decrease in ascorbic acid content but a decrease in E_h followed by an increase. At the same time, an oxidized flavor became detectable and increased in intensity in most of the milks, the rate of increase being greatest

in the control. When a sample which had been fortified by the addition of 60 mg. of ascorbic acid per l. of milk and then deaerated before it was frozen was examined after 160 days in storage, no trace of an oxidized flavor was found. After 51 days in storage, the older these samples were when they were thawed, the greater was the separation of insoluble solids.

Two and 3 days and again 4 to 7 days after each set of thawed milks had been placed at 2 to 4° C., beaker samples were obtained, warmed to 30° C. and the concentration of ascorbic acid and the oxidation-reduction potentials re-determined. These data are plotted in figures 3 and 4. Figure 3 also shows additional

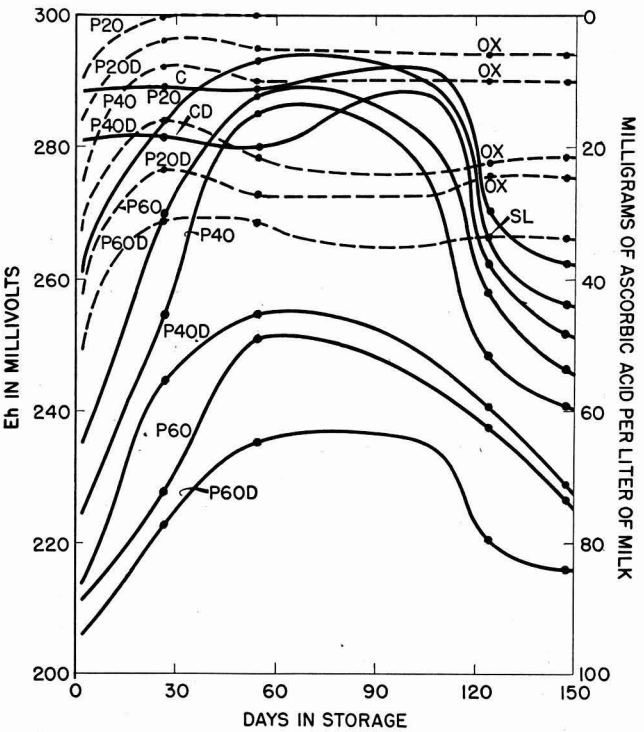

FIG. 3. Ascorbic acid content, E_h and intensity of the oxidized flavor of the thawed samples of figure 2 after 2 to 3 days at 2 to 4° C. (Flavor designations as in fig. 1.)

data on the flavor of the samples. In general, as the amount of ascorbic acid in these thawed refrigerated samples decreased, the oxidation-reduction potential increased, and the intensity of the oxidized flavor increased. A decrease in the intensity of the oxidized flavor never was noted. Having once developed, it always became more pronounced.

Figure 5 shows the effect of the storage temperature upon the retention of ascorbic acid, the oxidation-reduction potential and the onset of an oxidized flavor in samples of the same milk containing increasing amounts of added ascorbic acid. Methods used in earlier experiments were followed in preparing

and examining the samples. As soon as the various milks were ready, several cans of each, representing controls and the variables, were placed in compartments maintained at –10, –16 and –27° C. When 9 days old, a set of refrigerated fluid samples included no milk that tasted oxidized or contained titratable ascorbic acid. The E_h values ranged from 297 to 305 millivolts. Samples stored at –10° C. for 44 days and then thawed were flaky; those held at –16° C. for 98 days also were flaky, but all samples that were thawed after storage at –27° C. were homogeneous. As in other experiments with but few exceptions, the more

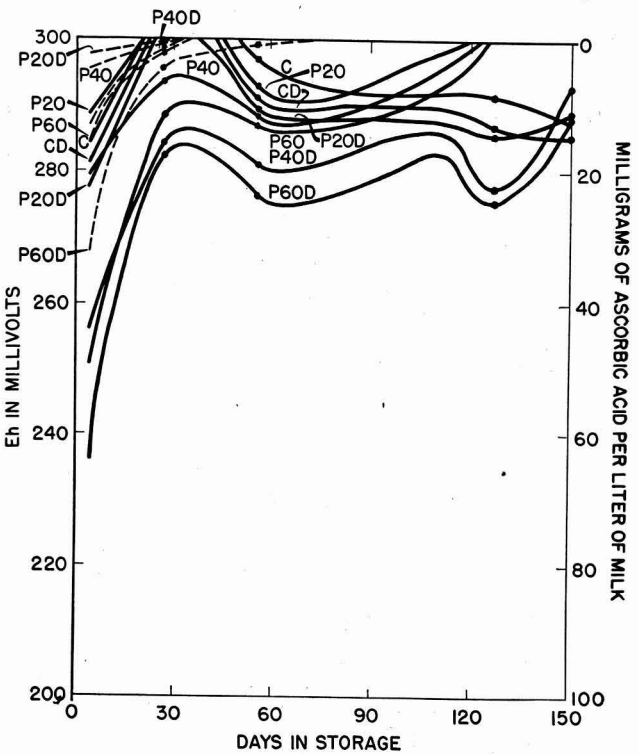

FIG. 4. Ascorbic acid content and E_h of the thawed samples of fig. 2 after 4 to 7 days at 2 to 4° C.

the milk was fortified with ascorbic acid and the lower its storage temperature, the greater was the resistance of the milk to the onset of an oxidized flavor.

The evidence in figure 5 indicates that oxidation of ascorbic acid and the onset of the oxidized flavor in milk may be accompanied by a reduction in the electromotive force or chemical energy of the system. After 98 days in storage, during which there was a loss in ascorbic acid and the development of an oxidized flavor, most of the samples had a lower oxidation-reduction potential than they did when they were frozen.

DISCUSSION

Many who have referred to the off-flavor known as "oxidized" or "tallowy"

have taken it for granted that oxidation of the fat was the cause. The view that the true fat is involved is unsound. Milk fat is too stable to be affected by this mild reaction (5). There is considerable evidence that a phospholipid, a relatively unstable fat-like substance containing phosphorus and associated with the fat, is the constituent of milk that is affected.

Although oxidation of a phospholipid, probably lecithin, now quite generally is regarded as the cause of the defect, oxidation-reduction potential measurements have been used by only a relatively few investigators in studying the problem. This paper attempts to show oxidation-reduction changes during the onset of the off-flavor in frozen milk by examining its thawed product, and at the same time recording the decrease in ascorbic acid.

In the experiment on which figure 1 is based, it was demonstrated that a

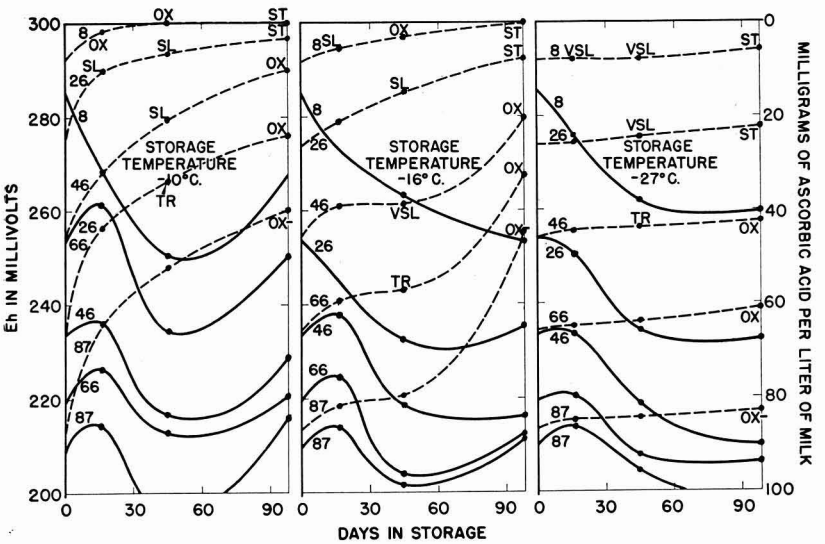

FIG. 5. Relationship between the retention of ascorbic acid, the oxidation-reduction potential and the onset of an oxidized flavor in milk stored at different temperatures. The corresponding ascorbic acid and E_h curves are indicated by numbers which also represent the mg. per l., of ascorbic acid in the fresh samples. Broken lines represent ascorbic acid, solid lines oxidation-reduction potential (E_h) of the samples. (Flavor designations as in fig. 1.)

strong oxidized flavor may develop in milk with but slight decrease in ascorbic acid content; also, the rate of development is much slower in milk that has been fortified with the acid. Deaeration aided in the preservation of the acid but only slightly retarded the onset of the off-flavor.

The storage temperature in the second experiment was not as cold as in the first; figure 2 shows that the retention of ascorbic acid was not as effective. Here again, fortification with ascorbic acid protected the fresh flavor of the milk. Although the E_h values, relative to their initial relationships, continued to follow approximately the same pattern, they did not continue to increase but rather decreased and then began to increase again.

Insofar as these data are concerned, it appears that during storage frozen milk may become more oxidized in flavor while its oxidation-reduction potential is decreasing. Whether or not the E_h values of the thawed samples at 30° C. accurately reflect the E_h values during frozen storage is not known. If they do, then opposing reactions were going on during a large part of the storage period. One reaction is a slow oxidation of ascorbic acid to dehydroascorbic acid and the other (or others) is reducing in character and of greater effect upon the oxidation-reduction potential of the system. If E_h values after thawing do not reflect the E_h values during storage, then constituents redissolved and soon approached a new equilibrium that substantially altered the oxidation-reduction potential. Dahle, *et al.* (2), working with frozen cream, obtained results that resemble these on frozen milk.

In the present study all E_h measurements were made in the same manner, and no reading was acceptable if there was drifting and the electrodes were not in agreement. The data, therefore, should be relative.

Swanson and Sommer (12) conclude from their studies on oxidation-reduction potentials in relation to the development of oxidized flavor in fluid milk that the E_h value of the medium does not seem to inhibit or accelerate the development of the off-flavor.

CONCLUSIONS

From the data of this paper, it may be concluded that a low E_h, obtained by adding ascorbic acid, greatly defers but does not prevent the development of an oxidized flavor in frozen milk. However, a low E_h does not increase the retention of vitamin C in the form of ascorbic acid.

In determining the flavor of the milks, the author had the benefit of the experienced judgment of C. J. Babcock.

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LIPID DETERIORATION IN DAIRY PRODUCTS. THE STABILITY OF MILK FAT AND FAT-SOLUBLE VITAMINS AS DETERMINED BY THE RE-EMULSIFICATION TEST

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The milk excreted by the mammary gland under proper sanitary and feeding conditions has a pleasant and sweet taste. Quite often, however, milk and its products develop flavors which render them distasteful to the human palate and are the cause of their rejection. There are rancid and oxidized flavors. While it is possible to reduce to a minimum, retard or prevent the lipolysis by a quick and prompt cooling of raw milk, storage at constant temperatures (2, 8) and subsequent pasteurization treatments (9), uniform control of the development of oxidized flavors is not possible.

The development of oxidized flavors in fresh milk could be practically retarded or prevented by the deaeration of milk (11), or depletion of milk of its total vitamin C content by rapid oxidative methods (6, 7). The retardation of oxidized flavors by deaeration and their stimulation in the presence of dissolved oxygen and vitamin C by the exposure of fresh milk to sunlight for a short period of time or by copper catalysis of the reaction (6. 7), indicates that oxygen plays an important part in the reaction which produces the oxidized flavors, and that the reaction is promoted by a catalyst. The prevention of the oxidized flavors by the complete depletion of the total vitamin C content of milk, irrespective of the oxygen and copper present, suggests that oxygen and copper, either alone or together, could not promote the reaction which produces the oxidized flavors (6, 7).

Thus, to develop the oxidized flavors in fresh milk, all components of the system must be present, namely, oxygen, vitamin C, catalysts such as copper, light or enzymes, oxidation-susceptible lipid fraction of the milk or, as recently reported, casein (13).

It long has been believed that vitamin C is an anti-oxidant, and that if it were increased in the milk, the development of the oxidized flavors would be prevented (1, 11, 12). Under certain conditions, vitamin C might exert a protective influence. However, the amounts of added ascorbic acid required for protection vary from sample to sample. Undoubtedly, this is due to the variation in the amounts of dissolved oxygen in the samples of milk. It has been shown that partial and quick oxidation of ascorbic acid in milk to dehydroascorbic acid, either by added hydrogen peroxide or by oxygen with light as a catalyst, brings the system more quickly to the point where the other reactions which produce the oxidized flavor in milk could be coupled to that involving ascorbic acid oxidation (6, 7). Consequently, the amount of ascorbic acid added to milk must be considerably in excess of that required to utilize all of the dissolved oxygen prior to

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the establishment of conditions favorable to the secondary reactions which result in the development of the oxidized flavor. The differences between samples of milk fortified with ascorbic acid in their abilities to resist the reaction which produces the oxidized flavor also could be attributed to variations in the ability of milk to promote ascorbic acid oxidation.

Ascorbic acid in milk is oxidized to dehydroascorbic acid which is a very unstable form of vitamin C and is readily destroyed by heat. Its accumulation in the milk is governed primarily by the rate of ascorbic acid oxidation. When dehydroascorbic acid is destroyed as rapidly as it is formed by the oxygenation of milk during pasteurization, the secondary reaction which produces the oxidized flavor is prevented. In this connection, it should be noted that the development of oxidized flavors in fresh milk apparently has nothing to do with the deterioration of milk fat, which is relatively stable. However, milk fat itself also undergoes oxidative deterioration in the presence of ascorbic acid, resulting in the development of metallic to fishy flavors. These flavors have been traced to oxidative processes which occur in the milk fat at the end of its storage life. Quite often they are accompanied by the extensive losses in vitamins A, E and carotene content of the milk fat. The susceptibility of milk fat to this type of deterioration depends on the type of product, the temperature of pasteurization and the conditions of storage. These factors were studied in some detail, and the data are presented to show that under certain conditions, namely, at the end of storage life of the milk fat, ascorbic acid plays an important part in the oxidative deterioration of fat, manifesting itself by the development of the objectionable oxidized flavors and the losses in the fat-soluble vitamins.

EXPERIMENTAL

In a recent study of the oxidation of vitamin A and its precursor, carotene, in milk fat (4, 5), it has been pointed out that the reemulsification test was found to be very useful in recognizing not only the flavor defects of milk fat, but also in detecting changes in the resistance of milk fat to oxidation, whether these changes are brought about during storage, by exposure to light, or any other factor. Consequently, in order to study the effects of different factors upon the ability of milk fat to resist deterioration in the presence of ascorbic acid, the following procedure was adopted.

At the end of various storage periods, milk fat, held either in the form of cream[1], butter[1] or pure fat[1], was re-emulsified in skim milk[2] depleted of its total vitamin C content by rapid oxidative method (7) to produce 4 per cent fat reconstituted milk. To aliquot portions of this milk, approximately 20 mg. of ascorbic acid and 0.1 mg. of copper per liter of milk were added, either alone or together. These portions of milk then were held at 0 to 5° C. up to 48 hours. Throughout the duration of the experiments, the samples were protected from

[1] Milk fats were obtained by churning of cream, oiling and centrifuging of butters prior to their use in the re-emulsification test, or prior to storage.

[2] In Club aluminum cream maker. The skim milk was depleted of its total vitamin C content by added H_2O_2 and the following pasteurization at 61.6° C (143° F) for 30 minutes (7).

TABLE 1

The effects of complete depletion of natural milk of its total vitamin C content by the oxygenation of milk during pasteurization upon the development of oxidized flavor in milk subsequently held at 0 to 5° C.

Held at 0–5° C.	Untreated control milk		(B) Oxygenated milk		Additions to (B) milk	
Days	Ascorbic acid	Flavor scores	Total vit. C	Flavor scores	Ascorbic acid	Flavor scores
	(*mg./l.*)		(*mg./l.*)		(*mg./l.*)	
0	12.3		00.0		20.8	
1	6.8	38 T	.....	42	13.0	42
2	0.0	25 T	.....	42	3.0	38
3	.....	Ex. T.	.....	42	0.0	25 T
			Copper added—0.1 p.p.m.			
1	00.0	25 T	.....	42	1.9	25 T
12		Ex. T.		42		Ex. T

light. They were scored for flavors; then the gravity cream was churned, and the butter obtained was centrifuged and the fat was analyzed for its fat soluble vitamin content. The judging was done on the basis of the score card recently revised by the American Dairy Science Association[3]. Vitamins A and E and peroxides were determined, using Koehen and Sherman (3), Quaife (10) and Volz and Gortner (14) methods, respectively.

It already has been remarked that when dehydroascorbic acid is destroyed as rapidly as it is formed, the secondary reaction which produces the oxidized flavors in milk is prevented. To test this point, a batch of fresh mixed milk was oxygenated during the pasteurization at 61.6° C. for 30 minutes. The oxygen was admitted in minute bubbles into milk undergoing treatment, using carborundum gas diffusion tubes.

TABLE 2

The effects of complete depletion of natural milk of its total vitamin C content by hydrogen peroxide and the following pasteurization and of added ascorbic acid and copper upon the development of oxidized flavors and the stability of fat soluble vitamins in milk subsequently held at 0 to 5° C.

Additions			Flavor scores of milk				At the end of 14 days holding period		
Ascorbic acid	Copper	Ascorbic acid[a]	Hours at 0 to 5° C.				Per 100 g. fat		
			3	6	9	14	Carot.	Vit. A	Vit. E
(*mg./l.*)	(*mg./l.*)	(*mg./l.*)					(μ*g.*)	(μ*g.*)	(μ*g.*)
.....	.....	.....	40	40	40	40	438	421	2125
.....	0.1	.....	40	40	40	40	433	419	2131
20.2	.....	20.9	40 –	30MT	25MT	Ex.MT	432	401	2183
20.2	0.1	20.9	25MT	25MT	Ex.M.	Ex.M.	432	399	2071

[a] Ascorbic acid re-added at the end of a 6 day storage period.

[3] Flavor scoring system: 40, no criticism; 35–40, acceptable to some consumers; 25, unsuitable for consumption. Symbols: Ex. = extremely; T. = tallowy; M. = metallic; F. = fishy; Oi. = oily.

The data presented in table 1 are rather conclusive in showing that the depletion of milk of its total vitamin C content by oxygenation prevents the development of the oxidized flavors, and that the oxidized flavors could be induced again by the addition of ascorbic acid to oxygenated milk. The results of this experiment are in agreement with the original one (6, 7).

The data of table 2 show that, although the oxidized flavors developed in samples of fresh milk containing added ascorbic acid prior to storage at 0 to 5° C., the vitamins A and E and carotenoid content of the fat remained practically unaffected. In this case, a sample of fresh mixed milk was first depleted of its total vitamin C content by hydrogen peroxide and the following pasteurization at 61.6° C. for 30 minutes.

The data presented in table 3 show the effects of the addition of ascorbic acid

TABLE 3

The effects of the addition of ascorbic acid and copper to reconstituted milks made of fresh skim milk depleted of its total vitamin C content or of skim milk powder and unstable fat (re-emulsification test) upon the development of oxidized flavors and losses of the fat soluble vitamins.

Reconstituted milk—4% fat							
Made of	Additions		Flavor scores of milk		Per 100 g. of fat		
	Ascorbic acid	Copper	Hours at 0 to 5° C.		Carotenoids	Vitamins	
			24	48		A	E
	(*mg./l.*)	(*mg./l.*)			(μg.)	(μg.)	(μg.)
	Control fat		. . . . .		671	498	2062
fresh skimmilk	.....	.1	40–oi	40–oi	640	441	1889
	20.7	.....	Extremely		416	308	1234
	20.7	.1	metallic and fishy		395	269	1127
skimmilk powder	.....	.....	40–	40–	599	438	2165
	.....	.1	40–	40–	610	428	2018
	19.7	.....	25 F	25 F	410	401	1074
	19.7	.1	Extremely metallic and fishy		380	305	1096

and copper to reconstituted milk (re-emulsification test) made of fresh skim milk depleted of its total vitamin C content, or of skim milk powder, and of fat susceptible to deterioration, upon the development of oxidized flavors and losses of the fat soluble vitamins. The results showed that extreme metallic to fishy flavors developed in samples of milk containing ascorbic acid added either alone or together with copper. The development of these flavors was also accompanied by considerable losses in vitamins A and E and carotenoid content of the fat.

These observations indicate, therefore, that the oxidized flavors in fresh milk are not associated with the milk fat, but with the unstable materials present either on the surface of the fat globules or in the plasma phase of the milk. This is clearly evident from a comparison of the data of tables 2 and 3.

Subsequently, it was thought of importance to obtain some idea concerning the effects of the following factors upon the development of the objectionable flavors and losses in the vitamins A and E and carotenoid content of the milk

fat in the re-emulsification test: the fat content of reconstituted milk, the amounts of added ascorbic acid, the exposure to light of fats susceptible and non-susceptible to oxidative deterioration, and the temperature of pasteurization of skim milk used in the test.

The data presented in table 4 show that in the re-emulsification test the development of the objectionable flavors was not retarded, and the losses in fat-soluble vitamins were not appreciably affected, either by the fat content or by the vitamin C content of the reconstituted milk products.

The data on the effect of the exposure of fat to light are presented in table 5. This experiment was prompted by the previous observations (4, 5), indicating

TABLE 4

The effects of the fat content of reconstituted milk (re-emulsification test) and of the amounts of subsequently added ascorbic acid upon the development of oxidized flavors and losses of the fat soluble vitamins

		Reconstituted milk made of skim milk and fat (A)							
		Additions		Flavor scores		Per 100 g. fat			
Type of fat (A)	Fat content	Ascorbic acid	Copper	hours at 0 to 5° C.		Carotenoids	Vitamins		Peroxides
				3	24		A	E	
	(%)	(*mg./l.*)	(*mg./l.*)		(*butm.*)	(μ*g.*)	(μ*g.*)	(μ*g.*)	(*Milliequiv.*)
Suscept.	Con.	fat	.....	.....	.....	628	500	1909	0.516
to oxi-	4.0	.....	.....	40–	40	612	482	1528	0.431
dative	4.0	.....	.1	40–	40	630	462	1737	0.397
deterio-	4.0	23.0	.....	25T	Ex.F.	554	354	1084	0.368
ration	4.0	23.0	.1	Ex.FT	Ex.FT	401	268	785	0.497
	4.0	106.3	.....	25T	Ex.FT	559	405	1265	0.371
	4.0	106.3	.1	Ex.FT	Ex.FT	331	245	721	1.116
	20.0	.....	.....	40–	40	594	480	2082	0.453
	20.0	.....	.1	40–	40	639	491	1714	0.511
	20.0	23.5	.....	Ex.FT	Ex.FT	564	412	1187	0.505
	20.0	23.5	.1	Ex.FT	Ex.FT	493	328	970	0.880
	20.0	82.1	.....	Ex.FM	Ex.FM	568	398	1403	0.354
	20.0	82.1	.1	Ex.FM	Ex.FM	420	359	1004	0.692

that vitamin A in the milk fat is readily photo-oxidized, whereas its precursor, carotene, remains unaffected. It was of interest, therefore, to find out also the effects of irradiation of stable and unstable fats upon the stability of tocopherols, and the susceptibility of fat to deterioration in the re-emulsification test. The irradiation experiment was performed following the same procedure as previously described (4, 5) with the exception that the temperature of the fat was maintained at 45 to 48° C. and the intensity of the light generated by the mercury vapor lamp at approximately 1400 foot-candles. The data in table 5, although not directly comparable, revealed that the susceptibility of fat to the foregoing type of deterioration depends primarily upon its ability to resist oxidation prior to irradiation, and to lesser degree upon the direct effect of irradiation. The data in table 5 also indicate that, irrespective of the ability of fat to

resist oxidation in the re-emulsification test, vitamin A was found to be photo-oxidized at a much faster rate than vitamin E, whereas the carotenoid content of the fat remained practically unchanged throughout the duration of the irradiation. Subsequently, it was thought of importance to obtain some idea concerning the effects of the temperature of pasteurization of skim milk used in the re-emulsification test upon the development of the objectionable flavors and losses in the vitamins A and E and the carotenoid content of the milk fat. For this reason, ascorbic acid and copper were added either alone or together to portions of reconstituted milks made of unstable fat and of fresh skim milks depleted of

TABLE 5

The effects of irradiation of fats susceptible and non-susceptible to deterioration with light generated by mercury vapor lamp upon the fat soluble vitamin content, and the susceptibility of fat to oxidative deterioration as determined by the re-emulsification test

		Reconstituted milk made of skimmilk and fat (A)					
Minutes irradiat. at 45–48° C.	Type of fat (A)	Additions		Flavor scores hours at 0 to 5° C.	Per 100 g. fat		
		Ascorbic acid	Copper		Carotenoids	Vitamins	
				6 & 48		A	E
		(*mg./l.*)	(*mg./l.*)		(μg.)	(μg.)	(μg.)
Control	Suscept.	Control fat		.....	634	492	2047
	"	.....	.1	40–Oi	602	416	1670
	"	20.0	.....	fishy	387	316	1026
	"	20.0	.1	ex. F.	204	186	906
60 minutes	Suscept.	Control fat		.....	585	160	1540
	"	.....	.1	40–Oi	553	167	1396
	"	20.0	.....	fishy	443	116	1164
	"	20.0	.1	ex. F.	229	73	1134
Control	Non-susceptible	Control fat average all sam.		.....	1024	883	3917
				40	1019	872	3966
60 minutes	"	Control fat		.....	1026	308	3777
	"	.....	.1	40–Oi	1015	240	3971
	"	24.3	.....	39–Oi	1010	224	3840
	"	24.3	.1	35–OiMT	971	201	2809

their total vitamin C content by added hydrogen peroxide, and the following pasteurization at 61.6 and 76.6° C. for 30 minutes.

A comparison of the data in table 6 suggests the possibility that the inactivation of a catalyst-enzyme by heat primarily was responsible for the retardation of inter-action between ascorbic acid and fat or fat-soluble vitamins in the milk, which was made of skim milk pasteurized at 76.6° C.

Although the evidence presented in the preceding paragraphs definitely indicates that the oxidative deterioration of milk fat could be catalyzed in the presence of ascorbic acid, nevertheless, it was apparent that the reaction might take place only at the end of the storage life of fat. Since it seemed possible that the changes in the ability of milk fat to resist the foregoing type of deterioration would be affected by the type of product held, the temperature of pasteuri-

TABLE 6

The effects of the temperature of pasteurization of skim milk used in the re-emulsification test upon the development of oxidized flavors and losses of the fat soluble vitamins.

Reconst. milk made of skim milk and unstable fat					Per 100 g. of fat			
Additions		Flavor scores			Carotenoids	Vitamins		Peroxides
Ascorbic acid	Copper	Hours at 0 to 5° C.						
		½ & 24	48			A	E	
(*mg./l.*)	(*mg./l.*)		(Skm.)[a]	(Butm.)[a]	(μg.)	(μg.)	(μg.)	(*milli-equiv.*)
Prior to re-emulsification					506	452	2431	0.196
After re-emulsification in the skim milks:								
			(I). pasteurized at 61.6° C.					
control		40	40	40	495	442	2197	.153
.....	.1	40	40	40	483	432	2080	.202
19.7	.....	25F	25F	Ex. F.	407	399	1808	.356
19.7	.1	Ex. F.	Ex.	F.	327	330	1294	.470
			(II). pasteurized at 76.6° C.					
control		40	40	40	473	436	2475	0.28
.....	.1	40	40	40	501	444	2284	0.18
22.1	.....	40	40	30F	477	451	2121	0.22
22.1	.1	40	35T	Ex. F.	449	389	1468	0.20

[a] Skm., gravity skim milk; Butm., buttermilk after the churning of gravity cream.

zation and the condition of storage, it was thought desirable to determine what effects these factors might have upon the storage life of fat as determined by the re-emulsification test.

For this reason, four lots of cream, butter and pure fat were prepared from mixed morning milks obtained from the Cornell University herd. Two portions of this milk were oxygenated continually during the pasteurization at 61.6 and 76.6° C. for 30 minutes. This was done to deplete the milk of the total vitamin C content prior to preparation and storage of the previously described milk products. The other two portions of the same milk were pasteurized at the same

TABLE 7

The effects of the depletion of milk of its total vitamin C content by oxygenation during the pasteurization, and the temperature of pasteurization upon the development of the tallowy flavor in cream during its storage at −17.7 to −16.1° C.

Milk		Flavor scores of cream and its buttermilk at the end of storage at −17.7 to −16.1° C.							
Treatment and Pasteurized at	Product	month							
		3	4	5	6	7	8	10	12
61.6° C.	Cream	38T	39T	39T	40	39T	38T	38T	30T
control	Buttermilk	38T	39T	40	40	39T	38T	40	35T
76.6° C.	Cream	40	40	40	38T	25T	25T	25T	25T
control	Buttermilk	38T	38T	39	ExTM	ExTM	ExTM	ExTM	ExTM
61.6 and 76.6° C. oxygenation	Cream and Buttermilk	40	40	40	40	40	40	40	40

temperatures, but without the oxygenation treatment (tables 7 and 8). In another experiment, a portion of mixed milk was first depleted of its ascorbic acid content by adding hydrogen peroxide. The aliquot portions of each cream, separated from the control and ascorbic acid depleted milks, then were pasteurized at 61.6, 68.3, 71.1 and 76.6° C. for 30 minutes prior to churning and storage of cream, butter and pure fat (table 9). Throughout the duration of the experiments, the samples of cream and fat were held in tightly sealed glass containers protected from the light. The butter samples were shaped in cylindrical forms and wrapped directly in two thicknesses of tinfoil. The samples of cream, butter and pure fat subsequently were held at −17.7 to −16.1° C.

The data presented in table 7 show that the creams separated from milks

TABLE 8

The effects of the depletion of milk of its total vitamin C content by oxygenation during the pasteurization, the temperature of pasteurization and storage of cream, butter and fat at −17.7 to −16.1° C upon the susceptibility of fat to oxidative deterioration (storage life of fat) as determined by the re-emulsification test.

Milk		Product held	The end of the storage life of fat
Pasteurized at	Treatment		
(°C.)			(months)
61.6	control	cream	4
	oxygen.	cream	4 to 5
76.6	control	cream	5
	oxygen.	cream	6
61.6	control	butter	4 to 5
	oxygen.	butter	5 to 6
76.6	control	butter	not sensitive to deterioration at the end of 12 months
	oxygen.	butter	
all temperatures	control & oxygen.	fat	"

Storage cream—56 to 58 per cent fat.

depleted of their total vitamin C content by oxygenation during pasteurization were found by judges to be perfect in flavor at the end of 12 months storage at −17.7 to −16.1° C. Moreover, it was found that the depletion of cream of its total vitamin C content by added hydrogen peroxide, and the following pasteurization at indicated temperatures resulted in the prevention of oxidized flavors, even at the end of 2 years storage at −17.7 to −16.1° C. It should be noted, however, that these creams developed a slightly "nutty" flavor at the surface, whereas the control samples of cream developed very strong tallowy flavor.

The data in tables 8 and 9 reveal that the temperature of pasteurization of milk or cream and the type of product held are the factors governing the susceptibility of fat to oxidative deterioration in the presence of added ascorbic acid. They also show that, irrespective of the temperature of pasteurization, the milk fat stored in the form of cream loses its resistance to oxidative deterioration at a much faster rate than the fat stored in the form of butter; and that only the

TABLE 9

The effects of the depletion of milk of its ascorbic acid content by hydrogen peroxide and the following pasteurization of cream, the temperature of pasteurization, and storage of cream, butter and fat for 2 years at −17.7 to −16.1° C. upon the susceptibilty of fat to oxidative deterioration as determined by the re-emulsification test.

Milk products obtained from cream			Reconst. milk made of skim milk and fat from (A)				Per 100 g. of fat				
			Additions		Flavor score			Vitamins			
Separat. from milk	Pasteur. 30 min. at	Product held (A)	Ascorbic acid	Copper	Hours at 0 to 5° C.		Carotenoids	A	E	Iodine No.	Peroxides
	(°C)		(mg./l.)	(mg./l.)	(½)	(24)	(μg.)	(μg.)	(μg.)		(milli-equiv.)
Both	61.6	butter	control fat				494	479	2397	.....	0.250
control	68.3		.....	.....	40	40	436	440	1384	32.70	0.160
and			.....	.1	40	40	430	450	1298	32.80	0.200
H_2O_2			19.5	.....	25F	Ex.F	240	251	784	33.00	0.432
			19.5	.1	Ex.F	Ex.F	231	240	878	33.10	0.561
"	71.1	butter	control fat				530	495	3005	.....	pres.
	76.6		.....	.....	40	40	476	475	2853	33.00	0.262
			.....	.1	40	40	480	486	2624	33.10	0.295
			18.3	.....	40	40	480	490	2869	33.00	0.208
			18.3	.1	40	40?	470	488	2985	33.16	0.201
"	All temp.		control fat				520	481	3047	.....	
		fat	all samples		40	40	495	468	2736	.....	
	All temp.	cream	control fat				447	446	2407		
			.....	.....	40	Ex.F	.....	.....	.....		
			20.0	.....	35F	40	.....	.....	.....		

All butter samples were given 93 flavor score at the end of 2 years storage.

fat from butter churned from cream pasteurized at 71.1 and 76.6° C. and the pure fat were found to be non-susceptible to oxidation resulting in the development of the objectionable flavors when re-emulsified in skim milk at the end of 1- and 2-year storage periods.

The re-emulsification test was found to be extremely useful in determining the sensitivity of fat to oxidation when the fat was obtained from products which as yet have not shown any apparent changes either in their flavors or in their fat soluble vitamin content. This is clearly evident from a comparison of data of tables 7 and 8. It shows that the cream separated from oxygenated milk did not develop any "off" flavors during the 12- month holding period. This period, during which neither organoleptic nor chemical changes could be accurately determined, often is referred to as the storage life of fat or of a food product. Yet, in the presence of ascorbic acid, with or without copper, the fat undergoes deterioration which is manifested by the development of the objectionable flavors and the losses of fat-soluble vitamins. This would explain the development of the distasteful flavors in food products made of storage cream plus products containing vitamin C, such as fresh milk or lemon juice.

In conclusion, it is of importance to point out that the development of "off" flavors in milk, as a result of ascorbic acid oxidation with copper as a catalyst, might not necessarily be connected with the deterioration of milk lipids. This was evident from the fact that occasionally a flavor which is difficult to describe developed at the end of a 48-hour storage period in the skim milk used as a control sample in the re-emulsification test. This would be in line with the experimental results obtained by Thompson *et al.* (13).

Although this flavor might be present in the gravity skim milk obtained from the reconstituted milk, it seldom was detected in the gravity cream buttermilk. This suggests that either some factor, unknown at present, prevents the development of this flavor in the gravity cream, or that the substance responsible for it was denaturated during the churning of cream.

Usually no objectionable flavors develop in the gravity cream buttermilk when fat used in the re-emulsification test is stable. The metallic to fishy flavor developed in reconstituted milk made of oxidation-sensitive fat and containing ascorbic acid, either alone or with copper, quite often was intensified by the churning of gravity cream. This flavor does not develop in the presence of copper alone.

The oxidized flavors which developed in the control samples of milk products during their storage might be carried into the portion of reconstituted milk containing copper alone. The churning of gravity cream from such milk may not result necessarily in the intensification of this flavor.

SUMMARY

(1) It has been shown that ascorbic acid plays an important part in the oxidative deterioration of milk fat at the end of its storage life, as determined by the re-emulsification test, resulting in the development of objectionable

flavors and losses in vitamins A and E and the carotene content of the fat. The susceptibility of fat to this type of deterioration is determined primarily by the treatment of milk, the temperature of pasteurization, the type of product, the conditions of storage, and to a lesser extent upon the direct and immediate effect of the exposure to light.

(2) The exposure of pure fat to light generated by mercury vapor lamp (1400 foot-candles) slightly affects its vitamin E content. However, it lowers the resistance of vitamin E in the stable fat to deterioration as determined by the re-emulsification test.

(3) The re-emulsification test was found to be useful in determining the end of the storage life of fat when the fat was obtained from products which have not as yet shown any apparent changes in their flavors. This view is supported by the observations showing that the depletion of cream of its total vitamin C content, either by oxygenation, or by hydrogen peroxide, has prevented the development of the objectionable flavors for 12 and 24 months at –17.7 to –16.1° C., respectively. In the re-emulsification test, however, the fat obtained from oxygenated milk pasteurized up to 76.6° C. lost its ability to resist the foregoing type of deterioration at the end of 4 to 6 months of storage, depending upon the conditions of processing.

(4) Only the fat from butter churned from cream pasteurized at 71.1 and 76.6° C. and the pure fat retained their abilities to resist deterioration in the re-emulsification test at the end of two years storage at –17.7 to –16.1° C.

ACKNOWLEDGEMENTS

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LACTATING FACTORS FOR DAIRY COWS IN DRIED GRAPEFRUIT PEEL

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Recent reports by Huffman et al. (2, 3) have shown that dairy cows fed alfalfa alone declined abnormally in milk production. A marked increase was obtained by supplementing the alfalfa with corn silage (2) or beet pulp or corn gluten meal (3). These feeds were claimed to contain unknown milk stimulating factors. Because of the interest throughout the citrus-producing states in the use of dehydrated citrus by-products in dairy rations (1, 4, 5), experimental work was conducted to ascertain the value of dried grapefruit peel as a source of these milk stimulating factors.

EXPERIMENTAL PROCEDURE

Two sources of dried grapefruit peel[1] were used in the feeding trials. One was sun-dried in the open desert. No juice was removed from the peel previous to drying. The other was dried mechanically. Part of the juice was removed before dehydration.

Four pairs of cows were placed into two lots. One of each pair eventually received sun-dried peel and its mate mechanically dried peel. Each pair was identical in breed and nearly identical in period of lactation and age. Numbers 56, 57, 60 and 61, Guernseys, were fresh, 40, 42, 16 and 17 days, respectively, when placed on experiment; 158 and 159, Jerseys, 41 days; and 257 and 272, Holsteins, 50 and 33 days, respectively. Numbers 60, 61 and 257 were first-calf heifers and the remainder were second-calf heifers.

As soon as the cows were placed on experiment, they were fed first-cutting alfalfa hay ad libitum in dry lot until milk production markedly decreased. This period was of 6 weeks duration for 158, 159 and 272 and 9 weeks for the remaining five animals. At the end of this period, the alfalfa was supplemented with 2 lb. of dried grapefruit peel twice daily for each cow for 4- to 5-week periods. After this, the dried citrus was replaced with an equal amount of a grain mixture consisting of six parts barley, six parts wheat bran, two parts cottonseed meal and two parts beet pulp. After 4 weeks on this ration they were supplemented with oat pasture. Four lb. of the grain mixture furnished approximately 2.91 therms of energy and 4 lb. of the dried grapefruit peel approximately 2.98 therms.

RESULTS AND DISCUSSION

The milk production records are given in table 1. In every case there was a decline in milk production during the alfalfa feeding period. When supple-

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[1] We are indebted to Mr. E. G. Harrington, Exchange Orange Products Company, Tempe, Arizona, for generous supplies of dried grapefruit peel.

TABLE 1

Effect of dried grapefruit peel on milk production (Av. daily production during test week)

Feeding period	Week of period	Sundried grapefruit peel fed: Guernseys (av. of 56 & 60)	Sundried grapefruit peel fed: Jersey 159	Sundried grapefruit peel fed: Holstein 257	Mechanically dried grapefruit peel fed: Guernseys (av. of 57 & 61	Mechanically dried grapefruit peel fed: Jersey 158	Mechanically dried grapefruit peel fed: Holstein 272
		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Alfalfa alone (*6–9 weeks*)	First	30.7	25.4	36.7	31.1	33.3	43.4
	Last	20.9	15.7	26.7	19.7	19.0	19.4
Alfalfa + 4 lb. citrus daily (*4–5 weeks*)	First	22.2	16.7	28.7	20.9	18.5	23.4
	Last	22.4	15.6	29.2	20.8	18.5	20.9
	Av.	22.4	16.1	29.0	20.8	18.2	21.7
Alfalfa + 4 lb. grain mixture daily (*4 weeks*)	First	21.4	15.5	27.0	19.9	18.4	17.5
	Last	20.5	13.6	23.6	17.4	15.3	16.4
	Av.	20.4	15.0	25.4	19.0	16.9	17.5
Alfalfa + grain mixture + pasture (*3 weeks*)	First	21.8	13.4	25.3	20.7	17.5	
	Last	23.0	13.9	27.3	21.4	17.2	
	Av.	22.6	13.8	26.0	21.4	17.3	

ments of either source of dried grapefruit peel were given, there was a small but definite increase in milk production for seven of the eight cows. In the case of Jersey cow 158, the dried peel only lessened the decline caused by the alfalfa ration. However, milk production remained constant for this cow during the 5-week period in which the peel was fed. There was no significant difference between the results received with either of the two sources of dried grapefruit peel.

In order to eliminate the effect of energy in the dried grapefruit peel upon milk production, the dried peel was removed and an equal amount of grain mixture given. This grain mixture was approximate in energy to the dried grapefruit peel. Milk production definitely fell off in the first week of this period except for the two Jerseys. During this total period of 4 weeks, there was a definite decline in milk production for all the cows. This proved that the increase caused by the dried peel was not caused by added energy. When the cattle were

TABLE 2

Weight records of cows

Period of feeding	Weight of cow no. 56	60	159	257	57	61	158	272
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Alfalfa alone								
At start	973	908	885	1133	942	780	843	1443
At end	982	853	873	1132	973	752	798	1490
End of alf. + 4 lb. citrus	985	878	890	1107	912	755	812	1527

placed on pasture there was a definite increase in milk production except for Jersey 159.

The weight records of the cows are given in table 2. During the alfalfa feeding period, there was no significant change in weight. Three of the cows gained weight, four lost weight and one remained the same. During the period of citrus feeding four of the cows definitely gained weight, two remained the same and two lost weight.

From the above results, it is apparent that both sundried and mechanically dried grapefruit peel contain the unknown milk-stimulating factors first demonstrated in certain feeds by Huffman et al. (2, 3). The grain mixture fed did not contain these factors in appreciable amounts, while oat pasture did. The findings give emphasis to the value of dehydrated citrus products in dairy rations.

SUMMARY

Four lb. daily of dried grapefruit peel added to an alfalfa hay ration increased milk production. An equal amount of a grain mixture did not maintain this increase. Supplementing a ration of alfalfa hay and concentrate mixture with oat pasture definitely increased milk production. It is concluded that dried grapefruit peel contains factors which stimulate milk production in dairy cows.

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THE NUTRITION OF THE NEWBORN DAIRY CALF
III. THE RESPONSE TO A PHOTOLYZED MILK DIET

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A series of reports from the Illinois Agricultural Experiment Station recently have contributed significantly to the fundamental information on the nutrition of the very young calf. Wiese *et al.* (17) prepared a "synthetic milk" which resulted in normal growth when fed to calves from 48 hours to 12 weeks of age. When riboflavin was omitted from this "synthetic milk", deficiency symptoms developed in from 2 to 6 weeks (18). Since these experiments have shown that the very young calf requires a dietary source of riboflavin, it was of interest to study the effects of feeding natural milk in which a major portion of the riboflavin had been destroyed.

Numerous reports in the literature (4, 5, 7, 13, 14, 19) have shown that significant amounts of the riboflavin of milk are destroyed by exposure to sunlight. Preliminary work showed that under proper conditions of sunlight exposure sufficient quantities of the riboflavin in colostrum and milk could be destroyed to yield a product which was extremely low in this vitamin. Studies then were begun to determine the effect of feeding photolyzed colostrum and milk to newborn or very young calves.

EXPERIMENTAL

Four purebred, male, Guernsey calves were used in this experiment. Two of these calves (*A* and *B*) were taken from their dams at birth and placed in 6 × 6-foot wooden box stalls with wire mesh flooring. Calf *A* was bedded with straw. All subsequent calves were bedded on burlap sacks to eliminate the possibility of the ingestion of straw stimulating rumen synthesis of riboflavin. Calves *A* and *B* were fed for 3 days on colostrum which had been photolyzed previously, frozen and stored[1] for over 3 weeks. The two remaining calves, *C* and *R*, twin males, were fed normal colostrum from their dam for 72 hours. Following the colostrum feeding period all calves received, as an exclusive diet, photolyzed whole milk from the Ohio State University herd at the rate of 10 per cent of their body weight. The milk intake was reduced during periods of scouring. Calf *R* received, in addition to the milk, an average of 2.99 mg. of crystalline riboflavin per day. The calves were fed twice daily from nipple pails. The milk was heated to 37° C. before feeding, and the volume of milk fed was recorded. The calves were housed in a steam-heated calf barn and weighed at weekly intervals.

Riboflavin estimations were made on samples of the milk from each feeding, using a modification of the fluorimetric technique reported by Hand (3). Blood plasma vitamin A determinations were made periodically using the procedure of

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[1] Stored in frozen food containers generously provided by by the Lily Tulip Cup Corporation, New York, N. Y.

Kimble (6). The method of Boyer *et al.* (1) was followed in estimating the vitamin A content of the milk and colostrum.

Approximately once each week, the experimental calves were placed on a 2 × 6-foot metabolism cage with a wire mesh floor and a 24-hour urine sample collected. A rubber feces-sack was attached to the calf to prevent contaminating the urine with feces. The urine was collected in a brown-glass bottle containing approximately 20 ml. of concentrated HCl. The final pH of the urine usually was about 1.5. The total volume was measured, and a representative sample was adjusted to pH 1.0, frozen and stored until assayed for riboflavin. The storage time averaged about 1 week, but in one case was as long as 6 weeks. Riboflavin determinations were made on the stored urine, using the fluorimetric technique of Slater and Morell (12). Recent work by these authors (11) has shown that human urine stored at a low temperature and a low pH gave lower riboflavin values upon analysis than when stored at a higher pH and at room temperature. No major discrepancies were observed in our results, however.

Since sunlight could not be depended upon as a constant source of photolyzing

TABLE 1

The effects of the photolysis treatment on the vitamin A, carotene and riboflavin of milk

Milk sample no. and treatment	Vitamin A		Carotene		Riboflavin	
	γ/liter	% loss	γ/liter	% loss	mg./liter	% loss
1. None	264		289		1.42	
1. Photolyzed	46	82.5	252	12.8	0.05	96.5
2. None	203		321		1.54	
2. Photolyzed	49	75.7	287	10.6	0.06	96.1

energy, a DH-1 400 watt mercury vapor lamp[2] was secured. This lamp emitted rays longer than 3000 Å, thereby eliminating excessive irradiation at the wave length of vitamin D activation. The lamp was used in conjunction with a highly polished parabolic aluminum reflector, and the rays were directed at a 2-gallon rectangular museum jar containing the milk. The milk was agitated slowly by continuous stirring. About 96 per cent of the riboflavin was destroyed by a 3-hour exposure. During the photolyzing period, the milk usually was allowed to reach a temperature of at least 60° C. for one-half hour as a bacteria-control measure.

The photolyzed milk was chalky white in appearance and had a pronounced "sunlight flavor". The milk seldom was over 36 hours old when fed and in all cases was kept under continuous refrigeration except during the period of light treatment. Vitamin A and carotene, as well as riboflavin, were destroyed in appreciable quantities, as shown in table 1. It will be noted that a treatment which destroyed over 96 per cent of the riboflavin also reduced the vitamin A by 75 to 80 per cent and the carotene by 10 to 12 per cent of their original quantities.

The biological inadequacy of the photolyzed milk was verified by a rat growth

[2] Purchased from the Westinghouse Electric Corporation.

test. Twenty weanling albino rats were divided equally into four groups as to size and litter. An attempt was made to distribute them equally as to sex, but one group (group 4) had three males and two females while all other groups had three females and two males. The groups were fed ad libitum the diets, as shown

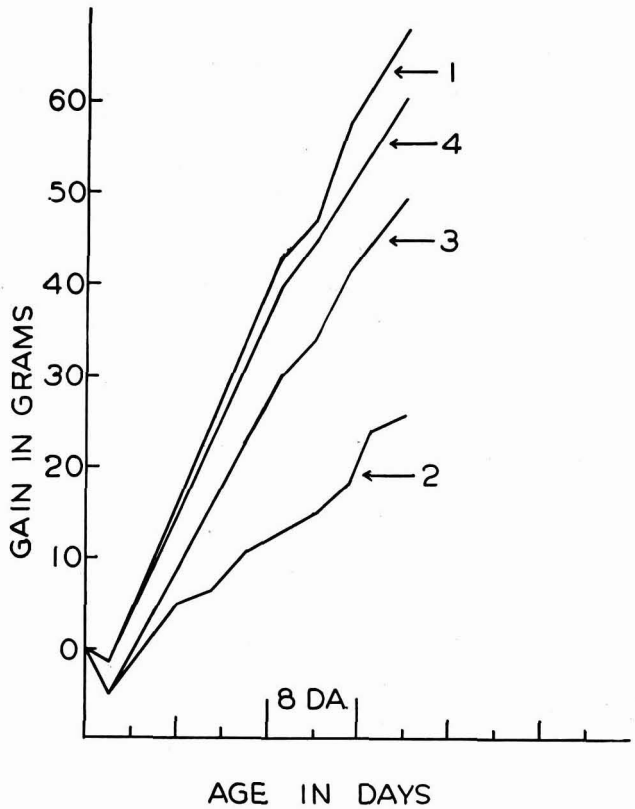

FIG. 1 Growth curves of rats fed the various mineralized milk diets:

Group	Diet	Grams gain/100 ml. consumed
1	Normal milk	6.10
2	Photolyzed milk	3.91
3	Photolyzed milk + riboflavin	5.22
4	Photolyzed milk + riboflavin + codliver oil	6.06

One drop of a mineral mixture solution containing 500 mg. each of ferric citrate, manganous sulphate and copper sulphate in 500 ml. was added to each feeding.
Riboflavin in sufficient quantity to approximate that of normal milk was added to the milk of groups 3 and 4.

in the legend of figure 1. The respective rates of growth are recorded graphically (fig. 1). The gain per 100 ml. of milk consumed also is shown. Obviously the photolyzed milk had a low biological value for growth, and when such milk was

supplemented with both riboflavin and vitamin A the response was similar to that obtained from normal milk. The amount of gain per 100 ml. of milk consumed was almost identical (6.10 g. and 6.06 g., respectively, for Groups 1 and 4) indicating that the difference in the rates of growth was not due to a difference in nutritive value of the milk consumed but to a difference in total consumption. As the photolyzed milk had a pronounced "sunlight flavor", it is probable that the palatability of the photolyzed milk was lowered, and this in turn reduced the consumption.

Because of the low vitamin A content of the photolyzed milk, supplemental vitamin A was fed to each calf. The vitamin A oil was mixed with soya-lecithin

TABLE 2

Average daily riboflavin intake in milligrams

Age	Riboflavin intake values for Calf						
				R			
	A	B	C	In photolyzed milk	Riboflavin added	Total intake	If fed normal milk[c]
	(γ)	(γ)	(γ)	(γ)	(γ)	(γ)	(γ)
1 day	1.24	2.05	?[b]	?[b]	.....	?[b]	.....
2 days	0.82	0.71	?[b]	?[b]	.....	?[b]	.....
3 "	0.64	0.29	?[b]	?[b]	.....	?[b]	.....
4–7 days	0.23	0.18	0.15	0.15	2.63	2.78	3.69
2 wks.	0.22	0.13	0.11	0.10	2.46	2.56	3.45
3 "	0.21	0.07	0.13	0.13	2.71	2.84	3.80
4 "	0.23	0.13	0.14	0.14	2.59	2.73	3.63
5 "	0.15	0.11	0.12	0.16	2.90	3.06	4.06
6 "	0.15	0.10[a]	0.17	0.18	3.01	3.19	4.22
7 "	0.17	0.15[a]	0.19	0.22	3.37	3.59	4.72
8 "	0.17	0.17[a]	0.15	0.17	3.61	3.78	5.05
9 "	0.12	0.20	0.10	0.18	3.47	3.65	4.86
10 "	0.22	0.23					
11 "	0.15	0.19					
12 "		0.16					
Average, 4th day to termination	0.18	0.15	0.14	0.16	2.99	3.13	4.16

[a] Calf B was fed 2 mg./day in addition to that present in the milk.

[b] Calves were fed normal colostrum from their dams. No record was kept of riboflavin intake.

[c] Calculated on the basis of the calf's intake of normal milk containing 1.4 mg. of riboflavin per liter.

(2) and a small amount of photolyzed milk in a Waring Blender. The amount of vitamin A fed varied from 5,000 to 25,000 I.U. daily.

The average daily riboflavin intake for each calf is recorded in table 2. During the colostrum feeding period, calves *A* and *B* received 2.7 and 3.0 mg. of riboflavin, respectively. This is less than 11 per cent of the amount found in an equal quantity of average Guernsey colostrum (16). Since calves *A* and *R* were not assigned to the experiment until 3 days of age, their riboflavin intake during the colostrum feeding period is unknown. From the fourth day to the termination of the experiment the daily riboflavin intake averaged about 4 per

cent of a normal intake with the exception of calf *R*. Assuming that normal milk contains about 1.4 mg./liter (an average found in our laboratory), this latter calf received approximately 72 per cent of the amount normally found in the milk prior to photolysis.

The growth rate of the calves is shown graphically in figure 2, with the Ragsdale standard (8) included for comparison. The growth of calf *A* was normal and uneventful for the first 4 weeks, except for a brief period of scours during the first week which responded to sulfathaladine medication. From the fourth week, the calf suffered from intermittent scours which showed little improvement on sulfathaladine treatment. The quantity of milk fed was reduced at each

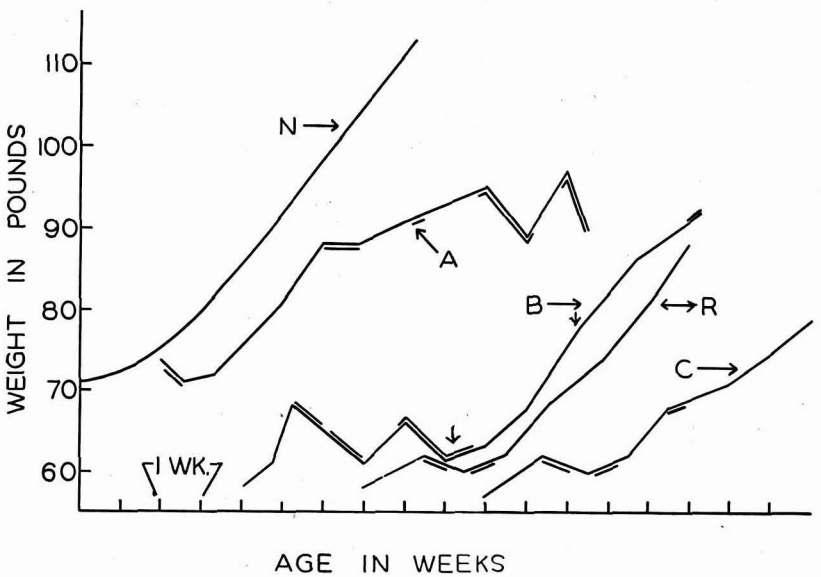

FIG. 2 Growth curves of calves used in this experiment. Double line indicates period of diarrhea. (N = Ragsdale standard, other letters refer to calves as designated in the text).

period of scours, and the calf never was able to consume the full allotment without scouring from the fourth week. An increasingly unthrifty condition developed, and obvious symptoms of riboflavin deficiency appeared at about 6 weeks. There were periods of excessive salivation and lacrimation. The haircoat became rough; there was evidence of a mild dry scaly dermatitis and a generalized alopecia which was more pronounced about the eyes and muzzle. A blood plasma ascorbic acid determination at 10 weeks showed 0.42 mg. per 100 ml., which was considered to be within the normal range. Periodic blood plasma vitamin A determinations (see table 3) showed normal levels, with the possible exception of the last two, which were taken towards the end of the experiment. These low values were considered to result from, rather than to be the cause of, the poor physical condition. Riboflavin excretions are shown in table 4. It will be noted that they remained low throughout the experiment.

TABLE 3

Blood plasma vitamin A of the calves used in the experiment

Age	Blood plasma vitamin A values for Calf:				
	A	B	C	R	Normal[c]
	(γ/100 ml.)	(γ/100 ml.)	(γ/100 ml.)	(γ/100 ml.)	(γ/100 ml.)
Birth	.....	0.41	.....	.....	3.3
2 day	4.86	.....	.....	.....	.....
3 "	.....	15.86	11.43	13.36	14.7
4 "	4.99	.....	.....	.....	.....
1 wk.	19.30	.....	.....	.....	13.1
2 "	.....	13.40	.....	.....	12.3
3 "	12.77	9.87	11.78	12.16	10.1
4 "	.....	.....	.....	.....	9.0
5 "	12.45	.....	10.75	10.75	.....
6 "	10.90	.....	8.90	8.99	.....
7 "	.....	12.60	8.79[a]	12.15[a]	.....
8 "	.....	.....	9.49	13.45	9.7[d]
9 "	.....	13.8	6.33[b]		.....
10 "	8.88	15.0			.....
11 "	9.96	12.1			.....
12 "					12.9[d]

[a] Vitamin A supplement was increased from 5,000 I.U. per day to 10.000 I.U. per day.
[b] Level determined 3 days after preceding determination while animal was in a state of collapse.
[c] Based on values obtained in this laboratory from nine Guernsey calves.
[d] Based on average values from seven calves.

Calf *A* was sacrificed at 10.5 weeks of age and a gross post mortem examination showed marked catarrhal enteritis, mild edema of the cerebrum and "white spotted" kidney. One cornea was pebbled in appearance similar to that reported by Street et al. in the riboflavin deficient dog (15). The rumen contained approximately 1 liter of macerated straw and fluid. The rumen fluid contained 0.22 mg. per liter of riboflavin, which is about three times the concentration found in the milk consumed. Incubation of a portion of the rumen contents for 16 hours

TABLE 4

Urinary excretion of riboflavin by the calves used in the experiment

Age	Urinary riboflavin excretion by Calf:			
	A	B	C	R
	(mg./day)	(mg./day)	(mg./day)	(mg./day)
24 hr.	0.30	.....	.....	.....
36 hr.	.....	0.10	.....	.....
1 wk.	0.05	.....	0.86	0.76
2 wk.	0.01	0.01	.....	.....
3 wk.	0.01	.....	.....	.....
4 wk.	0.07	.....	0.02	0.44
5 wk.	.....	0.01	.....	.....
6 wk.	0.03	.....	0.05	0.60
7 wk.	.....	0.05[a]	0.05	0.38
8 wk.	0.03	0.09[a]	.....	0.64
9 wk.	.....	0.04		
10 wk.	.....	0.03		
11 wk.	0.03			

[a] Received 2 mg. of crystalline riboflavin added to its diet during this period.

at 37° C. resulted in a 33 per cent increase in riboflavin concentration. These findings are considered as evidence of some microbiological synthesis, although insufficient in amount to fully protect the calf.

Calf *B* grew normally (fig. 2) for 1.5 weeks. Intermittent scours then resulted in poor growth until it was 5.5 weeks of age. At this time, the calf had been scouring for 9 days and was extremely unthrifty. It had a rough haircoat, a scaly dermatitis and mild alopecia which was more pronounced around the head. When a reduction in milk together with sulfathaladine treatment failed to improve the diarrhea, 2 mg. of crystalline riboflavin per day were added to the photolyzed milk for a period of 3 weeks. This addition to the diet resulted in the cessation of scours within 3 days, marked improvement in general appearance including the growth of new hair and the cessation of the excessive salivation and lacrimation. The amount of riboflavin excreted increased as shown in table 4. Growth was resumed almost immediately, and for the period of supplementary feeding it compares favorably with the Ragsdale standard. The

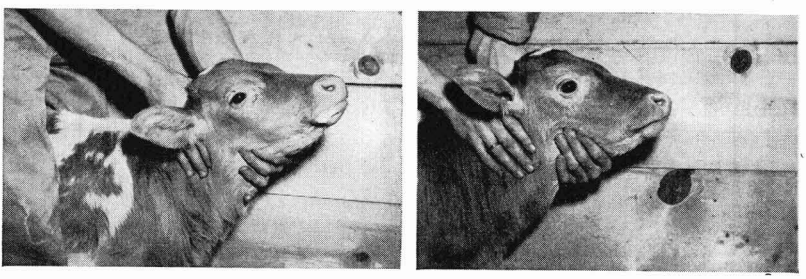

FIG. 3 Left, Calf *B* at 35 days of age, 3 days before 2 mg. of riboflavin were added to its daily diet. Note the excessive salivation. Right, The same calf at the conclusion of the 3 week supplement feeding period.

improvement of the deficiency symptoms following riboflavin therapy is shown in figure 3. Blood plasma vitamin A was within the normal range throughout the experiment, as shown in table 3. The blood plasma ascorbic acid at 9 weeks of age was 0.44 mg. per 100 ml. and was considered to be normal. Post mortem examination showed mild catarrhal enteritis as the only gross pathology.

The twin calves (*C* and *R*) differed by 1 lb. in weight at birth and critical body measurements were identical. They responded in an identical manner for the first 4.5 weeks, (fig. 1). At this point, the growth rate of calf *C* became slower while calf *R*, which was receiving a continuous daily riboflavin supplement, grew at a rate which approached the Ragsdale standard. No difference between these calves could be detected until after 4.5 weeks of age. From this time on, calf *C* was distinctly more lethargic, showed increased salivation and lacrimation, a dry scaly dermatitis and alopecia, particularly around the eyes and base of the ears. Calf *R* appeared completely normal.

About 2 weeks before calf *C* was sacrificed, it had marked difficulty in swallowing. It had to release the nipple several times during each feeding to clear its throat. About 48 hours prior to exsanguination it refused its feed. Thirty

hours later it was found in a state of acute collapse. It could not walk but was able to stand for a few seconds. A thin, watery salivation was excessive. The calf failed to respond to an intravenous injection of 500 ml. of "Intragel"[3]. Five mg. of riboflavin in physiological saline were injected intravenously 5 hours later. The calf was still alive 12 hours later but not noticeably improved in condition. At this point, the calf was destroyed and tissues obtained for microscopic study. The gross pathology consisted of marked catarrhal enteritis, a mild edema of the lungs and a few scattered spots on the kidney. No evidence of pneumonia was observed.

No gross pathology was found in calf *R* following exsanguination approximately 3 weeks later.

At 6 weeks of age the vitamin A supplement of both calves *C* and *R* was increased from 5,000 to 10,000 I.U. per day. It will be noted from table 3 that calf *C* failed to respond. This is further evidence that the low blood plasma vitamin A resulted from the riboflavin deficiency syndrome, probably the severe enteritis. Blood plasma ascorbic acid was within the normal range in both calves at 6 weeks of age (0.45–0.46 mg./100 ml.). Calf *R* gained 19.5 lb. per 100 l. of milk consumed, while calf *C* gained only 15.7 lb. on a similar amount. Thus, the difference in rate of growth can not be attributed entirely to the difference in the amount of milk consumed.

The residual effect of the high riboflavin intake during the colostrum feeding period is shown by a relatively high excretion in both calves at 1 week of age. Following this, the excretion in calf *C* decreased to a low level (see table 4) while that of calf *R* was maintained at a level 7 to 12 times higher for the remainder of the experiment. A single 24-hour urine collection from a 6 weeks old Guernsey calf which had been fed milk, hay and grain in the usual manner showed an excretion of 2.86 mg. per day. No clearcut lesions of the lips, gums or eyes, with the exception of one pebbled cornea, were observed in any of the calves in this experiment.

The process of photolysis no doubt destroyed other vitamins, particularly ascorbic acid and pyridoxine. The partial or complete destruction of these was not considered as a complicating factor in these experiments for the following reasons: It has been shown that calves do not require a dietary source of ascorbic acid (17). A marked poikilocytosis which responded to pyrodoxine treatment has been described in adult cattle (9, 10). A few poikilocytes were found in the blood of only one calf in this experiment (calf B), and the number was no larger than one might expect to find in the mild anemia from milk feeding.

An approximate riboflavin requirement for a very young calf was determined from the data presented. Calf *R* made normal response to a diet of 3.13 mg. per day and grew from a weight of 58 lb. to 89 lb. or a mean of approximately 70 lb. Therefore, this calf responded normally to an average daily intake of 94 γ per kg. of body weight. Calf B responded at 5.5 weeks of age to an average daily

[3] (8 g. of pyrogen-free gelatin per 100 ml. of 0.85 per cent saline) manufactured by Fort Dodge Laboratories Inc., Fort Dodge, Ia.

riboflavin intake of 2.15 mg. or, based on the weight of the calf (61 lb.), 77.4 γ per kg. of body weight. Since in vitamin deficiency diseases the amount required to cure the disease is usually larger than the amount required as a preventive, it seems logical that the growth requirement is even less than 77.4 γ per kg. of body weight. If calves were fed normal milk containing 1.4 mg. per liter at the rate of 10 per cent of the body weight, they would receive 140 γ per kg. which is almost twice the amount that was necessary to cure the symptoms of calf *B*. Thus, it seems that the possibility of a riboflavin deficiency developing during the milk feeding period is slight.

SUMMARY AND CONCLUSION

Approximately 96 per cent of the riboflavin in milk fed was destroyed by exposure to the radiations of a 400 W. mercury vapor lamp emitting light of wave lengths longer than 3,000 Å. Appreciable quantities of vitamin A and carotene also were destroyed. Four male Guernsey calves were fed this treated milk supplemented with adequate vitamin A. One of these calves also received approximately 2.99 mg. of added riboflavin daily.

Riboflavin deficiency symptoms consisted of erratic growth, intermittent diarrhea, a dry scaly dermatitis, alopecia, particularly about the head, periodic excessive salivation and lacrimation and in the acute stages, dysphagia and a peculiar collapse syndrome (one calf). Post mortem examinations showed evidence of catarrhal enteritis, mild edema of the lungs, (the collapse victim), pebbled cornea (one calf), mild edema of the cerebrum (one calf) and abnormalities of the kidney in two cases. These calves were extremely unthrifty. The addition of 2 mg. of riboflavin daily to the diet of one of these calves resulted in a prompt cessation of diarrhea, resumption of growth and a marked improvement in general appearance, including the growth of new hair. No other lesions of the lips or mouth or abnormalities of the eyes were noted.

The performance of the calf receiving 2.99 mg. of added riboflavin from the start was uneventful and approached the Ragsdale standard of growth.

Blood vitamin A and ascorbic acid levels were normal in all four calves. The urinary excretion of riboflavin varied from 0.01 to 0.06 mg. per day for calves receiving the treated milk with no added riboflavin and 0.38 to 0.64 mg. per day for the calf which received added riboflavin throughout the experiment.

The limited data of this experiment indicate that the minimum daily riboflavin requirement of the very young calf is somewhat less than 75 γ per kg. of body weight. The possibility of riboflavin deficiency during the milk feeding period is remote.

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SOME CHANGES IN DRY WHOLE MILK DURING STORAGE[1]

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Numerous changes other than the oxidation of the lipides are known to occur in dry whole milk during storage. Those in flavor are most apparent, although changes which can be measured more objectively may be important and would be most valuable as indices of deterioration if they could be shown to be associated with flavor.

The general factors influencing the development of a tallowy flavor in dry whole milk now are well known. Actual talloviness does not develop unless more than 0.3 ml. of oxygen per gram of powder is available, although Lea *et al.* (15) and Coulter (4) have shown that lower oxygen levels improve the keeping quality. Flavor deterioration is most rapid during the initial period of storage and once the oxygen has been exhausted from the free-space gas, low-moisture powder becomes almost stable in quality at normal storage temperatures (4, 15).

Stale and allied flavors in dry whole milk have never been exactly defined. Supplee (20), Tillmans and Strohecker (22), Hunziker (12) and many others in the industry have recognized stale, musty or gluey flavors believed to be associated with the protein fraction of the milk and have presented evidence that these flavors become more marked in high moisture powders. Lea *et al.* (15) noted the appearance, in powder stored at 47 and 37° C. for some time, of a flavor variously described as "heated," "burnt," "scorched" or "cooked". This flavor was considered to consist of two components, (a) a "burnt" or "caramel" taste associated with the protein or carbohydrate, and (b) a "butter-toffee" flavor associated with the fat.

Loss of solubility and browning accompany staling (12, 15). Doob *et al.* (8) have published extensive data on the influence of moisture on the browning of dried whey and skimmilk. Tarassuk and Jack (21) have reported on the browning reaction in whole milk powder and ice cream powder since this investigation was completed. McCreary (16) has noted a decrease in soluble lactose in dried milk stored for a year at room temperature.

Lea *et al.* (15) noted the disappearance of oxygen in sealed cans of both skim and whole milk powders and the production of carbon dioxide.

Chapman and McFarlane (3) developed a method for determining acid ferricyanide reducing substances in dry milk and reported that they increased in dry whole milk stored in contact with the atmosphere. Modifications of the method and indications as to the source of the reducing materials have been presented by Lea (14) and Crowe *et al.* (6).

The production of fluorescent materials has been demonstrated to be asso-

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ciated with the deterioration of certain foods. Jenness and Coulter (13) have reviewed the literature on this subject and have suggested a method for evaluating the fluorescence characteristics of dry milk based on successive extractions with (a) 67 per cent acetone, (b) 20:80 acetone—ether and (c) 10 per cent KCl, and determinations of the blue fluorescence of each solution.

The browning reaction recently has been reviewed by Cantor *et al.* (2). Maillard, in the original publications concerning the reaction which now bears his name (Maillard or browning reaction), reported that the evolution of carbon dioxide, production of water (12 moles of water for every mole of carbon dioxide) and the formation of water-insoluble products accompanied the browning. Maillard also concluded that atmospheric oxidation played no part in the reaction.

Pearce (18) compared the suitability of a number of objective tests with subjective scores of palatability, and concluded that palatability was the most precise. This conclusion is not surprising considering the complexity of the system and the deficiencies in information concerning the deteriorative changes which occur.

The purpose of this research was to study changes which occur in dry whole milk during storage other than those associated with the lipides, and to secure some information on the effects of moisture, oxygen and temperature on these changes.

MATERIALS

Some observations were made using simplified systems as described by Jenness and Coulter (13). Briefly, these were prepared from acid-precipitated casein, dialyzed-milk serum protein, filtered milk fat, a concentrate of fat-globule "membrane" from washed cream and commercial samples of lactose and ascorbic acid and were dried from the frozen state.

Unless otherwise noted the experimental work to be reported on dry whole milk involved two large lots of powder, one (lot 384) of commercial origin and the other (lot K33) manufactured in an experimental spray drier in the University laboratories, using a standard procedure comparable to commercial practice. Portions of each lot were adjusted by exposure to humid air to secure samples varying in moisture content from 2.0 to about 5.0 per cent. The samples were canned and nitrogen-packed to secure oxygen levels ranging from approximately 1.0 to 6.0 per cent. Samples at each moisture and oxygen level were stored at 20, 37 and 60° C. The samples of the commercial powder stored at 60° C. were lost due to failure of the heat regulator of the incubator. The samples stored at 60° C. were analyzed at 10-day intervals up to 50 days, those at 37° C. at 4-week intervals up to 16 or 20 weeks and those at 20° C. at 8-week intervals up to 32 weeks. Some data were secured on other lots of dry whole milk manufactured in the experimental drier.

EXPERIMENTAL

Change in flavor on storage. Over 1,000 samples of dry whole milk representing both commercial and experimental production were scored by a selected

panel of five judges. A flavor described as "burnt feathers" was recognized in 84 samples. In only 9 instances was this criticism used in describing the flavor of dry whole milk containing less than 2.0 per cent moisture. Unless obscured by tallowiness or the caramelized flavor of powder which had become "brown," it was recognized in virtually all samples of stored powder containing more than 3.0 per cent moisture. Although it appeared more rapidly at the higher temperature, it was recognized in dry whole milk stored at 20, 37 and 60° C.

The flavors of the stored powders in lots 384 and K33 are typical. After 10 days at 60° C. the powder at the two highest moisture levels (3.81 and 4.49 initial) was brown and had the characteristic caramelized flavor. After 8 weeks at 37° C. and 16 weeks at 20° C., all samples, regardless of oxygen level, which contained more than 2.5 per cent moisture, were criticized as having the burnt feathers flavor. At the lower moisture levels, the samples were criticized as stale and finally tallowy at oxygen levels above 4.0 per cent.

In an attempt to determine the source of the burnt feathers flavor, frozen-dried simplified systems were prepared consisting of calcium phosphocaseinate with and without the addition of one or more of the following constituents: lactose, butterfat, serum protein, fat-globule membrane material and ascorbic acid. These were stored at 37° C. over sulfuric acid-water mixtures to obtain vapor pressures comparable with dry whole milk of low (2.5 per cent) and high (5.0 per cent) moisture. The calcium phosphocaseinate systems remained virtually unchanged in flavor. Those containing the phosphocaseinate and lactose, either with or without the addition of the other constituents, acquired the characteristic burnt feathers odor at the higher vapor pressure, and a characteristic stale flavor at the lower vapor pressure. The burnt feathers flavor therefore appears to be associated with lactose-protein changes and appears in high-moisture but not in low-moisture powder. The characteristic stale flavor that develops in normal dry whole milk probably is a composite of flavors resulting from lactose-protein changes and oxidation of the lipids. The flavor designated in this study as burnt feathers is probably the same as that described by others as "gluey."

Relation of moisture content to flavor deterioration. The importance of moisture content to the overall deterioration of gas-packed dry whole milk is shown graphically in figure 1. The samples involved are those gas-packed at the two lowest oxygen levels (below 2.0 per cent) from lots 384 and K33. Although, as shown by Lea *et al.* (15) and Coulter (4), the rate of loss in score of adequately gas-packed dry whole milk is not a straight line function of time but decreases with time, the total loss in score at any time interval can be used as an index of the overall rate of deterioration. The average weekly loss in score was computed from the difference between the original (fresh) score and the score after storage for the longest period of time, or in the case of the higher moisture samples at the higher storage temperatures, from the difference between original score and that at the last period at which an actual score was given. The logarithms of the weekly loss in score at 20, 37, and 60° C. with

one lot and at 20 and 37° C. with the other lot are shown in figure 1 plotted against the moisture content. The rate of loss in score appears to increase logarithmically with increase in the moisture content. Considerable variability in the rates of loss in score between the different lots of powder at any given moisture content and temperature is evident. It is realized that since the rate of loss in score of dry whole milk is not a straight line function of time, the comparisons made are empirical; however, the conclusions are believed to be

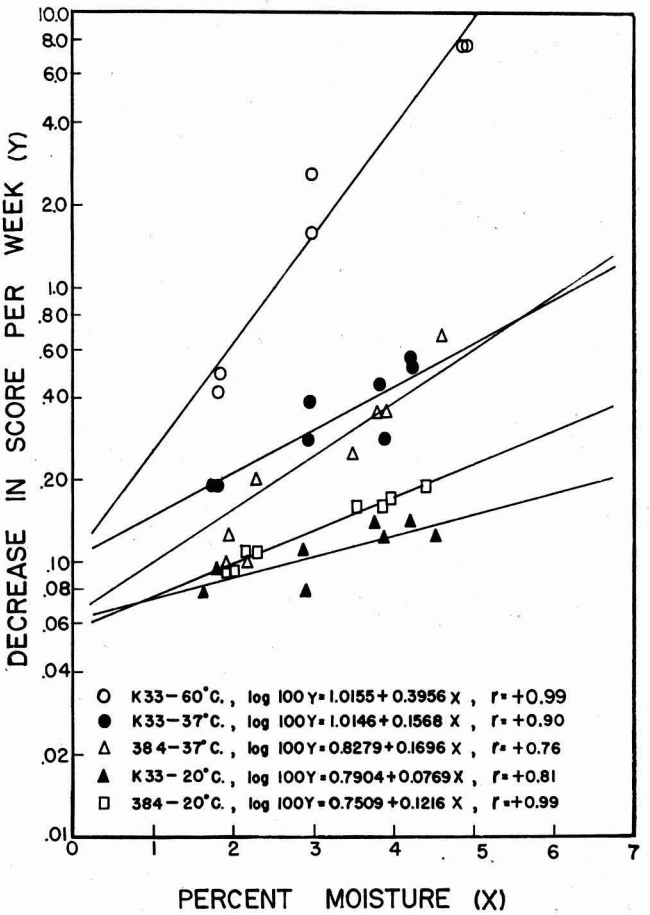

FIG. 1. The relationship of moisture content of dry whole milk to rate of loss in flavor score.

valid when dealing with adequately gas-packed dry whole milk. Holm and Greenbank (11) have shown that the minimum moisture content is not optimal in preventing talloviness of air-packed powder. Gyorgy *et al.* (9) and Williamson (23) have presented evidence indicating that certain antioxidants require moisture for effectiveness.

Acid ferricyanide reduction. The total and non-protein acid ferricyanide

reducing capacity was determined using the method described by Crowe *et al.* (6). Initial trials were made using 9 lots (162 samples) of spray-dried whole milk produced on the experimental drier. The moisture content of the lots was varied within the range of 1.32 to 4.78 per cent. In some instances moisture variation was secured by drying to different levels, in others by exposing portions of the powder in thin layers to a humid atmosphere. Part of the powder in each lot was packed in air in no. 2 cans, and part was gas-packed in

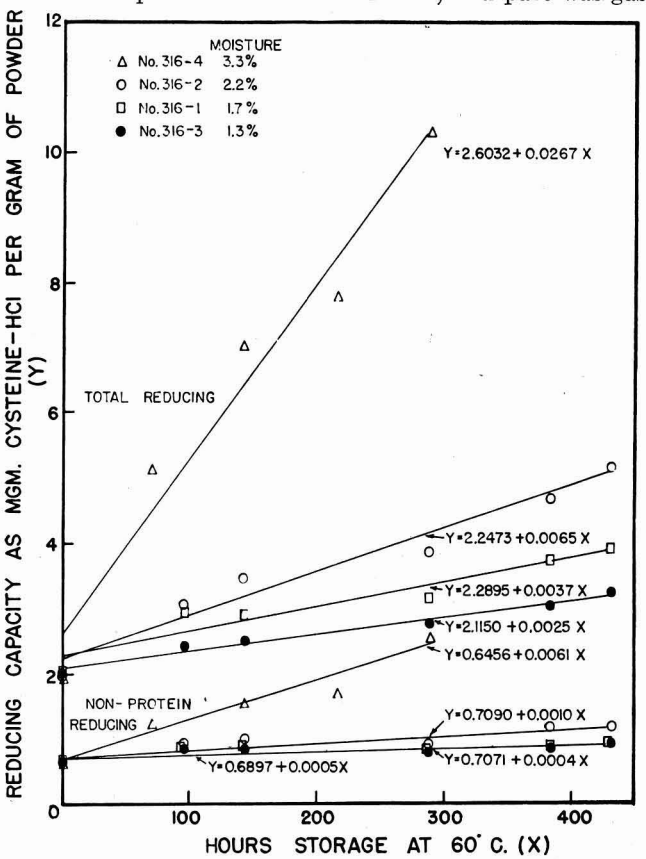

FIG. 2. Change in acid ferricyanide reducing capacity of dry whole milk stored at 60° C.

nitrogen to secure an oxygen level of less than 2 per cent. The powder was stored at 60 ± 1° C. and samples for analysis removed at intervals up to 432 hours.

The data for one group of samples which are graphed in figure 2 show an increase in both total acid ferricyanide reducing substances and non-protein acid ferricyanide reducing substances with time of storage at 60° C. Since the relationship appears to be essentially linear, the regression line for each powder was computed. The rate of production of acid ferricyanide reducing sub-

stances increases with increase in the moisture content of the powder. The data for the other samples (not presented) indicate a similar relationship.

To show in a more striking manner the relationship of the moisture content of the powder to the production of acid ferricyanide reducing substances, the log slopes of the regression lines were plotted against the average moisture contents of the samples (fig. 3). An increase in the moisture content of the

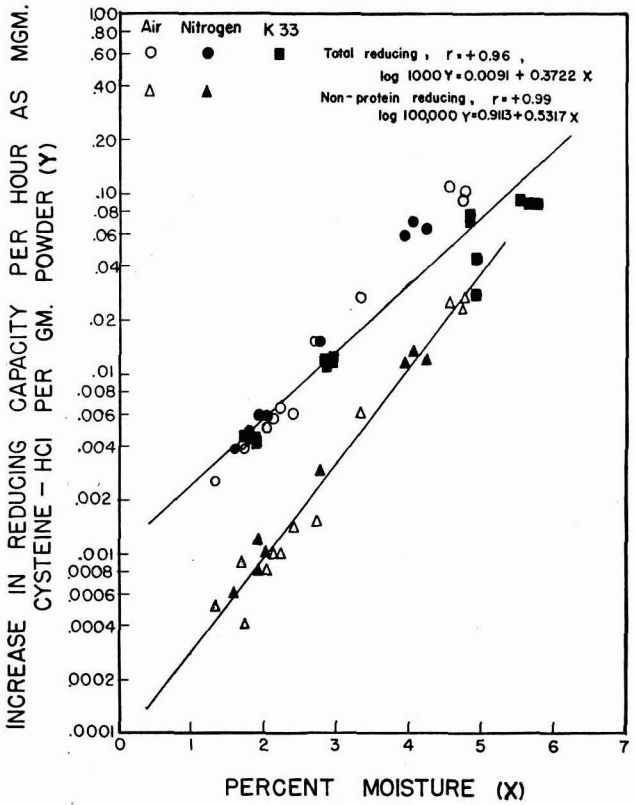

FIG. 3. The relationship of moisture content of dry whole milk to rate of increase in acid ferricyanide reducing capacity during storage at 60° C.

powder is accompanied by a logarithmic increase in the rates of production of both total and non-protein acid ferricyanide reducing substances. The data clearly indicate that the oxygen content of the atmosphere in contact with the samples is without effect on the rate of production of acid ferricyanide reducing substances.

The above observations were supplemented by data secured on lots 384 and K33. The results for the changes in the acid ferricyanide reducing capacity of the samples stored at 60° C. are plotted in figure 4. Since the oxygen level was without effect on the production of acid ferricyanide reducing substances, the

values shown for each moisture level are averages for the samples at the four oxygen levels.

The previously demonstrated linearity in the rate of production of acid ferricyanide reducing substances in dry whole milk stored at 60° C. is not maintained, particularly in the high moisture powders, on storage for longer than 10 days. In fact, a maximum may be reached followed by an actual reduction in the acid ferricyanide reducing capacity. This indicates a secondary reaction involving the non-oxidative utilization of the acid ferricyanide reducing substances.

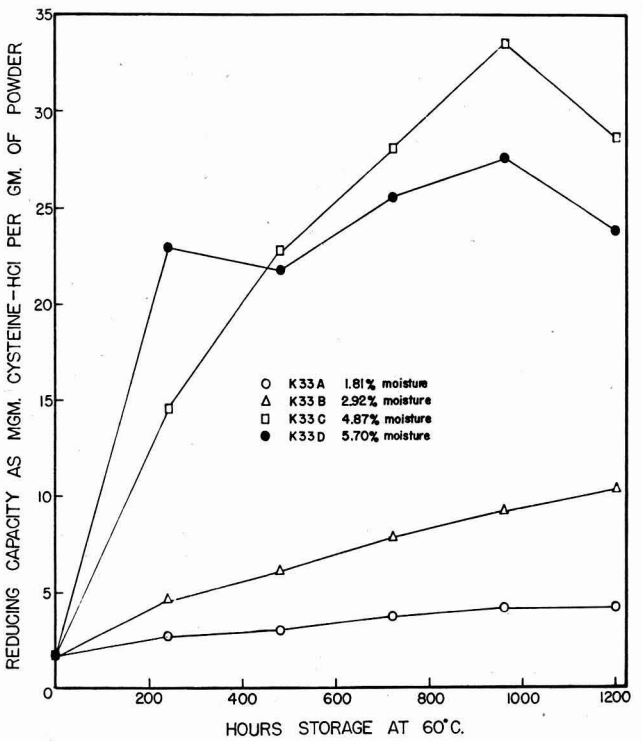

FIG. 4. Change in acid ferricyanide reducing capacity of dry whole milk stored at 60° C.

The rate of production of acid ferricyanide reducing substances in each sample was computed from the initial and 10-day values. These data plotted against moisture content in figure 3 confirm the relationship between the moisture content of the powder and the log of the rate of production of acid ferricyanide reducing substances during the initial storage period.

The data on the reducing capacity of the samples stored at 37 and 20° C. are summarized in tables 1 and 2. Since the oxygen level appears to have been without effect on the reducing capacity, only the average values for the samples at any given moisture level are shown. The initial reducing capacity of the

TABLE 1

Effect of storage at 37° C. on the acid ferricyanide reducing capacity of dry whole milk

% moisture	Reducing capacity (as mgms. cysteine—HCl/gm.)					
	Weeks storage					
	Initial	4	8	12	16	20
			Lot 384			
1.89	4.00	3.70	3.50	3.24	3.34	4.23
2.29	3.98	3.60	3.56	3.63	3.44	4.14
3.69	3.96	3.78	3.70	3.95	3.65	3.96
4.39	3.90	4.05	5.61	6.22	8.55	9.47
			Lot K33			
1.75	1.61	1.96	1.88	1.74	1.86	
2.82	1.75	1.97	1.86	1.91	2.08	
3.81	1.58	2.05	2.07	2.28	2.38	
4.33	1.61	2.28	2.16	2.86	2.72	

commercial powder (lot 384) was somewhat greater than twice that of the powder made in the experimental drier (lot K33). This difference may be due in part to a greater reducing capacity of the fluid milk, but probably is due primarily to differences in heat treatment during processing [see Crowe *et al.* (6)]. There was a slight decrease in the acid ferricyanide reducing capacity of the samples stored at 20° C., and except for the powder of the highest moisture content (4.39%), there was a slight decrease in the acid ferricyanide reducing capacity of the commercial powder up to 16 weeks storage at 37° C. The values at 20 weeks were about equivalent to those for the fresh powder. The acid ferricyanide reducing capacity of the samples highest in moisture increased materially during storage. The reducing effect of the low-moisture experimental powder samples was virtually unchanged during storage for 16 weeks at 37° C., but there was a definite, although minor, increase in the higher moisture samples.

TABLE 2

Effect of storage at 20° C. on the acid ferricyanide reducing capacity of dry whole milk

% moisture	Reducing capacity (as mgms. cysteine—HCl/gm.)				
	Weeks storage				
	Initial	8	16	24	32
		Lot 384			
1.90	4.00	3.46	2.97	3.32	3.11
2.32	3.98	3.49	2.94	3.32	3.05
3.81	3.96	3.51	3.28	3.19	3.10
4.46	3.90	3.49	3.30	3.30	3.11
		Lot K33			
1.75	1.61	1.61	1.56	1.49	1.45
2.82	1.75	1.61	1.64	1.47	1.47
3.81	1.58	1.72	1.55	1.49	1.49
4.41	1.61	1.71	1.77	1.53	1.56

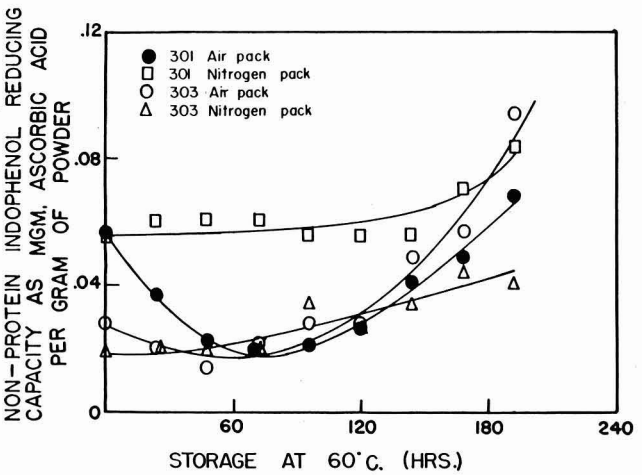

FIG. 5. Change in non-protein indophenol reducing capacity of dry whole milk stored at 60° C.

These data demonstrate the marked effect of temperature on the rate of production of acid ferricyanide reducing substances. At 20° C. the rate is so slow as to be negligible. At 37° C. the reaction is of minor importance except in powders of higher moisture content.

Ascorbic acid changes. The apparent ascorbic acid content of the samples

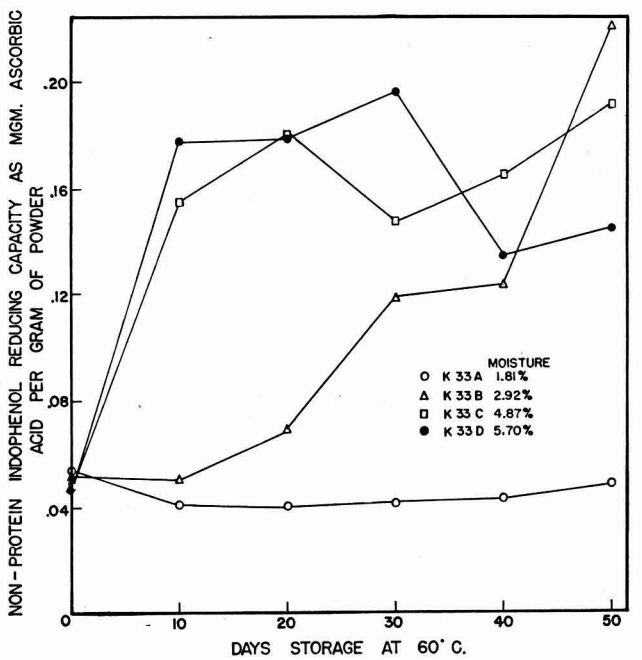

FIG. 6. Change in non-protein indophenol reducing capacity of dry whole milk stored at 60° C.

was determined on the protein-free filtrates titrating with 2,6-dichloro-benzenoneindophenol. The protein-free filtrates were secured by precipitation either with tungstic acid in the manner described by Crowe *et al.* (6), or with a mixture of trichloracetic and metaphosphoric acids according to the procedure outlined by Doan and Josephson (7). In the presence of oxygen, the ascorbic acid is gradually oxidized in dry whole milk. As shown in figures 5 and 6, substances which react with indophenol are produced particularly in powders of higher moisture.

Fluorescence changes. Fluorescence was determined by the method of Jenness and Coulter (13). Since the oxygen content appeared to be without effect on fluorescence changes during storage, the results at each moisture level have been averaged without regard to oxygen level.

No change in fluorescence of either extract I or II resulted from storage at 20° C., but sample 384 showed a marked increase in fluorescence of extract III which was not duplicated by sample K33. The greater susceptibility of sample 384 to development of fluorescence was even more apparent on storage at 37° C. Sample K33 exhibited scarcely any increase in fluorescence of extract I and only a small increase in extract III during storage for 16 weeks at this temperature. In sample 384, on the other hand, marked increases in fluorescence of both of these extracts occurred. The fluorescence of extract II was unaffected in either sample. Figure 7 shows the changes in fluorescence of sample K33 during storage for 50 days at 60° C. Under this more drastic condition, the fluorescence of extract I increased sharply if the moisture content was sufficiently high, and some increase was noted in fluorescence of extract II. The effect on extract III is interesting in that at moisture contents in excess of about 4 per cent, the initial sharp rise was followed by a pronounced decrease in fluorescence, due either to destruction or insolubilization of the fluorescing materials.

In general, then, it appears that temperature and moisture level determine whether and to what extent fluorescing materials are produced during storage. A considerable difference between powders in susceptibility to production of these materials is also evident.

Production of carbon dioxide. The head-space gas of the cans was analyzed for carbon dioxide and oxygen with a Fisher Precision Gas Analyzer equipped with a Continental Can Company sampling device. All readings were computed to standard temperature and pressure in the manner described by Coulter and Jenness (5).

Carbon dioxide was produced at rates varying with the temperature and the moisture content. Typical data for the powder held at 60° C. are shown in figure 8. Since the rate of production of CO_2 appears to be essentially a straight line function of time over the period studied, the regression line for each powder was computed. The log slopes of the regression lines for the samples held at 60 and 37° C. are shown in figures 9 and 10, plotted against the moisture content. The 60° C. data include those for lot K33 and those for the samples in

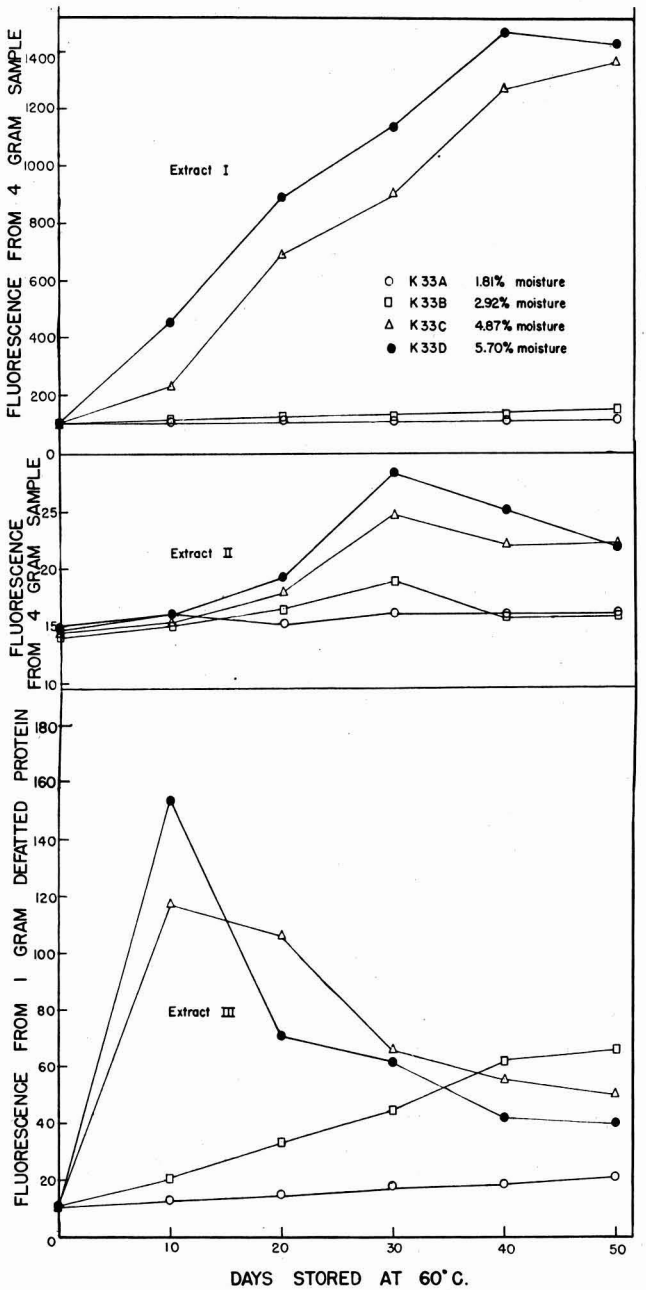

FIG. 7. Change in extractable fluorescence of dry whole milk stored at 60° C.

the initial 9 lots all of which were held for only 10 days. Although the data show considerable scatter, it is evident that production of CO_2 at any given temperature is an exponential function of the moisture content. The effect of oxygen level on the rate of production of CO_2 is not entirely clear. As shown in figure 9, the rate of production of CO_2 in the air-packed samples appears to be slightly higher than in the gas-packed samples. However this difference is far from uniform, and the effect of the oxygen level, if it is a factor, is minor in comparison to that of the moisture content. Production of CO_2 in the samples held at 20° C. was so slow that consistent rate data were not obtained.

Since the powder absorbs some of the carbon dioxide, Coulter and Jenness (5) and Pearce (19), the total carbon dioxide produced cannot be computed from the volume and analysis of the gas.

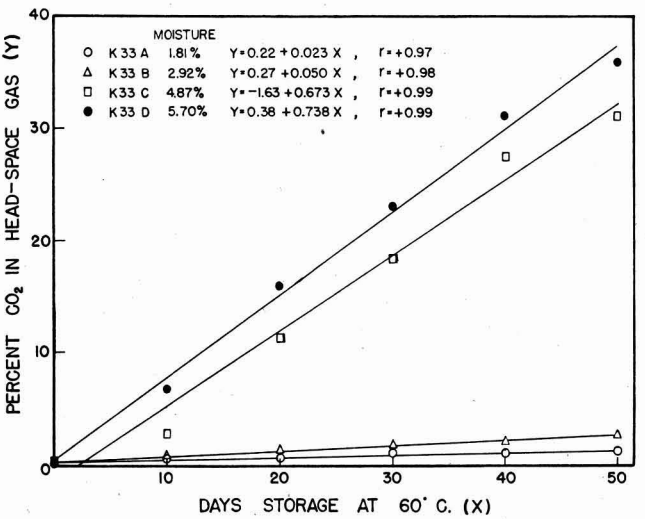

FIG. 8. Change in per cent CO_2 in head-space gas of dry whole milk stored at 60° C.

Oxygen utilization. Only a limited amount of data were secured which were adequate to establish rates of oxygen utilization. These were for the initial nine lots which were held at 60° C. for 10 days. The regression lines for oxygen utilization for each of the air-packed samples were computed and log slopes of the regression lines are shown plotted against the per cent moisture in figure 11. These data indicate that the rate of oxygen utilization increases logarithmically with increase in the moisture content. With these samples there was a very high correlation (+0.92) between the rate of oxygen utilization and carbon dioxide production. Since the rate of carbon dioxide production was only slightly greater in the air-packed than in the nitrogen packed samples, direct oxidation involving the free-space oxygen can play only a minor role in carbon dioxide production.

Production of water. The moisture content of the samples was determined at each examination period using the American Dry Milk Institute toluene dis-

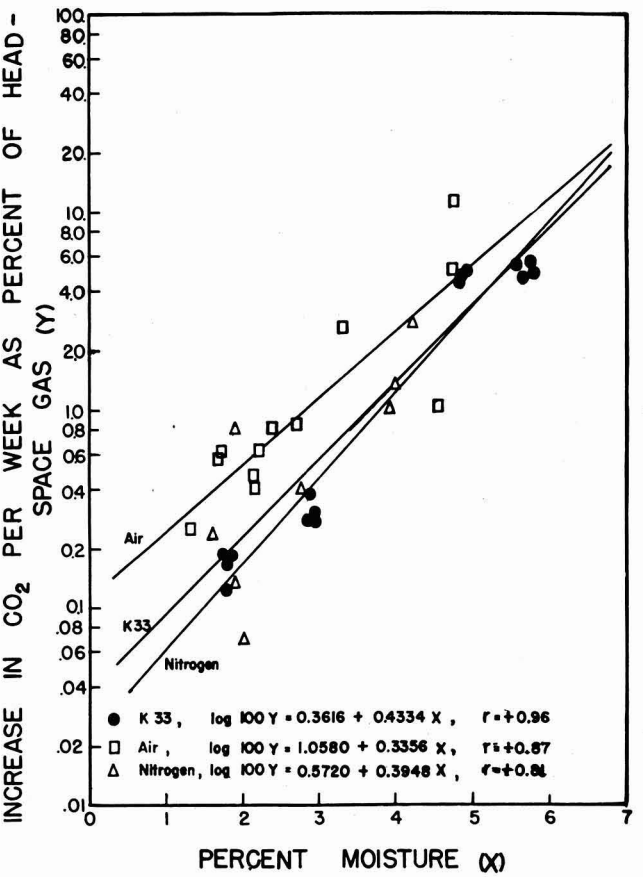

FIG. 9. Relationship of moisture content to rate of increase in CO_2 in head-space gas of dry whole milk stored at 60° C. in air and nitrogen. Sample K33 was stored at oxygen levels ranging from about 1 to 6 per cent.

tillation method. Since the oxygen content appears to be without effect on changes in the moisture content during storage, the results at each moisture

TABLE 3

Effect of storage at 60° C. on the moisture content of dry whole milk

Days of storage					
0	10	20	30	40	50
% moisture					
1.90	1.90	1.60	1.80	1.76	1.90
2.88	3.00	2.95	2.95	2.89	3.02
3.81	4.13	4.95	5.13	5.25	5.95
4.49	5.15	5.94	5.78	6.24	6.53

level have been averaged without regard to oxygen level. The data for the samples stored at 60° C. are shown in table 3. There was a definite increase in the moisture content of the samples having initial moisture levels of 3.81 and 4.49 per cent but not in lower moisture content samples. There was no change in the moisture content of any of the samples stored at 37 and 20° C.

Change in protein solubility. The soluble nitrogen of the samples was de-

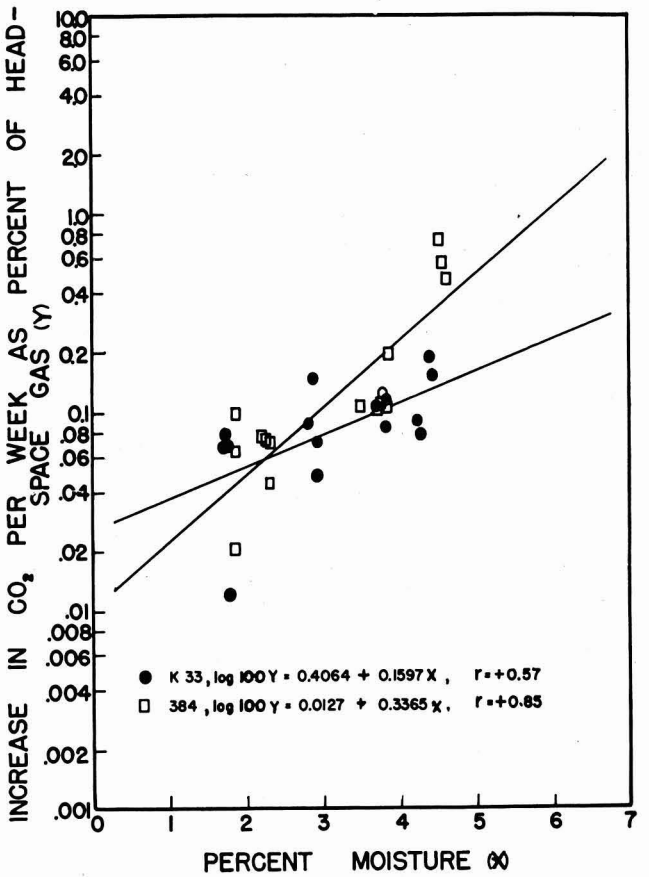

FIG. 10. Relationship of moisture content to rate of increase in CO_2 in head-space gas of dry whole milk stored at 37° C.

termined on an aliquot taken from the center portion of the centrifuge tube following treatment of the milk according to the American Dry Milk Institute method for solubility index. This method was not entirely satisfactory for those samples which had become brown, since the treatment did not effect a sharp separation of the insoluble material. Filtration of the brown samples was found more satisfactory. The results for the sample stored at 60° C. are summarized in table 4. Since the oxygen level was without effect on protein

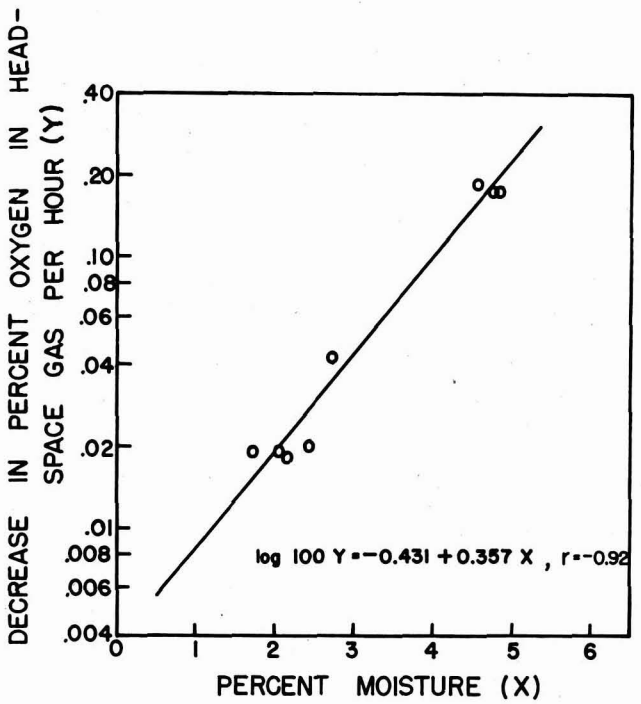

FIG. 11. Relationship of moisture content to rate of decrease in oxygen content of headspace gas of dry whole milk stored at 37° C.

insolubilization, the values reported are the averages at each moisture level. At the end of 50 days storage, the soluble nitrogen in the samples containing 4.49 per cent moisture had been reduced to 6 per cent of the original value. This means that virtually all of the protein was rendered insoluble by the experimental conditions. The solubility of the nitrogenous substances was not

TABLE 4

The effect of moisture level and storage at 60° C. on the lactose, soluble nitrogen and pH of dry whole milk

Storage period in days	% lactose[a]				Soluble N (Mg. per 100 ml.[a])				pH			
	Initial % Moisture											
	1.90	2.87	3.81	4.49	1.90	2.87	3.81	4.49	1.90	2.87	3.81	4.49
0	36.30	36.65	36.05	36.03	503	502	505	508	6.47	6.49	6.49	6.41
10	36.05	34.30	33.67	33.27	506	495	90.2[b]	70.3	6.49	6.46	6.17	5.87
20	35.30	33.30	33.17	32.40	504	482	52.7	39.7	6.47	6.32	5.63	5.64
30	35.20	33.23	32.57	31.57	502	458	38.6	33.0	6.45	6.48	5.43	5.38
40	34.85	33.06	32.20	31.37	489	452	36.4	30.3	6.42	6.40	5.32	4.97
50	35.75	33.55	32.56	31.57	495	468	29.5	30.4	6.48	6.32	5.10	4.99

[a] Moisture-free basis.

[b] The average of two filtered samples, the average of two centrifuged samples being 394.

affected by storage at 60° C. at the 1.90 per cent moisture level and only slightly affected at 2.87 per cent moisture. The solubility of the protein was unchanged in all of the samples stored at 37 and 20° C.

Change in lactose content. The lactose was determined by a modification of the chloramine-T method of Hinton and Macara (10), which is based on the stoichiometric oxidation of the aldehyde group of the sugar. Zinc hydroxide was used for the deproteinization of the milk as suggested by McDowell (17).

The per cent of lactose in the samples stored at 60° C. is shown in table 4. Since the oxygen level did not affect the lactose content, the values shown are the averages at each moisture level. Storage of dry whole milk with an initial moisture content of 4.49 per cent at 60° C. for 40 days resulted in a maximum loss of 15 per cent of the lactose as measured by this method. The lactose decreases most rapidly during the first 10 or 15 days of storage under these conditions. The tendency for an increase in the lactose content at 50 days over that at 40 days may be due to a reduction of the chloramine-T reagent by the increased concentration of competitive reducing systems in the browned milk. The decrease in reducing sugar at the 1.90 per cent moisture level was only 4 per cent.

The lactose content of the samples stored at 37 and 20° C. was unchanged.

Changes in pH. The pH of the reconstituted samples was determined using a Leeds and Northrup glass-electrode pH meter. The pH values for the samples stored at 60° C. are shown in table 4. The data reported are average values at each moisture content, as the oxygen level had no affect on the pH of the reconstituted milk. There was a marked decrease in the pH of the samples having an initial moisture content of 4.49 per cent; a lesser decrease at the 3.80 and 2.87 per cent moisture levels; but none at 1.90 per cent. The pH of the samples stored at 37 and 20° C. for 20 and 32 weeks, respectively, was unchanged.

DISCUSSION

Minimal oxygen levels are considered desirable for the storage of dry whole milk to prevent oxidative changes. Minimal moisture levels have not been considered optimal, because some moisture is necessary for the effectiveness of certain antioxidants. In adequately gas-packed powder, however, oxidation cannot occur.

Numerous changes take place in dry whole milk during storage which are accelerated by increase in the moisture level. These include development of stale, or, at higher moisture levels, a burnt feathers flavor, production of acid ferricyanide reducing substances, production of carbon dioxide and utilization of oxygen. The rate of change of each of these, at least during the initial stages, has been shown to increase logarithmically with increase in moisture content. Other changes also increasing in rate with increase in moisture content but for which adequate rate data were not secured are: production of indophenol reducing substances, production of water, production of extractable fluorescent materials, browning, loss of lactose, increase in acidity and loss of

protein solubility. Thus, in adequately gas-packed dry whole milk, a minimal moisture content appears to be desirable; however, the rates of change in every instance are very slow in powders containing 2 per cent or less moisture.

Barker (1) in 1933 observed a logarithmic increase in the rate of heat denaturation of egg albumin with increase in the relative vapor pressure. He explained his observations on the basis that the relative humidity affected the freedom of the water molecules to move between and among the relatively immobile protein molecules and aggregates. He considered this interpretation pertinent regardless of whether the water was a reactant or merely a medium in which the reaction occurred. A similar relationship appears to hold for the reactions involved here.

Although vapor pressure determinations were not made on the samples involved in these trials, determinations on other samples covering the same range in moisture content showed that there is an essentially linear relationship between the per cent moisture and the relative vapor pressure.

All of the reactions mentioned are accelerated greatly by increase in temperature. The magnitudes of the various changes are unimportant except in samples which are higher in moisture than is considered desirable in commercial practice. Therefore, none of the objectively measurable changes can be considered as an effective index of deterioration in commercial dry whole milk.

SUMMARY AND CONCLUSIONS

The following non-lipid changes occur in dry whole milk during storage: development of a stale or burnt feathers flavor, production of acid ferricyanide and indophenol reducing substances, production of carbon dioxide, utilization of oxygen, production of water, production of extractable fluorescent materials, browning, loss of lactose, increase in acidity and loss in protein solubility. All of these changes increase in rate with increase in moisture content and temperature but appear to be relatively unaffected by oxygen.

The rate of change in those variables for which adequate data were obtained increased logarithmically with increase in the vapor pressure of the water in the system, at least during the initial stages.

ACKNOWLEDGMENTS

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