JOURNAL OF DAIRY SCIENCE

Contents

Vol. XXVIII, No. 1, January, 1945

Published by the

AMERICAN DAIRY SCIENCE ASSOCIATION

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WHERE QUALITY PREDOMINATES

THE Puerto Rico Research

In the two-billion-gallon ice cream year to come, over one billion gallons will be vanilla! Naturally, with one flavor affecting such a large percentage of all ice cream sales, the entire industry has a vital interest in vanilla raw material research.

Studies in growing and curing at the Hacienda Miguel vanillery in Puerto Rico have been showing considerable promise of late—certainly sufficient to warrant giving the ice cream industry these highlights.

It was in 1919 that vanilla cuttings from Mexico were first reported as a "promising new crop for Puerto Rico." The soil and climate gave evidence that high quality beans could be developed on this island possession. However, the 1928 hurricane wiped out much of the progress that had been made.

Vanilla growing again received renewed attention in 1933. Five years later (1938) David Michael & Co. bought a plantation and started to work directly with the growing and curing of vanilla beans.

The choice of Puerto Rico for the vanillery had special advantages. It is the only spot in the world where technical control can go hand in hand with the growing and curing. The planters of Madagascar must look to far off France for technical guidance. In Mexico, the plantings are far removed from any possible laboratory control.

But in Puerto Rico, the Federal Experiment Station at Mayaguez, and the facilities of the University of Puerto Rico, with all their scientific laboratories and research staffs are "right around the corner" from Hacienda Miguel.

At present, much work is being done with experiments in curing. You could not be closely associated with a plantation for very long without having it cross your mind that ALL PRESENT CURING METHODS ARE QUITE PRIMITIVE. The alternate sunning and sweating of the harvested beans over a period of months certainly lacks modern scientific advantages. And so, independently and in cooperation with the other agencies, we have been finding that much better results can be obtained in the cured beans by methods using scientific controls. It remains for further experiments to reveal which of these scientific methods will produce the best results. But it is already clear that improved products will come about from this work in Puerto Rico.

These modern curing methods will make it possible to harvest beans at the peak of their perfection—whereas present primitive curing necessitates the picking of beans before maturity.

The third point of interest is that experimentation is being carried on with hybrid vanilla. This cross pollinating might in itself result in an important future variety.

David Michael & Co. feels that in Mixevan it has produced the finest vanilla flavoring possible—designed for the special requirements of the ice cream industry. Years of experience have gone into the processing of the added vanillin and the best vanilla beans that the markets could provide. The next step must be the improvement of the beans themselves.

And that is the reason for the Puerto Rico research.

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

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

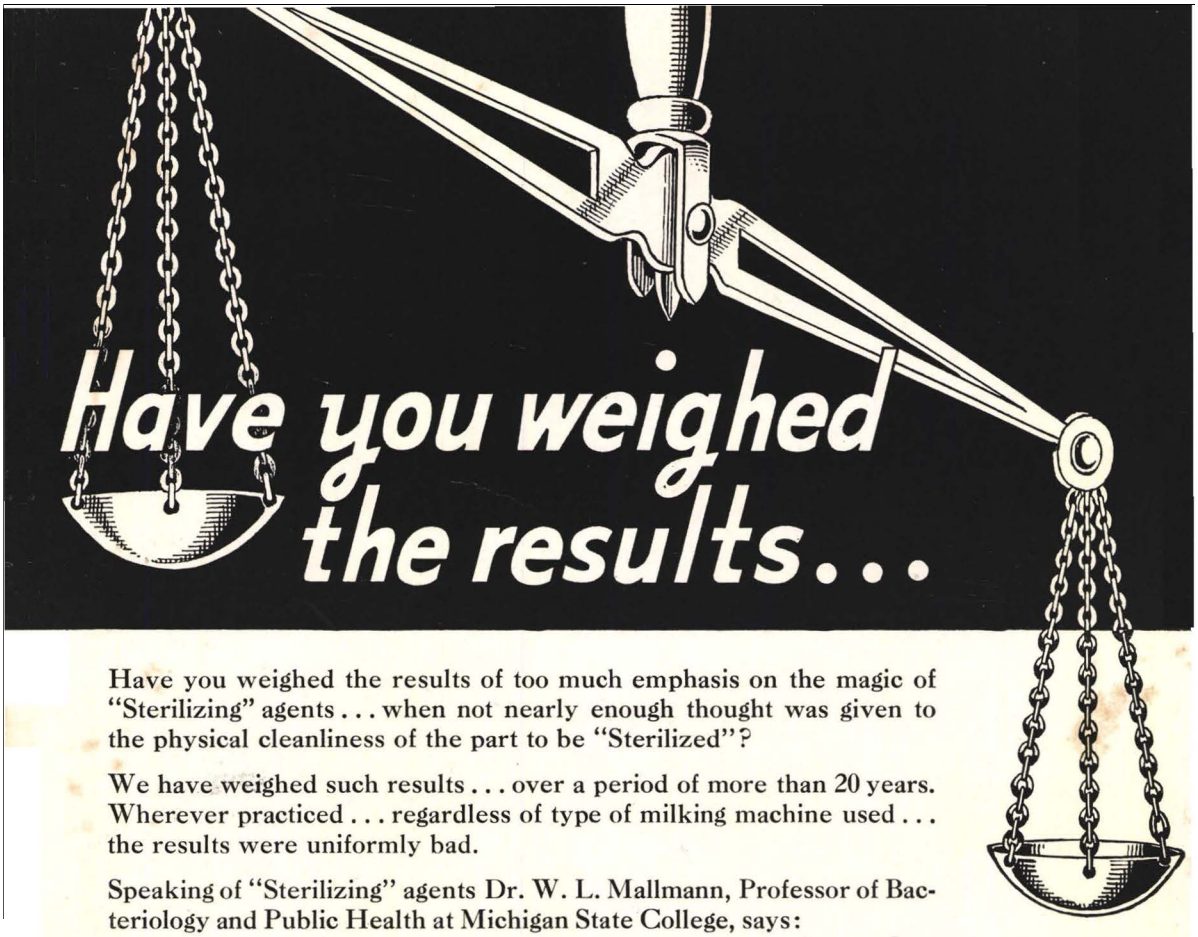
Subscriptions. Price; $6.00 per volume in North and South America; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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

Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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



Have you weighed the results of too much emphasis on the magic of "Sterilizing" agents . . . when not nearly enough thought was given to the physical cleanliness of the part to be "Sterilized"?

We have weighed such results . . . over a period of more than 20 years. Wherever practiced . . . regardless of type of milking machine used . . . the results were uniformly bad.

Speaking of "Sterilizing" agents Dr. W. L. Mallmann, Professor of Bacteriology and Public Health at Michigan State College, says:

> ***"Based on our present day knowledge of present sanitizing agents, it can be definitely stated that sanitization is possible ONLY when the surface is free of soil and surface film of either organic or inorganic nature."***

In farm language this means that a dirty milk utensil . . . though chlorine, lye or heat treated is still dirty . . . and unfit for use in the production of safe, high quality milk.

To the processing plant it means that the greatest source of Thermoduric bacteria must be *physically removed* . . . not merely surface treated.

There is still no magic compound which will take the place of thorough scrubbing.

You can be a vital force in building a greater postwar market for all dairy products, by preaching, encouraging and insisting that all milk utensils . . . particularly milking machines, be scrubbed clean for every use . . . regardless of the "Sterilizing" method or agent you think best.

JOURNAL OF DAIRY SCIENCE

VOLUME XXVIII JANUARY, 1945 NUMBER 1

STUDIES ON THE STABILIZATION OF CAROTENE IN DEHYDRATED FEEDS AND FOODS*

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Dehydrated alfalfa products, because of their carotene content, are used to a large extent to supply the vitamin A activity of mixed animal and poultry feeds. The relatively rapid loss of carotene from some dehydrated plant materials during storage makes difficult the maintenance of adequate vitamin A activity in feeds throughout the year, and any practical method of stabilizing the carotene would be of great importance.

Numerous reports have appeared on the instability of carotene in dried plant tissues and the conditions of storage which affect it. Fraps and Treichler (6) reported that alfalfa leaf meal stored at room temperature lost 50 per cent of its carotene in 11 months, and most investigators since then have found similar losses. The temperature of storage is an important factor in determining the rate of carotene loss. Smith (27), Taylor and Russell (29), Wall (30), Fraps and Kemmerer (5), and Wiseman, Kane and Cary (33) all found that the loss of carotene from alfalfa meal was fairly rapid during the summer and very slow during the winter. Guilbert (8) stated that a 10-degree rise in temperature approximately doubled the rate of loss of carotene. Russell, Taylor, and Chichester (24) found no loss of carotene in alfalfa stored *in vacuo* at 0° C. Wilder and Bethke (31) found that losses of carotene from machine-dried alfalfa meal ranged from 10 per cent in 6 monthes at – 23 to – 26° C. up to 98 per cent in 16 days at 80° C. They also found that the kind of container (burlap or paper bags) made no difference in the rate of loss, as did Kon and Thompson (13), and that pellets lost carotene at practically the same rate as loose meal. Storage in sealed tin cans *in vacuo* or under nitrogen, either at room temperature

Received for publication June 22, 1944.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

We wish to thank Cerophyl Laboratories, Inc., Kansas City, Mo., for the samples of dehydrated alfalfa and oats; Hoffman-LaRoche, Inc., Nutley, N. J., for the samples of glucoascorbic acid, d-isoascorbic acid, and Na-isoascorbate; the Warriner Starch Co., St. Francisville, La., for the dehydrated sweet potatoes and Prof. P. B. Pearson, Agricultural and Mechanical College of Texas, College Station, Texas, for the dehydrated diced carrots.

or at 37° C., resulted in little or no loss of carotene. A report from the New Jersey Experiment Station (20) stated that dehydrated alfalfa meal mixed with blackstrap molasses lost 44 per cent of its carotene in 102 days, while the untreated meal lost 70 per cent. In a preliminary report Silker, Schrenk, and King (26) found that there was a less pronounced loss of carotene when thiourea, hydroquinone, diphenylamine, or sodium cyanide was added to the alfalfa before dehydration. The only really effective ways of retaining carotene in dehydrated plant materials are to store at low temperatures or to remove the air and store *in vacuo* or under inert gases.

The stabilization of carotene in solution, on the other hand, can be successfully accomplished by the addition of any of a number of antioxidants. Among those used up to date are hydroquinone (Olcovich and Mattill (21)), alpha-tocopherol (Sullmann (28)), a combination of hydroquinone and alpha-tocopherol (Quackenbush, Cox, and Steenbock (23)), diphenylamine (Williams, Bickoff, and Van Sandt (32)), and phenyl-alpha and beta-naphthylamine (Morgal, Byers, and Miller (19)).

The present studies were undertaken to determine the effect of various treatments, reducing agents, and antioxidants upon the stability of carotene in dehydrated alfalfa, unjointed cereal grasses, carrots, and sweet potatoes.

METHOD OF CAROTENE ANALYSIS

The method of carotene analysis used throughout these experiments is a combination of the rapid extraction method of Moore and Ely (18) using a Waring blender and a foaming solvent, and the method of Hegsted, Porter, and Peterson (10) for the removal of impurities from the petroleum ether solution by means of aqueous diacetone. The method is as follows:

The weighed sample of dry material, usually one gram, is placed on a filter paper in a funnel and wet with hot tap water. The excess water is removed from the sample by applying mild suction, and the sample is washed into the container of a Waring mixer with 100 cc. of 95 per cent ethyl alcohol. Sixty-five cc. of Skelly-solve B are added, and the blender run for 5 minutes. (If the mixture does not foam during the stirring, additional alcohol is added until foaming does occur.) The residue is allowed to settle, and the extract is poured into 30 cc. of 10 per cent alcoholic KOH. The residue is extracted again for 2 minutes with a mixture of 40 cc. of alcohol and 30 cc. of Skelly-solve B. After settling, the extract is poured off and combined with the first extract. The residue is washed into a beaker with about 30 cc. of Skelly-solve B. The basic extracts are filtered, and the filter paper washed with the Skelly-solve wash. The filtrate is added to 40 cc. of water in a 500-cc. separatory funnel and shaken. The upper Skelly-solve layer is removed, and the alcohol layer is reextracted twice with 25-cc. portions of Skelly-solve.

The Skelly-solve extracts are combined, and washed with three 150-cc.

portions of water. The Skelly-solve solution is then washed with 20-cc. portions of aqueous diacetone (100 cc. acetone-free diacetone plus 6 cc. water) until the lower diacetone layer is colorless (4–6 washings). The Skelly-solve layer is then carefully washed once by pouring water through (without shaking) until all cloudiness disappears. The volume of the Skelly-solve solution is then measured, and the concentration of carotene determined with an Evelyn photoelectric colorimeter, with the 440 mμ filter. A standard curve was made, using crystalline carotene (90 per cent beta and 10 per cent alpha), and the concentration of carotene in the unknown solution is read directly from the curve. The calculation is as follows:

$$\mu\text{g. carotene/gm.} = \frac{\mu\text{g./cc.} \times \text{volume of extract in cc.}}{\text{wt. of sample in gm.}}$$

The sample is wet before extraction because better extraction results, as shown by Moore and Ely (18). No further carotene can be removed from the sample after the two extractions, and extraction with an alkaline solution gives values no higher than with the neutral solvents, as shown in table 1. Crystalline carotene can be carried through the entire procedure without appreciable loss.

The method as described gives results reproducible within 5 per cent. When results obtained by this method are compared with those obtained by passing the Skelly-solve solution through a column of activated $CaHPO_4$ (Moore (17)) according to the method of Moore (16), there is no appreciable difference (table 1). Diacetone and the $CaHPO_4$ column leave the same amount of chromogen in solution, and the $CaHPO_4$ removes no chromogen from the diacetone-washed solution. Hauge *et al.* (9) reported that extraction of the non-carotene pigments from solutions from alfalfa hay with 94 per cent diacetone alcohol gave carotene values in close agreement with those obtained by adsorption upon a dicalcium phosphate column. Berl and Peterson (3) obtained similar results when determining carotene in butter.

It has been shown by Kemmerer and Fraps (11) that the carotene solutions secured from plants by any of the usual methods of carotene determi-

TABLE 1

Effect of analytical results of variations in procedure of analysis of dehydrated oats

	Carotene
	μg./gram
1. Assay as described—old sample oats	219
2. Final solution from 1 passed through $CaHPO_4$ column	212
3. Assay as described, except KOH added before extraction instead of after	224
4. Assay as described—new sample oats	617
5. Duplicate of 4	612
6. Final solution from 4 passed through $CaHPO_4$ column	625
7. Shelly-solve solution passed through $CaHPO_4$ column instead of being extracted with diacetone	626

nations, except chromatographic separation with calcium hydroxide, usually contain three or more yellow pigments in varying percentages. These pigments were classified as beta-carotene, neo-beta-carotene, impurity A, carotenoid X, and occasionally alpha-carotene and neo-alpha-carotene. Carotenoid X, which was later identified by these authors (12) as neo-beta-carotene U of Polgár and Zechmeister (22), has no vitamin A potency. The neo-beta-carotene of Beadle and Zscheile (1) was identified as neo-beta-carotene B of Polgár and Zechmeister, and has approximately one-half the vitamin A activity of beta-carotene. Impurity A has very little, if any, vitamin A activity as shown by Wiseman, Kane, Shinn, and Cary (34).

Because of these impurities, it must be recognized that results obtained by the method used here give only approximate values, both as to beta-carotene content and biological activity. However, the same criticism applies to most other methods of carotene determinations. Where comparison of carotene contents of various samples of the same type is desired, rather than the absolute values, the method is entirely suitable. It was used in preference to an adsorption method because, in the authors' experience, it was more rapid and convenient.

EXPERIMENTAL

In the experiments reported here the materials used were samples of dehydrated alfalfa and young unjointed oats obtained from the Cerophyll Laboratories, Kansas City, Missouri. The materials to be added to the dehydrated products were dissolved in water, except where indicated, in such concentration that 60 cc. of water contained the amount to be added

TABLE 2

Stability of carotene in dehydrated oats and alfalfa moistened with various solutions and dried at 45° C.
Stored at room temperature

	pH	Carotene—μg./gm. (dry basis)			Per cent loss in 3 months
		Initial	1 month	3 months	
Oats + H_2O	6.1	338	146	83	75
Oats + 1% $Na_2S_2O_3$	6.1	362	175	101	72
Oats + H_2SO_4	3.9	350	87	33	90
Oats + H_2SO_4 + 1% $Na_2S_2O_3$	3.9	343	292	210	39
Oats + H_2SO_4 + 0.5% $Na_2S_2O_3$	3.9	360	211	73	80
Oats + H_2SO_4	2.8	378	62	19	95
Oats + H_2SO_4 + 1% $Na_2S_2O_3$	2.8	415	322	41	90
Alfalfa + H_2O	6.3	162	132	72	55
Alfalfa + 1% $Na_2S_2O_3$	6.4	187	123	67	64
Alfalfa + H_2SO_4	4.5	149	98	50	66
Alfalfa + H_2SO_4 + 1% $Na_2S_2O_3$	4.5	183	144	75	59
Alfalfa + H_2SO_4	3.5	178	134	58	67
Alfalfa + H_2SO_4 + 1% $Na_2S_2O_3$	3.5	197	180	105	47
Alfalfa + H_2SO_4 + 0.5% $Na_2S_2O_3$	3.5	189	144	65	66

to 100 gm. of dry matter. This solution was sprayed upon the oats or alfalfa, and the sample was thoroughly mixed by hand. The materials were then dried at the temperatures indicated, and stored in the dark at room temperature in loosely stoppered bottles. Carotene analyses were run on the materials immediately after drying and 1, 3, and 6 months afterwards.

The results of drying overnight at 45° C. after various treatments are shown in table 2. Sodium thiosulfate was used as a reducing agents in the hope that it would be effective, because it is cheap and easy to apply. Sulfuric acid, alone and in combination with the thiosulfate, was added in order to partially duplicate the conditions presented in mineral acid legume silage, where carotene is fairly stable. Only the combination of acid and 1 per

TABLE 3

Effect of heating on carotene stability in dehydrated alfalfa and oats
Stored at room temperature

	Carotene—μg./gm. (dry basis)				Per cent loss in 6 months
	Initial	1 month	3 months	6 months	
Oats—unheated	274	.....	141	84	70
Oats + H_2O—dried 5 hrs. at 95° C.	300	.....	181	141	52
Oats—heated dry at 95° C. for 2 hrs., then H_2O, and dried 5 hrs. at 95° C.	222	.....	197	172	22
					3 months
Oats—unheated	212	174	130	.....	39
Oats + H_2O—dried 3 hrs. at 95° C.	187	184	149	.....	20
Oats—heated dry 60 min. at 93° C.	202	172	144	.....	29
Alfalfa—unheated	167	.....	117	.....	30
Alfalfa + H_2O—dried 5 hrs. at 95° C.	143	.....	96	.....	33
Alfalfa—heated dry 2 hrs. at 95° C., then H_2O, and dried 5 hrs. at 95° C.	129	.....	90	.....	30
Alfalfa—heated dry 60 min. at 93° C.	156	131	102	.....	33

cent sodium thiosulfate was of any help in maintaining the carotene level in the oats, while none of the treatments were effective on the alfalfa. The figures given as pH are an attempt to represent the actual acidity resulting from the additions. The figures are obtained by suspending 10 gm. of the dry material in 200 cc. of distilled water, letting stand for one hour, and determining the pH of the supernatant with a glass electrode.

In all other experiments the samples were dried at 95° C. for a shorter period of time. It was consistently found, with the oats, that this drying at high temperatures caused markedly increased stability of the carotene (see tables 3 and 4, and controls in the following tables). The usual loss of carotene from the untreated oat control samples was about 70 per cent in 6 months at room temperature. Loss from the heated samples ranged from 22 to 61 per cent depending upon the temperature and duration of the heat treatment. The increased stability after heat treatment is probably

caused by heat inactivation of the carotene oxidase, which evidently is not entirely destroyed by the original commercial dehydration. In table 3 are given the results of various heat treatments. In six months' storage, the control sample lost 70 per cent of its carotene; the sample dried five hours at 95° C. lost 52 per cent; and a sample which was heated dry for two hours at 95° C., then moistened and dried at 95° C. for five hours, lost only 22 per cent. Although the additional heating resulted in less loss of carotene during storage, it destroyed more carotene during the heating so that the actual carotene content at 6 months was only 172 μg. per gm., as compared with 141 μg. for the five-hour drying, and 84 μg. for the unheated sample. Sixty minutes dry heating at 93° C. was required to give appreciable improvement in stability.

TABLE 4

Effect of various acids on carotene stability in dehydrated oats and alfalfa
Stored at room temperature

	Carotene—μg./gm. (dry basis)			Per cent loss in 6 months
	Initial	3 months	6 months	
Oats—unheated and untreated	274	141	84	70
*Oats + H_2O	245	223	173	29
*Oats + 0.3% glucoascorbic acid	253	226	184	28
*Oats + 0.3% glucoascorbic acid + H_2SO_4	277	203	140	49
*Oats + 0.3% Na-isoascorbate	250	224	183	27
*Oats + 0.3% d-isoascorbic acid	242	234	193	20
*Alfalfa + H_2O	133	104	79	40
*Alfalfa + 0.3% glucoascorbic acid	131	103	75	43
*Alfalfa + 0.3% Na-isoascorbate	131	107	73	44
*Alfalfa + 0.3% d-isoascorbic acid	139	113	76	45

* Dried 5 hrs. at 95° C.

The effect of heat treatment on alfalfa depended on the sample used. No improvement was obtained with the first sample used (tables 3 and 5), while with a different sample (table 7) the loss of carotene was decreased from 69 to 49 per cent by moistening with water and drying at 95° C. for 3 hours. Evidently the second sample was subjected to less rigorous heat treatment during its original dehydration.

When glucoascorbic acid, sodium isoascorbate, and d-isoascorbic acid were added to oats and alfalfa at levels of 0.3 per cent of the dry matter of the samples, there was no increase in stability over that resulting from drying with water alone (table 4).

The addition of 0.1 per cent gum guaiac to oats, in alcohol solution, dilute NaOH solution, or water suspension, had no beneficial effect on carotene stability when the samples were stored at 37° C. (table 5). At this temperature the loss of carotene from the untreated samples was 83 per cent in six months—considerably more than at room temperature. Guaiac, citric acid, tartaric acid, and phosphoric acid, at levels of 0.1 per cent, were all

TABLE 5

Effect of guaiac and various acids on carotene stability
Stored at 37° C.

	Carotene—μg./gm. (dry basis)				Per cent loss in 6 months
	Initial	1 month	3 months	6 months	
Oats—unheated and untreated	236	171	86	40	83
*Oats + H_2O	226	195	124	63	72
*Oats + 0.1% guaiac in 0.005 N NaOH	231	187	118	54	77
*Oats + 0.1% guaiac in H_2O	240	181	114	54	78
*Oats + 0.1% guaiac in EtOH	244	179	104	46	81
Alfalfa—unheated and untreated	150	120	73	43	71
*Alfalfa + H_2O	134	98	67	34	74
*Alfalfa + 0.1% guaiac in EtOH	151	113	61	30	80
*Alfalfa + 0.1% citric acid	125	87	71	36	71
*Alfalfa + 0.1% tartaric acid	119	89	76	35	71
*Alfalfa + 0.1% H_3PO_4	121	92	74	36	70

* Dried 5 hrs. at 95° C.

ineffective in stabilizing the carotene in dehydrated alfalfa at 37° C. These acids and guaiac were tried because they have been reported by Grettie (7) and Mitchell and Black (14) to be effective in preventing or delaying the appearance of rancidity in fats and oils.

It was thought that making the dehydrated materials alkaline might have a stabilizing effect on the carotene. Consequently sodium bicarbonate, disodium phosphate, ammonia, and urea were added to oats and alfalfa (table 6). None of these samples had appreciably more carotene after storage than did those samples dried with water alone.

Diphenylamine was also tried on the oats after the preliminary report of Williams *et al.* (32) on its activity in stabilizing carotene in solution. The diphenylamine was added in alcohol solution at the rate of 0.9 per cent,

TABLE 6

Effect of various compounds on stability of carotene
Stored at room temperature

	Carotene—μg./gm. (dry basis)				Per cent loss in 6 months
	Initial	1 month	3 months	6 months	
Oats—untreated and unheated	609	388	225	144	77
*Oats + H_2O	576	411	298	246	57
*Oats + 0.5% $Na_2S_2O_3$	598	414	316	250	58
*Oats + 0.9% diphenylamine in EtOH	576	445	376	338	41
*Oats + 0.3% d-isoascorbic acid	556	415	303	256	54
					3 months
*Oats + 0.5% $NaHCO_3$	584	405	284	.....	51
*Oats + 0.5% Na_2HPO_4	612	387	291	.....	52
*Oats + 0.1% NH_3	585	408	292	.....	50
*Oats + 0.5% urea	576	412	305	.....	47

* Dried 3 hrs. at 96° C.

and dried in the same manner as the other samples. After six months, the diphenylamine sample had lost 41 per cent of its carotene, as contrasted to 57 per cent for the water control and 77 per cent for the unheated control. That the stabilizing effect was not caused by the alcohol can be seen by looking at the alcohol control in table 8. The diphenylamine was later tried on alfalfa and again had a considerable effect (table 9).

It was thought worthwhile to pellet the materials, and then to protect the pellets by coating with some material impervious to air. The pellets were made in a small hand pelleting machine. By using weighed amounts of either oats or alfalfa, the finished pellets were of uniform weight, about

TABLE 7

Effect of pelleting and coating with wax on the stability of carotene
Stored at room temperature

	Carotene—μg./gm. (dry basis)				Per cent loss in 6 months
	Initial	1 month	3 months	6 months	
Oats—untreated, loose	576	373	242	151	74
Oats—untreated, pellets	581	396	285	201	65
Oats—pellets coated with Flexowax	603	433	416	331	45
Oats—pellets coated with gelatin	604	410	292	208	66
*Oats + H_2O—loose	578	374	290	216	61
*Oats + H_2O—pellets	556	388	300	232	58
*Oats + H_2O—pellets + Flexowax	545	402	322	264	51
*Oats + H_2O—pellets + paraffin-beeswax	555	389	310	224	60
Alfalfa—untreated, loose	272	220	134	85	69
Alfalfa—untreated, pellets	274	208	124	81	70
Alfalfa—untreated, pellets + Flexowax	279	260	145	99	64
*Alfalfa + H_2O—loose	272	209	171	138	49
*Alfalfa + H_2O—pellets + Flexowax	270	206	183	148	45
Dehydrated sudan grass	395	287	192	144	63
Sudan grass—pellets	399	271	186	132	67
Sudan grass—pellets + Flexowax	390	316	218	240	39

* Dried 3 hrs. at 95° C.

1.2 gm. The coating materials tried were gelatin, a 60–40 paraffin-beeswax mixture, and Flexowax C (Candy Co., Chicago), a high-melting (126° F.), fairly flexible wax which does not readily crack. The Flexowax was found most satisfactory.

Results of preliminary experiments are shown in table 7. Pelleting alone caused little or no decrease in carotene loss, while the Flexowax-coated pellets lost considerably less carotene in six months, especially with oats and Sudan grass. The loss in unheated oats was reduced by the Flexowax from 74 to 45 per cent in six months, giving final carotene contents of 151 and 331 μg. per gm. respectively. The loss in Sudan grass was reduced from 63 to 39 per cent. Similar results are seen in table 8. However, the results with alfalfa showed only a slight difference between the coated pellet and the controls.

TABLE 8

*Effect of hydroquinone and alpha-tocopherol on stability of carotene in dehydrated oats**
Stored at room temperature

	Carotene—μg./gm. (dry basis)			Per cent loss in 3 months
	Initial	1 month	3 months	
Oats—untreated, loose	515	413	250	52
Oats—untreated, pellets + Flexowax	527	435	346	34
Oats + 0.1% alpha-tocopherol—loose	545	303	204	63
Oats + 0.1% alpha-tocopherol—pellets + Flexowax	540	462	234	57
Oats + 0.5% hydroquinone—loose	545	378	263	52
Oats + 0.5% hydroquinone—pellets + Flexowax	538	465	348	35
Oats + tocopherol + hydroquinone—loose	527	332	246	53
Oats + tocopherol + hydroquinone—pellets + Flexowax	531	453	341	36
Oats + EtOH—loose	535	348	219	59
Oats + EtOH—pellets + Flexowax	546	436	272	50
Oats—untreated, pellets, washed with N_2 before dipping in Flexowax	502	411	312	38

* All additions in this series were made in alcoholic solution—30 cc./100 gm., and the grass was then dried at 108° C. for 40 minutes.

The results of attempting to remove oxygen from the pellets before coating are shown in tables 8 and 9. The pellets were placed in a vacuum desiccator and evacuated and washed with nitrogen twice before dipping in the Flexowax. This treatment was fairly effective with alfalfa, but not with the oats.

TABLE 9

Effect of various antioxidants on stability of carotene in dehydrated alfalfa
Stored at room temperature

	Carotene—μg./gm. (dry basis)			Per cent loss in 3 months
	Initial	1 month	3 months	
Alfalfa—untreated	262	189	104	60
Alfalfa—untreated, pellets + Flexowax	262	197	127	51
*Alfalfa + H_2O	263	198	167	36
*Alfalfa + H_2O—pellets + Flexowax	263	199	166	37
†Alfalfa + 0.9% diphenylamine	282	232	203	28
†Alfalfa + diphenylamine—pellets + Flexowax	282	232	184	35
†Alfalfa + 0.1% alpha-totcopherol	276	138	108	60
†Alfalfa + alpha-tocopherol—pellets + Flexowax	276	154	119	57
†Alfalfa + 0.5% hydroquinone	276	190	152	45
†Alfalfa + hydroquinone—pellets + Flexowax	276	191	154	44
†Alfalfa + alpha-tocopherol + hydroquinone	282	170	122	57
†Alfalfa + alpha-tocopherol + hydroquinone—pellets + Flexowax	282	174	138	51
Alfalfa—untreated, pellets, washed with N_2 before dipping in Flexowax	268	184	181	34

* Dried 2 hrs. at 108° C.

† Additions made in alcoholic solution—30 cc./100 gm., and then the material dried at 108° C. for 1 hr.

It was thought advisable to try treating the materials with various antioxidants, and then pelleting and coating with wax. Results with oats are shown in table 8, and with alfalfa in table 9. The addition of 0.5 per cent hydroquinone, 0.1 per cent alpha-tocopherol, or a combination of both, under the conditions of this experiment, resulted in no better retention of carotene than occurred in the controls. The addition of diphenylamine to alfalfa resulted in considerably less loss than in the controls. Pelleting and coating with Flexowax resulted in no additional retention.

Several samples of dehydrated sweet potatoes and carrots were also obtained, and carotene analyses run on them during storage. Results are shown in table 10. When sweet potatoes were stored in the form in which they were dehydrated (slices), only 5 per cent of the carotene was lost in six months' storage. However, when the sweet potatoes were ground in a Wiley mill and then stored, very rapid loss of carotene occurred, 65 per cent

TABLE 10

Stability of carotene in dehydrated carrots and sweet potatoes
Stored at room temperature

	Carotene—μg./gm. (dry basis)				Per cent loss in 6 months
	Initial	1 month	3 months	6 months	
Dehydrated sweet potatoes—ground	166	133	88	58	65
Dehydrated sweet potatoes—ground—pellets + Flexowax	162	132	74	49	70
Dehydrated sweet potatoes—sliced	164	160	148	156	5
Dehydrated carrots—diced	650	630	654	488	25
Dehydrated diced carrots—pellets	670	547	341	227	66
Dehydrated diced carrots—pellets + Flexowax	647	530	396	338	48
Dehydrated carrots—flakes	685	496	400	.....	.....

being lost in six months. Mitchell and Lease (15) have reported that sweet potato flour lost 98 per cent of its carotene when stored for a year in contact with the air. Scoular *et al.* (25), on the other hand, reported that sweet potatoes lost only 17 per cent of their carotene in a year at room temperature. The difference can be explained by our results with grinding.

Similarly, dehydrated carrots (diced) lost only 25 per cent of their carotene during the six-month storage period. However, when the diced dehydrated carrots were compressed into pellets, thus breaking the outer coating, 66 per cent of the carotene was lost in six months. Dehydrated carrot flakes showed a fairly rapid loss of carotene as contrasted to the diced form. The rate of carotene loss for diced carrots is not as extensive as the values reported in the literature. Cruess and Joslyn (4) reported about a 66 per cent loss in four months at room temperature. Beardsley, Prindle, and Stevens (2) reported a 39 per cent loss when stored at 70° F. for six months in air, and no loss when stored under nitrogen or CO_2 in sealed cans.

DISCUSSION

The results show that additional heating of the commercially dehydrated oats increased stability of the carotene in the oats, probably because of heat inactivation of the enzyme carotene oxidase. In some cases the heat treatment is also effective on dehydrated alfalfa, probably depending on previous treatment. From these results it would seem that slightly more heat during the original dehydration process would be effective in decreasing the loss of carotene from these materials during storage, without causing excessive loss of carotene during the dehydration.

The addition of antioxidants to the dehydrated materials was relatively unsuccessful except for diphenylamine, which considerably increased the stability of carotene. If it proves to be nontoxic to animals, and an economical means of application can be found, diphenylamine may become important commercially in stabilizing carotene in dehydrated materials. The difference in levels of diphenylamine and hydroquinone used may account for the activity of the diphenylamine and inactivity of the hydroquinone, since Silker, Schrenk and King (26) found hydroquinone to be active when added to alfalfa before dehydration.

Protection of the materials from the air by pelleting and coating with wax was effective—oats which had been pelleted and coated with wax had twice as much carotene after six months as had the loose material. This method of stabilization may be of commercial application. Pellets of about one pound weight were made in a Carver laboratory press under a pressure of 12,000 pounds per square inch, and then coated with wax. Under commercial conditions even larger blocks could undoubtedly be made. This treatment, however, was not very effective with dehydrated alfalfa.

Loss of carotene from dehydrated carrots and sweet potatoes during storage is much less than from dehydrated alfalfa and oats, as long as the original sizable dices or slices of the carrot and sweet potato pieces are not changed. However, when the dehydrated materials are ground or compressed into different shapes, the loss of carotene is very rapid. When commercially dehydrated sweet potatoes are to be stored for longer periods of time, they should be stored in the shape in which they are dehydrated, rather than as flour. If flour is desired, it should be ground immediately before use.

SUMMARY

1. The effects of various physical and chemical treatments upon the stability of carotene in dehydrated alfalfa and oats were studied.

2. Additional heat treatment was effective in decreasing the loss of carotene in dehydrated oats from 70–80 per cent to 30–50 per cent in six months. The loss in one sample of dehydrated alfalfa was reduced from 70 to 50 per cent in six months.

3. The addition of 0.9 per cent diphenylamine to dehydrated oats reduced the loss of carotene from 77 to 41 per cent in six months. The loss of carotene from dehydrated alfalfa was reduced from 60 to 30 per cent in three months by the addition of diphenylamine.

4. In the concentrations and conditions used, the following were ineffective in reducing the loss of carotene during storage: sodium thiosulfate, sulfuric acid, glucoascorbic acid, d-isoascorbic acid, sodium isoascorbate, guaiac, citric acid, tartaric acid, phosphoric acid, sodium bicarbonate, disodium phosphate, urea, ammonia, alpha-tocopherol and hydroquinone.

5. Protection of dehydrated oats from the air by pelleting and coating with Flexowax reduced the loss of carotene from 74 to 45 per cent in six months.

6. Dehydrated sweet potatoes, stored as slices, lost only 5 per cent of their carotene in six months. When ground and stored, the loss of carotene was 65 per cent in six months.

7. Dehydrated diced carrots lost no carotene during the first three months of storage, and lost 25 per cent during the second three months; when the dehydrated diced carrots were compressed into pellets, breaking the outer surface, the loss was 66 per cent in six months. The loss of carotene from dehydrated sliced carrots was much more rapid than from dehydrated diced carrots.

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THE USE OF THIAMIN DISULFIDE FOR THE ESTIMATION OF REDUCING SUBSTANCES IN PROCESSED MILK[1]

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Sulfhydryl groups in milk have been recently studied by workers interested in oxidized and cooked flavors (4, 5, 6, 8, 11, 12) and by those concerned with baking quality of non-fat milk solids (2, 10).

There is a lack of agreement in results on the amount of sulfhydryl groups in raw and heated milk. Jackson (7) could not obtain a positive nitroprusside test for sulfhydryl groups in fresh milk except after denaturation of the milk proteins with sodium cyanide. These results have been substantiated by Gould and Sommer (6) and Josephson and Doan (8). The latter workers and Gould (4) found that milk yielded a positive nitroprusside test when heated to approximately 70° C. for 30 minutes. Stamberg and Bailey (10), using the Heyrovsky micropolarograph, found the combined total of sulfhydryl groups and disulfide linkages in raw skimmilk to be equivalent to approximately 248 mg. of cysteine-hydrochloride per liter. Their method indicated that boiled milk contained no measurable amount of sulfhydryl groups. Gould (5), using a KIO_3 titration method, found a heat labile substance in fresh skimmilk.

The purposes of this investigation were to adapt the use of thiamin disulfide to the determination of certain reducing substances in milk, to study the effect of heat treatment on these reducing substances, and to correlate the quantity of such reducing substances with baking quality of non-fat milk solids.

EXPERIMENTAL MATERIALS AND METHODS

The fresh skimmilk was obtained from a Grade A supply of pasteurized milk. The non-fat dry milk solids were obtained from certain Pacific Northwest plants using the spray process. The baking quality of the non-fat milk solids was determined by the test baking laboratory of the Consolidated Dairy Products Company, Seattle, Washington. The dried milk was stored in tightly stoppered test tubes and held at temperatures of 0°–5° C.

Heat treatment was done in a rapidly circulating, thermostatically controlled water bath. The use of 15 × 120 mm. thin-walled Pyrex test tubes and the rapid circulation of the bath made it possible to heat the 10-ml. samples of milk to the desired temperature in approximately one minute. Following heat treatment, the milk was rapidly cooled in cold running water.

Received for publication June 26, 1944.

[1] Scientific Paper No. 610, College of Agriculture and Agricultural Experiment Stations, State College of Washington.

[2] American Dry Milk Institute Research Grant and in cooperation with the Washington State Dairy Products Commission.

The cysteine hydrochloride used as a standard was obtained from the Eastman Kodak Company. Determinations of both nitrogen and sulfur gave 96 per cent of the theory. The iodometric titration method of Virtue and Lewis (13) for SH-groups yielded 106 per cent recovery, which indicated that the substance assayed contained little or no cystine. For the purposes of this investigation, the cysteine was assumed to be 96 per cent pure.

The method used for the determination of reducing substances in milk is one proposed by Zima, Ritsert and Moll (14) for the estimation of substances in blood capable of reducing thiamin disulfide. According to Zima and his co-workers, the disulfide may be reduced to thiamin by hydrogen, hydrogen sulfide, glutathione and cysteine. Their results show that cysteine can be quantitatively recovered by the use of thiamin disulfide. Throughout this paper, all of the substances in milk that reduce thiamin disulfide have been calculated as cysteine hydrochloride.

The thiamin disulfide was prepared according to the procedure of Zima and Williams (15). Thiamin hydrochloride (Eastman Kodak, No. 5180), 10 g., was dissolved in 13 ml. of water and treated with a solution of 3.43 g. NaOH in 20 ml. of water. The yellow solution was cooled to near freezing in an ice bath, and exactly an equivalent amount of iodine (3.77 g. of iodine and 5 g. KI in 40.0 ml. solution) was added slowly with stirring and cooling. The reddish color of the mixture, due to a very slight excess of iodine, indicates the proper end-point. The solution was evaporated in a vacuum to a thick sirup and the residue extracted with N-butanol at 40° C., first 30 ml. and then two 10-ml. portions. The iodides remain behind. The extract was evaporated in a vacuum to a thick sirup and the residue extracted with 15 ml. acetone. The acetone extract was diluted with another 15-ml. portion of acetone and the mixture centrifuged. The acetone solution was then diluted with 200 ml. additional acetone and the solution stored at 0° C. for crystallization of the oxidation product, thiamin disulfide. After three days, the mother liquor was decanted off and the product dried on filter paper. The dried product was then purified by dissolving in 2 ml. of water per gram and diluted with 25 volumes of acetone. The disulfide crystallized out as colorless crystals during 24 hours at 0° C. After a second recrystallization, the product was practically free of interfering substances. A solution of the disulfide was prepared by dissolving 200 mg. in 75 ml. of a solution containing 10 ml. of N/10 HCl. The solution of thiamin disulfide was placed in a brown glass bottle and stored in a refrigerator. Under those conditions the solution showed no signs of deterioration.

Preliminary trials indicated that 2 ml. of fresh skimmilk or 200 mg. of non-fat milk solids was a suitable quantity for the determination of those substances in milk capable of reducing thiamin disulfide. One ml. of the disulfide solution was added to the milk in a test tube and, after mixing and covering with 3 drops of isobutanol, allowed to stand 2 hours at room tempera-

ture. When other substances were added, they were restricted to a volume of 1 ml.; otherwise 1 ml. of distilled water was added to each sample. Two ml. of 10 per cent trichloracetic acid was added to the digestion mixture at the end of the two-hour period. The proteins were separated from the liquid by centrifuging the tubes at high speed for five minutes and decanting into a second test tube through a funnel containing a small wad of cotton. Two ml. of the clear liquid was diluted to 100 ml. in a volumetric flask, and a suitable aliquot of from 1 to 5 ml. taken for the determination of thiamin by the thiochrome method. Blanks were prepared by the addition of the thiamin disulfide reagent following the trichloracetic acid. There is no reduction of the disulfide after the addition of the trichloracetic acid.

Any of the several slightly differing procedures for the direct thiochrome method may be used for the latter part of this method. Suggestions from methods of Conner and Straub (1) and Mason and Williams (9) were used in the procedure followed in this work. The aliquot for oxidation was made up to 5 ml. with distilled water in a 20 × 150 mm. test tube, and exactly 15 ml. of redistilled isobutanol added. After the isobutanol and aqueous layers were caused to appear homogeneous by stirring with a jet of air for a few seconds, 2 ml. of a solution containing 0.5 g. NaOH and 2 mg. potassium ferricyanide was quickly added from a pipette. The stirring was continued for 2 minutes. The aqueous layer that quickly separated was drawn off and discarded. The isobutanol was shaken briefly with approximately 2 g. of anhydrous sodium sulfate, and the mixture centrifuged a few seconds at high speed. The alcoholic solution was decanted into specially selected test tubes and the fluorescence determined in a Coleman Model 12 Electronic Photofluorometer. The instrument was set with a standard quinine sulfate solution and the oxidation of the thiamin to thiochrome checked with a standard solution of thiamin hydrochloride. According to the theory, one gram molecule of cysteine hydrochloride is equivalent to 2.14 gram molecules of thiamin hydrochloride.

EXPERIMENTAL RESULTS

Preliminary experiments showed that the pH of the reaction mixture of thiamin disulfide and material containing – SH groups must be between 4.8 and 6.2 for optimum results. High alkalinity caused some of the disulfide to revert to thiamin. Very little reduction of the reagent occurred at a pH of 4.0 or less. The pH of the mixture of 2 ml. of milk and 1 ml. of the reagent was around 5.4.

Quantitative recovery of cysteine-HCl from unheated skimmilk proved to be somewhat difficult. This fact was to be expected since Gould (3) found that glutathione was destroyed by unheated milk. It was considered possible that the addition of an excess of ascorbic acid would protect the cysteine and make recovery possible. The results presented in table 1 show that the addition of 5 mg. of ascorbic acid per ml. of milk is sufficient to permit the

TABLE 1

The effect of ascorbic acid on the recovery of cysteine hydrochloride from skimmilk

Ascorbic acid added (mg. per ml.)	Cysteine-HCl added (mg. per ml. of milk)	Total –SH groups calculated as mg. cysteine-HCl per liter		Per cent recovery of added cysteine-HCl	
		Unheated	Heated*	Unheated	Heated
0.0	0.0	Nil	19.6	.....	.....
0.0	9.6	3.4	29.5	35	103
2.5	9.6	6.8	28.9	71	96
5.0	9.6	9.3	29.0	97	97
7.5	9.6	8.3	.....	86	.....
5.0	0.0	Nil	19.7	.....	.....

* 97–97.5° C. for 5 minutes.

quantitative recovery of cysteine hydrochloride from unheated skimmilk. It may be noted from the data in table 1 that the addition of ascorbic acid is unnecessary for good recovery of cysteine-HCl from heated milk.

The data presented in table 2 show the improvement caused by ascorbic acid on the recovery of cysteine-HCl from 12 samples of non-fat milk solids of variable sulfhydryl content. Without ascorbic acid the recovery of cysteine is roughly proportional to the amount of sulfhydryl groups originally present.

The sulfhydryl contents of good and poor baking samples of non-fat milk solids from four different milk-drying plants were determined. The samples were collected throughout the year and represent extremes in baking quality. However, the actual range in baking quality was very small in most cases. The results in table 3 vary from 3.8 to 10.8 mg. of cysteine-HCl per 100 g. of non-fat milk solids; however, the average values for the good and poor baking samples are the same. The analysis of variance of data shown in table 3 is presented in table 4 and indicates much greater variability between flumes than within flumes for both the good and poor baking samples.

TABLE 2

The effect of ascorbic acid on the recovery of 9.6 mg. cysteine hydrochloride per 100 grams of non-fat milk solids

Sample No.	Original –SH groups in milk (mg. per 100 grams) expressed as cysteine-HCl	Without ascorbic acid	With 5 mg. ascorbic acid per 100 mg. milk solids
19	3.5	50	91
15	4.3	50	95
27	4.3	33	96
43	4.8	68	101
3	6.1	82	98
22	6.3	65	97
9	6.7	67	97
17	8.8	69	105
16	11.0	80	104
48	11.5	70	104
44	11.6	92	93
8	11.7	88	88

TABLE 3

Comparison of the sulfhydryl content of good and poor baking non-fat milk solids

	Total No. of samples	–SH content expressed as mg. cysteine-HCl per 100 g.				
		Flume A	Flume B	Flume C	Flume D	Average
Good baking	48	7.0	6.6	3.8	10.4	6.95
Poor baking	39	6.3	5.6	4.7	10.8	6.85

The lack of uniformity in the amount of sulfhydryl groups found in the non-fat milk solids from the several sources made it desirable to have more information on factors that might be responsible for this variability. The stability of the substances in milk that reduce thiamin disulfide was studied

TABLE 4

Analysis of variance of the data from table 3 for the sulhydryl content of non-fat milk solids manufactured throughout the year in four flumes

Source of variation	Degrees of freedom	Sum of squares	Mean square
Good baking			
Total	47	40,779.3	
Between flumes	3	26,157.7	8,719.2
Within flumes	44	14,621.6	332.3
Poor baking			
Total	38	36,570.1	
Between flumes	3	19,019.1	6,339.7
Within flumes	35	17,551.0	501.5

as indicated by the results in table 5. The non-fat milk solids were quickly reconstituted by shaking 2 g. of the milk with 20 ml. of water in a 125-ml. Erlenmeyer flask. Two-ml. portions were removed at the indicated time

TABLE 5

Stability of sulfhydryl groups in freshly heated (97° C., 5 min.) skimmilk and reconstituted non-fat milk solids

Holding time at 20° C.	Per cent loss of sulfhydryls	
	Non-fat milk solids	Fresh skimmilk
10 min.	12.6	3.0
30 "	16.6	3.0
60 "	23.3	21.0
2 hours	28.3	32.0
4 "	54.7	51.0
6 "	62.7	66.5
24 "	84.7	85.2
Original content of – SH groups expressed as cysteine-HCl	10.8 mg. per 100 grams	20.9 mg. per liter

intervals for the determination of sulfhydryl groups. The rate of loss was similar for both reconstituted non-fat solids and freshly heated skimmilk except that in the case of the latter there was a lag during the first 30 minutes. After 24 hours at 20° C. approximately 85 per cent of the –SH groups had disappeared.

The effects of time and temperature of heat treatment on the sulfhydryl groups in pasteurized skimmilk are illustrated in figure 1. The minimum heat treatments required for the liberation of detectable amounts of –SH

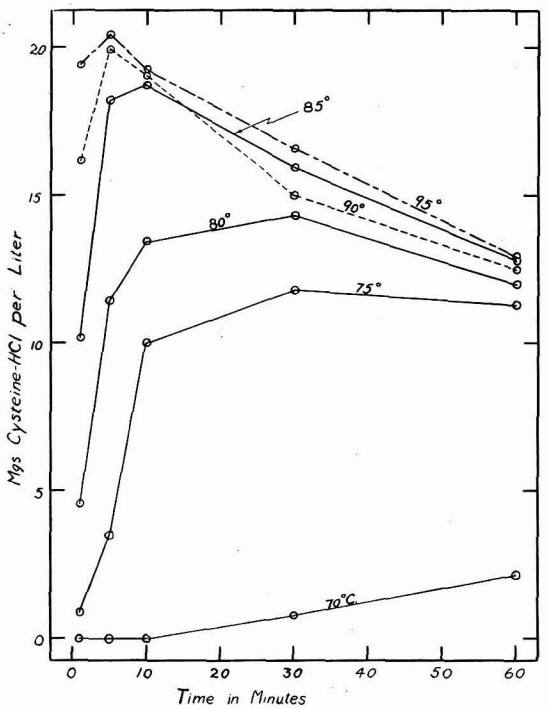

FIG. 1. The effects of time and temperature of heat treatment on the number of sulfhydryl groups in pasteurized skimmilk. Results expressed as cysteine hydrochloride.

groups are 70° and 75° C. for approximately 30 minutes and 1 minute respectively. Heating the milk for 60 minutes at 70° C. results in the liberation of the equivalent of only 2.2 mg. of cysteine-HCl per liter. The increase in the amount of liberated sulfhydryl is very rapid and reaches a maximum of approximately 20 mg. cysteine-HCl per liter during the first 10 minutes of heating at temperatures above 80° C. After reaching a maximum, the values for a period of one hour in the temperature range 75°–95° C. vary between the narrow limits of 11.3 and 12.9 mg. cysteine-HCl per liter.

DISCUSSION

The exact nature of the substances liberated by skimmilk heated to temperatures above 70° C. that are capable of reducing thiamin disulfide cannot be definitely stated. However, it has been shown by other methods (4, 8, 11) that both H_2S and – SH are liberated at those temperatures. The presence of – SH groups has been confirmed by the nitroprusside test in this laboratory. It has also been substantiated by us that thiamin disulfide is reduced by both – SH and H_2S. Unreported results show a better than 50 per cent recovery of H_2S by the thiamin disulfide reagent. Perhaps further investigation would reveal a more quantitative recovery of the H_2S.

The findings of others (2, 4, 6, 8) that certain reducing substances are liberated following heat treatment of milk were substantiated by the new method used in this investigation. On the other hand, Stamberg and Bailey (10), using the dropping mercury electrode, found that unheated fresh skimmilk contained a relatively large amount of reducing substances that were destroyed during heat treatment at boiling temperatures. Gould (5), using a KIO_3 titration method, obtained results qualitatively similar to those of Stamberg and Bailey. It appears that, as one reducing system is destroyed by temperatures above 70° C., another is liberated.

Although skimmilk is subjected to rather drastic heat treatment previous to drying when the product is intended for baking purposes, in no case was it possible to find more than approximately 60 per cent as much reducing substances in dry skimmilk as in an equivalent amount of laboratory heated skimmilk. A part of the reducing substances may be either oxidized or liberated as H_2S during the drying process. The experimental results showed a rather rapid disappearance of the reducing substances in freshly heated skimmilk and freshly reconstituted non-fat solids with standing. Gould's work (4) indicates that the amount of contaminating copper in the milk may cause rather large variations in the rate of disappearance of the sulfhydryl groups in milk. For this reason the results presented in table 5 may not apply quantitatively to all lots of similarly heated skimmilk and reconstituted non-fat milk solids. Time of storage did not appear to greatly affect the sulfhydryl groups in non-fat milk solids, however, since samples of dry milk produced 5 years ago exhibited a range of values for reducing substances similar to those manufactured more recently.

The wide range of time and temperatures, in which rather high values for reducing substances in the heated milk occur, seems to partially account for the lack of correlation between those substances and baking quality in non-fat milk solids.

The use of the method described in this report for the quantitative estimation of reducing substances liberated by heat treatment of milk is of value to those interested in cooked and oxidized flavors in milk. The use of a more highly purified disulfide reagent and very careful standardization of the

estimation of the liberated thiamin would result in even greater precision than is indicated by the results presented in this paper. This method has the advantage of requiring only small quantities of the substance being examined. It would be possible to get reliable results with much less than the 2 ml. aliquot used in this investigation.

CONCLUSIONS

Thiamin disulfide is an excellent reagent for the quantitative estimation of certain reducing substances liberated by milk during heat treatment at temperatures above 70° C.

The addition of 5 per cent of ascorbic acid to unheated skimmilk permits the recovery of as little as 10 mg. cysteine-HCl per liter of skimmilk.

Reducing substances equivalent to approximately 20 mg. of cysteine-HCl per liter are liberated when pasteurized skimmilk is heated to 90°–95° C. for 5 minutes. Unheated milk does not reduce thiamin disulfide.

Spray process non-fat milk solids contain substances that reduce thiamin disulfide equivalent to 0.0–11.5 mg. of cysteine-HCl per 100 grams.

According to the method used in this work, there is no correlation between the quantity of thiamin disulfide reducing substances and the baking quality of non-fat milk solids.

Heating of skimmilk longer than 10 minutes at temperatures of 80° C. or above results in a loss of reducing substances. Both reconstituted non-fat milk solids and freshly heated skimmilk rapidly lose their reducing substances on standing at 20° C.

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THE EFFECT OF CHLOROBUTANOL ON CERTAIN MEMBERS OF THE B COMPLEX IN THE RUMEN AND BLOOD PLASMA ASCORBIC ACID LEVELS*

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The work of Phillips *et al.* (5, 6) has shown that ascorbic acid was associated with reproduction in the bovine. Bortree and associates (2) demonstrated that chloretone increased vitamin C levels of both the cow and the bull. Scheidenhelm and co-workers (7) showed that chloretone had a beneficial effect on slow-breeding bulls. More recently Bortree *et al.* (1) concluded that feeding chlorobutanol will increase blood plasma ascorbic acid levels in calves, heifers, cows and bulls. Throughout these latter studies, a dose of 5 grams fed with the grain per day and continued for 105 days was quite satisfactory and produced no harmful effects.

During the past few years, ascorbic acid in amounts necessary for use in prophylactic treatment of "hard to settle" cows and "slow breeding" bulls has been difficult to secure. In some instances this has led to the use of chlorobutanol for that purpose. It was felt that, if this were to become a common practice, investigation of its effect on the synthesis of the B complex in the rumen of the cow was necessary.

EXPERIMENTAL

The observations reported herein were made on a 966-pound Holstein heifer with a rumen fistula. The ration consisted of timothy hay, corn silage, and a grain mixture of corn, oats, linseed meal, iodized salt and $CaCO_3$. This ration maintained normal blood plasma vitamin A concentrations.

Samples from the rumen were taken four times at two-hour intervals on test days, beginning two hours after the morning feeding. The sampling consisted of removing approximately one kilogram of material from the central portion of the rumen from which 100 grams were accurately weighed and diluted with an equal weight of 95 per cent ethyl alcohol. It was then stored in a refrigerator between sampling, and in a dark, cold room at 35 to 36° C. overnight. The composite sample was then placed in a large, flat pan and dried for 48 hours at 50° C. The dry matter content ranged between 15 and 17 per cent. The material was then finely ground in a Wiley mill and stored in the cold room until the analyses were made.

Received for publication June 26, 1944.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

The chlorobutanol was so mixed into the grain ration that the heifer received five grams per day. New batches were prepared each sixteenth day.

Analyses of the samples for riboflavin were made by the microbiological assay of Snell and Strong (8) as modified by Strong and Carpenter (10). Pantothenic acid was determined microbiologically by the method of Strong *et al.* (11) and niacin by the method of Snell and Wright (9). Thiamine determinations were by the chemical thiochrome method of Hennessy (3). Analyses of blood plasma ascorbic acid were by the method of Mindlin and Butler (4).

RESULTS

The only analyses available for the vitamin content of the rumen contents after the addition of chlorobutanol were obtained on the 68th day. Samples were collected on the 115th day and on the 155th day, but they were inadvertently destroyed before analyses were completed.

The results indicate that riboflavin, niacin, thiamine and pantothenic acid syntheses were not materially affected by additions of 5 grams of chlorobutanol daily to the ration (Table 1). There was no noticeable outward

TABLE 1

The effect of chlorobutanol on the rumen content of certain of the B complex vitamins

Days	Niacin	Riboflavin	Thiamine	Pantothenic acid
	μg./gm.	μg./gm.	μg./gm.	μg./gm.
− 11	51	13.2	1.6	14.3
− 1	47	11.0	1.3	7.8
0	chlorobutanol added to ration			
+ 68	51	11.8	2.2	8.1
+160	chlorobutanol removed from ration			
+201	55	13.8	2.2	16.3
+231	68	13.0	3.5	6.4
+260	61	11.9	2.0	5.4

effect on the heifer, except that it seemed to stimulate appetite and thus increase body weight. This increased from 966 to 1228 pounds during the chlorobutanol administration and dropped to 1200 pounds after withdrawal of the drug. The chlorobutanol was removed from the ration after it had been fed for 160 days.

The rise in blood plasma ascorbic acid by the addition of chlorobutanol was definite but not marked. However, the higher level was maintained throughout the chlorobutanol feeding period (fig. 1). Following this feeding period, blood plasma ascorbic acid dropped to a rather low level within two weeks and required nearly one month to regain concentrations which were characteristic for this heifer. No detrimental effects were noted. Since there was no apparent reason for this drop other than the after effect of long-time chlorobutanol feeding, we felt it was necessary to determine the effect of feeding chlorobutanol for only a three-week period. The results of

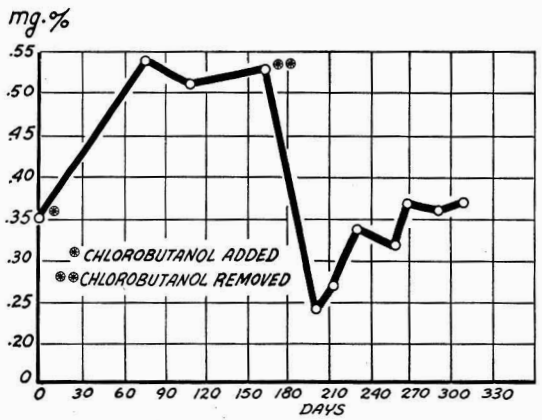

FIG. 1. The effect of feeding chlorobutanol for 160 days on blood plasma ascorbic acid concentrations.

this experiment (fig. 2) indicate that no unusual drop below normal in blood plasma ascorbic acid occurred.

CONCLUSIONS

The results of this experiment indicate that chlorobutanol, when fed to a 966-pound heifer at the rate of 5 grams per day, had no detrimental effect on the rumen synthesis of thiamine, riboflavin, niacin and pantothenic acid.

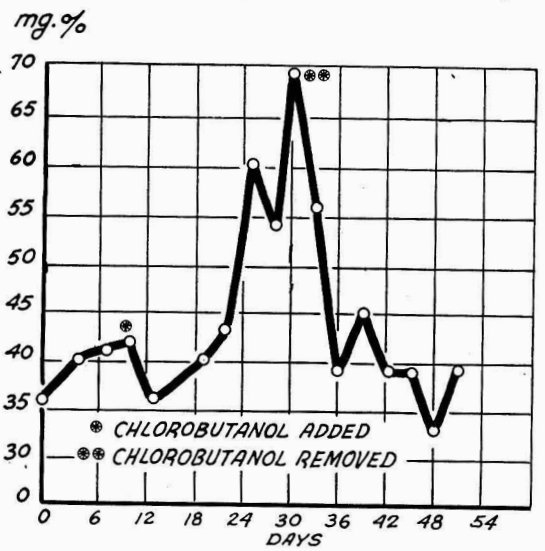

FIG. 2. The effect of feeding chlorobutanol for 21 days on blood plasma ascorbic acid concentrations.

The work of previous workers (1, 2, 7) was confirmed in that chlorobutanol will increase blood plasma ascorbic acid. The administration of chlorobutanol at the rate of 5 grams daily was without obvious harmful effects for a period up to 160 days. During this interval the drug sustained increased concentrations of blood plasma ascorbic acid. This dropped sharply and below normal upon removal of the drug. It required approximately one month for the heifer to regain normal concentrations of this vitamin in its blood plasma. A subsequent three-week period of chlorobutanol administration and withdrawal brought about an increase in blood plasma ascorbic acid without the sub-normal drop accompanying the withdrawal of the drug.

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EFFECT OF HIGH-TEMPERATURE-SHORT-TIME PASTEURIZATION ON THE ASCORBIC ACID, RIBOFLAVIN AND THIAMIN CONTENT OF MILK*

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Earlier papers by Holmes, Tripp, Woelffer and Satterfield (7), Holmes, Jones, Wertz, and Kuzmeski (5), and Holmes (3) contain data concerning the effect of the holding method of pasteurization on the ascorbic acid, riboflavin, and thiamin content of milk. This process consists of pasteurizing milk by holding it at about 142–144° F. for 30 minutes. Since the conditions existing for the high-temperature-short-time pasteurizing process are somewhat different from those of the holding process which is generally used for the commercial pasteurization of milk, a question naturally arises concerning the effect of the high-temperature-short-time pasteurizing process upon the vitamin content; and this study was undertaken to accumulate data regarding this point.

EXPERIMENTAL

Equipment. The high-temperature-short-time pasteurization of milk is accomplished by different means and with various types of equipment. An electro-pure pasteurizer,[1] which was part of the equipment for instructing students in various pasteurization processes, was employed for pasteurizing the twenty lots of milk which were included in the experiments reported here. According to the manufacturer, the electro-pure pasteurizer operates on regular single phase, 220-volt, 60-cycle current and requires about one kilowatt-hour for pasteurizing 90 pounds of milk. Pasteurization takes place in the electrode chamber, which is a vertical, rectangular compartment through which the milk ascends at a constant uniform flow between flat carbon electrodes on opposite walls of the chamber. The temperature range of the equipment was reputed to be from 160.5° to 180.0° F. with a fixed, invariable holding time of about 22 seconds. In these experiments an attempt was made to pasteurize milk at various points within the reputed temperature range of the pasteurizer.

Source of milk. The milk used in these experiments was produced by the College herd of about seventy Ayrshire, Guernsey, Holstein, Jersey, and Shorthorn cows. The conditions of feeding, management, composition of the ration, and stage of lactation were quite similar to those previously de-

Received for publication June 26, 1944.

* Contribution No. 530, Massachusetts Agricultural Experiment Station.

[1] Manufactured by the Trumbull Electric Manufacturing Co., Plainville, Conn.

scribed by Holmes and Holmes (4). The cows were milked by machine at 3 : 30 P.M. and 3 : 30 A.M. The afternoon milk was promptly cooled and held at 40° F. until the morning milk was cooled and mixed with it. About four hours later a sample of the raw milk was taken for assay, the milk was pasteurized and again sampled for assay. The milk was pasteurized by the high-temperature-short-time process at irregular intervals during eighteen months. It is felt that the twenty samples of mixed-herd milk collected over such a long period are fairly typical of milk produced by the commercial dairies of this area.

Assay procedures. The assays of the raw and pasteurized milk were commenced within 30–60 minutes after the milk was pasteurized. The ascorbic acid was determined by the 2–6 dichlorophenolindophenol titration method described by Holmes, Tripp, Woelffer, and Satterfield (6). The riboflavin content of the milk was determined by the fluorescence procedure discussed in detail by Holmes, Jones, Wertz, and Kuzmeski (5). The thiochrome method reported by Hennessy and Cerecedo (2) was used to determine the thiamin content of the raw and pasteurized milk.

RESULTS

Of the twenty samples of milk included in this study, Table 1, three represented milk pasteurized at 161°; two at 162°; two at 164°; and one each at 167°, 168° and 177°; two at 180°; and eight at 181° F. The amount of ascorbic acid in the raw milk varied from 11.7 mg. to 18.7 mg. per liter and in the pasteurized milk from 11.6 mg. to 20.1 mg. per liter. The riboflavin in the raw milk varied from 1.32 mg. to 1.71 mg. and in the pasteurized milk from 1.19 mg. to 1.75 mg. per liter. The thiamin in the raw milk varied from 0.30 mg. to 0.41 mg. and in the pasteurized milk from 0.29 mg. to 0.40 mg. per liter. The average values obtained for the twenty samples of milk were ascorbic acid of raw 16.4 mg. and of pasteurized milk 16.6 mg. per liter, riboflavin of raw milk 1.50 mg. and of pasteurized milk 1.50 mg. per liter, and thiamin of raw milk 0.36 mg. and of pasteurized milk 0.35 mg. per liter.

Thus in these experiments conducted with a commercial-size pasteurizer there was no loss of ascorbic acid or riboflavin and only about a three per cent loss of thiamin. Hence, considered from a practical standpoint, milk pasteurized by this type of high-temperature-short-time procedure has as satisfactory ascorbic acid, riboflavin, and thiamin content as it had before pasteurization.

It is of interest to compare the results obtained in these experiments with those obtained in similar experiments conducted with another procedure. Holmes, Tripp, Woelffer, and Satterfield (6) determined the ascorbic acid, before and after pasteurization of milk (Guernsey and Holstein cows), drawn by a milking machine into aluminum pails and subsequently pasteur-

ized at 143° F. for 30 minutes in a 300-gallon stainless steel vat equipped with stainless steel agitators, which kept the milk gently in motion during the pasteurization period. They assayed 30 samples representative of 300-gallon lots of milk pasteurized at irregular intervals during an eighteen months' period, identical with the experimental period of this study, and found 17.3 mg. of ascorbic acid in the raw milk and 14.0 mg. per liter in the pasteurized milk. Thus 18.7 per cent of the ascorbic acid was lost during pasteurization. Subsequently, Holmes, Jones, Wertz, and Kuzmeski (5)

TABLE 1

Effect of high-temperature-short-time pasteurization on the ascorbic acid, riboflavin and thiamin content of milk

(Milligrams per liter of milk)

	Temperature, °F.	Ascorbic acid		Riboflavin		Thiamin	
		Raw	Pasteur.	Raw	Pasteur.	Raw	Pasteur.
1	161	18.7	18.7	1.39	1.58	0.32	0.31
2	161	18.2	.....	1.50	1.43	0.35	0.33
3	161	15.7	15.7	1.47	1.37	0.33	0.35
4	162	16.9	16.4	1.48	1.46	0.35	0.33
5	162	15.9	20.1	1.35	1.43	0.32	0.30
6	164	11.7	13.4	1.32	1.37	0.35	0.33
7	164	17.1	17.1	1.71	1.75	0.40	0.37
8	167	16.7	17.1	1.58	1.60	0.39	0.39
9	168	15.5	15.5	1.68	1.70	0.41	0.40
10	177	17.6	17.6	1.56	1.62	0.38	0.38
11	180	16.7	16.5	1.58	1.46	0.39	0.38
12	180	15.9	19.6	1.35	1.36	0.32	0.30
13	181	18.7	18.7	1.39	1.37	0.30	0.29
14	181	18.2	.....	1.50	1.57	0.35	0.34
15	181	16.9	16.9	1.48	1.19	0.35	0.35
16	181	11.7	13.2	1.32	1.43	0.35	0.32
17	181	17.6	17.6	1.56	1.61	0.38	0.38
18	181	15.5	15.7	1.68	1.66	0.41	0.36
19	181	17.1	17.1	1.71	1.66	0.40	0.40
20	181	15.7	11.6	1.47	1.31	0.33	0.35
Average	.....	16.4±1.9	16.6±2.2	1.50±0.13	1.50±0.15	0.36±0.03	0.35±0.03

repeated the determination, using a different herd of cows, different herdsmen, dairymen, and pasteurizing equipment. Again the milk (Ayrshire, Guernsey, Holstein, Jersey, and Shorthorn cows) was drawn by milking machine and pasteurized in a stainless steel vat (200-gallon) equipped with stainless steel agitators. Thirty-two samples of milk were assayed and averaged to contain 19.7 mg. per liter in the raw milk and 15.9 mg. in the pasteurized milk, a loss of 18.3 per cent. As might be anticipated, the ascorbic acid content of milk produced in 1938 and in 1943 by different herds of cows which received different rations was not identical. However, it is interesting to note that when the milk produced in different localities was pasteurized for the same length of time, at the same temperature, and by the same type of equipment, the loss of ascorbic acid was for all practical purposes identical, 18.7 per cent and 18.3 per cent.

In a previous paper Holmes, Jones, Wertz, and Kuzmeski (5) reported a 2 per cent loss of riboflavin during the pasteurization of milk for 33 minutes at 144° F. in a stainless steel pasteurizer. In this study, using the high-temperature-short-time pasteurization process, there was no loss of riboflavin; and this is in accordance with the findings of Javillier (9), who reported no loss of riboflavin during preheating and sterilizing of milk, and Houston, Kon, and Thompson (8), who found slightly more riboflavin in milk after pasteurization or sterilization. However, these differences have no practical significance.

The loss of thiamin, about 3 per cent, by the high-temperature-short-time pasteurization procedure is appreciably less than the 9.1 per cent loss reported by Holmes, Jones, Wertz, and Kuzmeski (5) for milk pasteurized 33 minutes at 144° F. and much less than the 25 per cent loss reported by Elvehjem (1) and Krauss *et al.* (10).

SUMMARY

Twenty lots of milk were pasteurized at irregular intervals during eighteen months by the high-temperature-short-time process. The milk was assayed for ascorbic acid, riboflavin, and thiamin just before and immediately following pasteurization. The average values obtained were 16.4 mg. per liter of ascorbic acid for the raw, and 16.6 mg. for the pasteurized milk; 1.50 mg. per liter of riboflavin for the raw, and 1.50 mg. per liter for the pasteurized milk; and 0.36 mg. per liter of thiamin for the raw, and 0.35 mg. per liter for the pasteurized milk.

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BROWN ALFALFA HAY—ITS CHEMICAL COMPOSITION AND NUTRITIVE VALUE IN DAIRY RATIONS[1]

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Alfalfa hay which contains 30 per cent or more of moisture when placed in the mow or stack is likely to become brown during storage (14, 24, 26). This color varies from brown to black, depending upon the amount of heat developed during storage (10). Excessive heating may result in brown hay which has a pleasing sweet, tobacco-like aroma, and which may be more palatable to cattle than green hay (18). More intensive heating may result in black or charred hay.

Brown hay occurs most frequently in Kansas from the first cutting of alfalfa because of less favorable hay-curing weather. This problem was discussed by Cottrell (9) as early as 1902 in a bulletin from the Kansas Agricultural Experiment Station. The proportion of the total hay crop which cures into brown hay each year is unknown. It was estimated recently that 15 per cent of the hay in 34,500 carlots inspected at Kansas City, Missouri, was sample grade because of damage associated with spontaneous heating (22). An earlier statement placed losses from spontaneous heating at fully one-tenth of the hay harvested in the United States (3).

Although practically all the brown hay in Kansas is produced inadvertently, many farmers regard it as equal, if not superior, to green hay for cattle-feeding purposes. Published results of feeding tests are not in agreement on the value of brown hay, but the majority indicate decreased digestibility and lowered productive value (26).

REVIEW OF LITERATURE

The composition and feeding value of brown hay has been studied by several workers. Watson's review (26) shows that brown hay may resemble normal hay in chemical composition, but that the browning process is associated with losses in organic matter and in feeding value proportional to the intensity of brown color developed by the hay. Percentage losses in weight attributed to the browning of legume hay were—dry matter 19, nitrogen-free extract 23, fiber 22, and crude protein 9. Up to two-thirds of the starch disappeared during the heating of stored hay. Depressions in digestibility were greatest in the case of protein, with true protein being affected more than crude protein. Only "slight heating" of hay reduced the apparent digestibility of protein 50 per cent. A total loss of digestible protein occurred in "very badly-heated" hay. The nitrogen-free extract was less

Received for publication July 5, 1944.

[1] Contribution No. 156, Department of Dairy Husbandry.

severely affected. The digestion coefficient for fiber was depressed only where the hay was badly heated. Milk production deceased when heated hay displaced one-half of the normal hay in dairy rations. The health of the cows was disturbed in a few cases when large proportions of "badly-heated" hay were fed.

Boekhout and deVries (2) in 1904 reported that spontaneous heating of hay in stacks with interior temperatures of 185° and 205° F. decomposed some of the pentosans and nitrogen-free extractives. Very black heated hay was examined microscopically. The epidermal cells and fibrovascular bundles were not darkened. The protoplasm of other cells was darkened or wholly black, and appeared to account for the darkening noted in adjacent cell walls.

At the Kansas Agricultural Experiment Station (25), wilted alfalfa containing an average of 53.28 per cent moisture underwent the following percentage losses during a storage period of several months in a stack: Dry matter 39.07, protein 32.71, ether extract 63.57, crude fiber 46.54, and nitrogen-free extract 45.36. Material removed from the stack varied from light-brown to black in color. The percentage of ash in the burned samples exceeded that in the original hay because of losses in organic matter during burning. Calculated losses of organic matter, based on these differences in ash percentage, agreed closely with those obtained by weighing and sampling each kind of hay removed from the stack.

According to Dodd (10), stacked hay will retain some green color if no perceptible heat is present, but will appear light-brown at 120° F., "warm brown" at 140° to 150° F., dark brown at 160° F., and more or less charred beginning at approximately 190° F. During these changes, sugars are thought to be decomposed into glucic acid and other unsaturated compounds (4, 15). These in turn may unite rapidly with oxygen to produce additional heat, leading eventually to black hay containing 50 per cent or more of carbon. Lignin also may be decomposed and lead to increased heat liberation (21).

In a series of large-scale experiments from 1929 to 1934, Hoffman and Bradshaw (16) studied the extent and nature of losses from spontaneous heating in six samples of alfalfa and one sample of clover. The moisture content of the samples at the time of storage in a barn varied from 28 to 70 per cent. The storage interval extended from one to seven and one-half months. Losses during storage varied with the moisture content of the hay and with the maximum temperature recorded during storage. The principal losses in percentage were: Dry matter 4 to 22, ether extract 6 to 47, sugars 59 to 94, and hemicelluloses 14 to 52. Spectrophotometric methods showed that losses in ether extract were accompanied by complete destruction of carotene. Some cellulose and crude protein were lost where storage conditions favored relatively heavy destruction of other organic constituents.

Lignin appeared to be unaffected in these experiments. The highest temperature recorded in these tests was equivalent to 172.4° F. Roethe (24) reported similar results with respect to losses of dry matter, ether extract, sugars, hemicelluloses, protein, cellulose, and carotene. Others (17, 27) have stressed the seriousness of losses in chemical constituents during the spontaneous heating of hay.

In Kansas studies of the production of yearling beef (19), normal alfalfa hay was compared with similar hay browned in a stack, and with black hay made by stacking fresh green alfalfa. Daily weight gains were approximately equal in the brown-hay and normal-hay groups. Steers fed the black alfalfa gained 25 per cent less and their final market value per hundredweight was appreciably lower. Stack-browned alfalfa hay was compared with field-cured alfalfa for short periods during each of two years in single reversal feeding trials with dairy cows at the same station (6, 7, 8). Each type of hay was used with a basal ration which included sorgo silage and a grain mixture of corn chop, wheat bran, and linseed oil meal. The cows consumed 5.5 per cent more grain and produced 4.3 per cent less milk when fed brown hay than when fed normal green hay.

Brown hay slightly surpassed green hay of the same cutting for the growth of lambs fed fattening rations of alfalfa hay, barley grain, and salt (18). In digestion experiments with sheep (11), the coefficients of digestibility for dry matter, crude protein, nitrogen-free extract and crude fiber were approximately the same for brown hay of excellent quality as reported by other investigators for ordinary green hay.

The palatability of brown hay has been considered by several workers. Maynard, Esplin, and Boswell (18) reported that cattle usually consumed brown-cured alfalfa with less waste than green-cured alfalfa. Willard (28) found less sugar in stack-browned alfalfa but the palatability of the hay was retained. Folger (11) suggested that the good results with brown alfalfa hay reported by livestock feeders may be explained on the basis of the animals eating more brown hay than ordinary hay. Cannon, Collins, and Espe (5) observed no difference in palatability between brown hay and green hay, but blackened hay was less palatable to lactating cows. Reed (23) reported the results of dairy feeding tests, from which it was concluded that alfalfa hay was not made more palatable by browning in the mow.

MATERIALS AND METHODS

This investigation consisted of two parts: 1. A series of digestion trials, and 2. A double reversal feeding test.

Six cows, divided into three groups of two cows each, were used to determine the apparent digestibility of: 1. Normal alfalfa hay, 2. Brown alfalfa hay, and 3. Black or charred alfalfa hay. All the hay represented first cutting, the normal and brown hays being from the same source and from

different sections of the same stack, but the charred hay was from a different source. The digestion trial extended over a 10-day feces-collection period beginning January 28, 1941. The trial was preceded by a 13-day adjustment period during which the animals were shifted from normal herd feed (alfalfa hay, silage, and a grain mixture) to alfalfa hay supplemented only with drinking water. The cows received all the alfalfa hay they would eat, and were fed and watered twice daily. The normal and brown hays were fed as long hay, but the charred hay was chopped when stacked. The feeds were weighed and sampled daily. Feed refusals were obtained from only one cow, and 5 per cent of each portion of her waste feed was sampled for analysis. The freshly voided feces were accumulated in covered metal containers by 24-hour periods. Five per cent by weight of each day's collection of feces was accrued as a composite sample, preserved by daily additions of 5 cubic centimeters of chloroform. The average body weight of each cow was obtained at the beginning of the adjustment period, and at the beginning and end of the 10-day collection period, from weights taken on three consecutive mornings before feeding, watering, or milking. The cows remained in stanchions throughout the feces-collection period.

Normal alfalfa hay and brown alfalfa hay were compared in a 90-day double-reversal feeding test, starting January 21, 1941. The test consisted of three 30-day periods, each divided into a 10-day preliminary period and a 20-day test period. Two groups of four cows each were balanced at the beginning of the test in so far as possible with respect to breed, stage and level of lactation, and milking frequency. The cows in Group I were fed normal hay during Period 1, brown hay during Period 2, and normal hay during Period 3. The Group II cows were fed just the reverse, being started on brown hay, changed to normal hay, and then back to brown hay. Four cows were used in each group during the feeding test, but two cows in each group were so low in production that it seemed unjustifiable to include data from them in interpreting the results. The authors recognize the limitations of the small groups but present the results only as supplemental data to the digestion trials. The normal hay and brown hay (same stack of brown hay as used in digestion trials) fed to these cows were from different sources but were both first cutting. All hay was chopped prior to feeding after the first week of the experiment in order to obtain more accurate feeding data. The normal hay was fed at a daily rate of approximately 20 pounds per 1,000 pounds of body weight; and sufficient brown hay was fed to equal the dry matter intake of normal hay, based on moisture analyses made every five to seven days during the test. In addition to hay, both groups of cows were fed Atlas sorgo silage and a grain mixture (400 parts by weight of ground yellow corn, 200 ground oats, 200 wheat bran, 100 soybean oil meal, 10 salt, and 10 steamed bone meal). The number of pounds of silage fed was the same as that of hay, all necessary adjustments being

made at the beginning of each 30-day test period. Proportionately more hay was fed than normally, since hay was the variable being tested. The grain mixture was fed at the daily rate of 0.4 of a pound for each pound of milk in excess of 16 pounds for Holsteins and 0.6 of a pound for each pound of milk in excess of 10 pounds for Jerseys. Grain feeding was adjusted according to the milk production of each cow at the beginning of each 20-day test period. Wood shavings were used for bedding. The cows were kept in stanchions except for brief daily periods of exercise. Rations fed and the milk produced were recorded, and samples of each were composited at regular intervals for chemical analysis. Feed refusals were weighed once daily and discarded without sampling for analysis. The average body weight of each cow was obtained at the beginning and the end of each 20-day test period from weights taken on three consecutive mornings before feeding, watering, or milking.

All samples, except those for which moisture only was determined, were analyzed under the supervision of the Department of Chemistry in the Kansas Agricultural Experiment Station.

RESULTS AND DISCUSSION

Chemical composition. The chemical analyses (table 1) show that the normal and brown hays from the same stack, and the black hay from another source, each contained more than 21 per cent of protein (oven-dry basis) whereas Morrison's (20) average for 632 samples from many sources is 16.3 per cent. The crude fiber content of the normal and brown hays also was lower than Morrison's average. The experimental hays were of excellent quality from the standpoint of leafiness which, together with the high protein content shown by the analyses, is to be expected since hay stacked with sufficient moisture to cause "browning" usually retains a higher percentage of leaves than hay more thoroughly cured in the field.

The ash content of the hay appears to have increased as the degree of excessive heating increased; however, the percentages of calcium and of phosphorus were approximately the same in the heated as in the normal hay. Swanson, Call, and Salmon (25) reported that the ash content was a measure of loss of organic matter and nutrients. On that basis the 10.60 per cent of ash in brown hay would be equivalent to the ash in 109 pounds of normal hay (of 9.72 per cent ash), or a loss in organic matter of 8 per cent more than normal hay during the storage period. If the black hay containing 11.66 per cent ash is assumed to be of the same original quality, the ash would be equivalent to 120 pounds of normal hay, and the loss would be 17 per cent greater than in the normal hay during storage. Both losses are less than reported by Swanson *et al.* (25) for similar types of hay. The three hay samples (normal, brown, and black) used in the digestion trials had approximately the same protein content; but if adjustments are made,

as above, for differences in losses of organic matter during storage, the protein content of the brown hay would be equivalent to 19.3 per cent in terms of material having the same organic matter and ash content as the normal hay (a loss of 10.3 per cent more than normal hay) versus 17.5 per cent of protein in the black hay (a loss of 18.6 per cent).

Crude fiber and nitrogen-free extract were the constituents most markedly affected by storage conditions as indicated by analyses of samples of hay used in the digestion trials. Using the ash factor for storage-loss adjustment, however, the brown hay was found to contain only 3 per cent more fiber and the black hay 15 per cent more than normal hay. As the degree of excessive heating increased, the nitrogen-free extract content was lowered

TABLE 1

Chemical composition of alfalfa hays used in digestion trials and in double-reversal feeding test, compared with data reported by Morrison (20)

Description of alfalfa hay	Moisture	Composition of dry matter[a]						
		Crude protein	Ether extract	Crude fiber	Ash	N-free extract	Ca	P
Used in digestion trials								
Normal[b]	20.4	21.51	2.40	25.20	9.72	41.16	1.53	0.29
Brown[c]	11.4	21.04	1.93	28.17	10.60	38.27	1.56	0.30
Black[d]	16.9	21.03	1.85	35.03	11.66	30.42	1.57	0.28
Used in double-reversal feeding test								
Normal	12.5	15.01	2.19	34.47	9.46	38.87	.....	.....
Brown	14.6	22.09	2.06	27.83	11.02	37.00	.....	.....
Analyses reported by Morrison								
Normal[e]	9.6	16.3	2.2	32.1	9.2	40.2	1.58	0.23
Brown[f]	10.2	16.3	1.4	27.5	10.4	44.4	.....	.....

[a] Dry matter of experimental hays determined by drying in an oven at 100° to 105° C.
[b] Good quality, leafy hay from outer portion of stack on College farm.
[c] Similar to the normal hay, except selected from inner portion of the same stack.
[d] Coarsely-chopped hay selected from the remains of a partially-burned stack on a nearby farm.
[e] Representing 632 samples.
[f] Representing one sample.

according to analyses of the samples; when adjusted for ash changes, the loss during storage in the brown hay was 15 per cent and the black hay 39 per cent more than in normal hay. When similarly computed, the loss in ether extract was 26 per cent more for the brown hay and 36 per cent more for the black hay.

Comparisons of analyses for the two hays used in the double-reversal feeding test indicate that the brown hay originally was much higher in protein and lower in fiber than the original normal hay. However, the sample of the brown excessively-heated hay contained more ash, less nitrogen-free extract, and somewhat less ether extract than the sample of normal hay.

Digestion trials. The digestibility-coefficients (table 2) show that excessive heating lowered the apparent digestibility of all the measured constituents, except ether extract, and the greater the apparent degree of heating the greater the reduction in digestibility. Of the dry matter in normal hay, an average of 61 per cent was digestible, while in brown hay an average of 41 per cent was digestible, and in black hay 27 per cent. Thus, excessive heating during storage resulted in an average reduction in digestibility of dry matter in brown hay of 33 per cent, and 56 per cent in black hay compared with normal hay. The black hay was 33 per cent less digestible than brown hay in dry matter. Digestibility of crude protein was affected more than any of the constituents—averaging 67 per cent for normal hay, 17 per cent for brown and 3 per cent for black, or a reduction of 75 per cent in digestibility for brown hay and 96 per cent for black hay. The average digestibility of crude fiber was reduced from 41 per cent for normal hay to 36 for brown and 14 for black, or a reduction of 22 per cent for brown and 66 per cent for black hay compared with the normal hay. Nitrogen-free extract averaged 72 per cent in digestibility for the normal hay, 59 per cent for the brown, and 53 per cent for the black. Thus, the reduction from normal hay was less than for the constituents previously mentioned—being 18 per cent for brown hay and 26 per cent for black. Ether extract was the exception, in that the apparent digestibility averaged higher for the excessively heated hays than for normal (normal hay 25 per cent, brown hay 33, and black 43). The coefficients of digestibility for the various constituents of the normal hay are in reasonable agreement with the average of 242 trials reported by Morrison (20), considering the fact that only one source of hay is involved, and that it was of a better than average quality.

By applying the digestibility-coefficients to the analyses of the different hays, it was found that the normal hay contained 14.4 per cent digestible protein, the brown hay 3.4 per cent, and the black only 0.6 per cent (table 2). Thus, the brown hay contained 23 per cent as much as normal hay, and black hay only 4 per cent as much, or a reduction caused by excessive heating during storage of 77 and 96 per cent, respectively. Similarly, the content of total digestible nutrients in normal hay was 55.7 per cent, in brown hay 37.5 per cent, and in black hay 23.4 per cent. The brown hay, therefore, contained 67 per cent as much and the black hay 42 per cent, or respective reductions of 33 and 58 per cent.

A measure of the relative palatability of the hays is indicated by the fact that the cows fed normal hay consumed an average of 20.2 pounds of dry matter daily per 1,000 pounds of body weight, while the cows fed brown hay averaged 15.0 pounds, and those fed black hay averaged only 10.0 pounds. Therefore, the cows consumed 26 per cent less dry matter as brown hay and 50 per cent less as black hay than in the form of normal hay. The daily intake of digestible protein per 1,000 pounds of body weight averaged

TABLE 2

Summary of data obtained in digestion trials with dairy cows fed rations of normal, of brown, and of black alfalfa hay

	Group 1			Group 2			Group 3		
Description of hay	Normal			Brown			Black		
Cows, herd numbers	K392	402A	Av.	286	K391	Av.	311A	486	Av.
Body weight (lbs.)									
Beginning, preliminary period	1073	770	922	1166	1027	1097	772	1014	893
Beginning, collection period	1088	761	925	1144	977	1061	702	941	822
End, collection period	1090	773	932	1134	943	1039	678	904	791
Daily milk production[a] (lbs.[b])	Dry	25.6	.....	Dry	16.8	.....	Dry	8.4	.....
Daily intake (lbs.[b])									
Hay (on dry matter basis)	25.1	22.0	.....	17.7	17.7	.....	8.3	11.4[c]	.....
Tap water	92.6	101.9	.....	62.3	71.8	.....	27.8	50.8	.....
Total water	99.1	107.5	.....	64.6	74.1	.....	29.5	53.7	.....
Feces voided									
Per day (lbs.[b] of dry matter)	10.0[d]	8.7[e]	.....	10.5[f]	10.4[g]	.....	6.4[h]	7.9[i]	.....
Moisture (%)	81.0	82.4	.....	76.2	75.9	.....	66.3	70.9	.....
Composition, dry matter in feces (%)									
Crude protein	16.9	18.3	.....	30.0	29.8	.....	28.1	26.3	.....
Ether extract	4.6	4.4	.....	2.2	2.2	.....	1.5	1.3	.....
Crude fiber	37.1	37.4	.....	30.9	30.7	.....	39.3	44.1	.....
Ash	12.6	12.3	.....	10.3	9.9	.....	9.8	8.4	.....
Nitrogen-free extract	29.8	28.0	.....	26.6	27.1	.....	21.3	19.3	.....
Digestion coefficients[j]									
Dry matter	60	61	60	41	42	41	23	31	27
Crude protein	68	66	67	16	17	16	−3	8	3
Ether extract	23	27	25	32	33	33	36	49	42
Crude fiber	41	41	41	35	36	36	14	14	14
Nitrogen-free extract	71	73	72	59	59	59	46	59	53
Composition of dry matter in hay (%)									
Digestible crude protein		14.4			3.4			0.6	
Total digestible nutrients		55.7			37.5			23.4	

[a] Expressed as 4% fat-corrected milk during collection period (12).
[b] Original data recorded in grams.
[c] Small amounts of hay were refused. These were weighed and analyzed to compute the net intake of nutrients.
[d] Medium consistency and green colored, when voided.
[e] Moderately firm and green colored, when voided.
[f] Medium consistency and dark to black colored, when voided.
[g] Moderately firm and dark colored, when voided.
[h] Voided in the form of black, hard, dry pellets. Pelleted form of feces began to disappear within five days, after cow was changed to normal rations. Cow 311A developed habit during trial of sucking various wood, metal, and canvas objects attached to manger.
[i] Freshly voided feces not as dry, but otherwise similar to those of 311A in appearance.
[j] Averages based on coefficients of individual cows calculated to first decimal.

2.91 pounds for normal hay, 1.51 pounds for brown hay, and only 0.06 of a pound for black hay. The daily intake of total digestible nutrients per 1,000 pounds of body weight averaged 11.26 pounds for normal hay, 5.63 for brown hay, and 2.34 pounds for black hay.

Water consumption was less for the cows fed excessively heated hay. The cows fed normal hay drank an average of 105 pounds of free (tap) water daily per 1,000 pounds of body weight, those fed brown hay 64 pounds, and those fed black hay 49. After water in the milk produced was subtracted from the total water intake (free water plus water in hay), the net water consumption averaged 99 pounds for the cows fed normal hay, 58 pounds for those fed brown hay, and 47 pounds for those fed black hay. Total water per 1,000 pounds of body weight, less water in the milk produced, was consumed at the average rate of 4.9 pounds per pound of dry matter intake by the cows fed normal hay; similarly, the cows fed brown hay consumed an average of 3.9 pounds of water per pound of dry matter, and the cows fed black hay 4.7 pounds of water. The total water intake appeared low for the brown hay and black hay groups, particularly the latter, averaging 32 per cent less for the cows fed brown hay and 60 per cent less for those fed black hay than for those fed normal hay. The ratio of water intake to dry matter intake per 1,000 pounds of body weight, however, was higher than those calculated from data reported by Atkeson and Warren (1) for cows fed more complete rations.

The cows fed normal hay received two and a half times their protein requirements and approximately the required amount of total digestible nutrients, according to Morrison's (20) feeding standard for good cows. The cows fed brown hay received approximately half the needed protein and total digestible nutrients, while the cows fed black hay received one-fifteenth of the needed protein and one-fourth the total digestible nutrients, in spite of the fact that all cows were fed all the hay they would consume readily. The deficiency in nutrient intake in the groups fed brown and black hays, particularly the latter, would indicate that the coefficients for these hays are conservative because of the effect of endogenous nitrogen under such conditions.

During the digestion trials, all cows fed the brown and black hays lost weight, especially during the preliminary period, and became gaunt in appearance; and the cows fed black hay showed signs of uneasiness and a craving for something. Feces voided by the cows fed excessively heated hay were dark to black, those from the black hay ration being hard, dry, flattened pellets (fig. 1). Whether these conditions of the cows, particularly those fed black hay, were due to lack of digestible nutrients, lack of water, or a combination of both, is unknown.

The excessively heated alfalfa hays used in these trials were produced unintentionally and varied somewhat in texture and color intensity. The

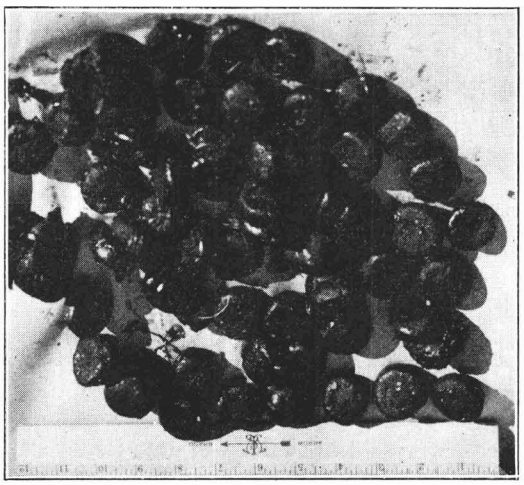

FIG. 1. Feces voided by cow 311A on February 6, 1941, following a period of feeding on chopped, black, alfalfa hay. (Photo by courtesy of Paul Dittemore.)

brown alfalfa varied from a light-brown, soft-stemmed grade to a rather dark-brown, moderately crisp hay, and was regarded as averaging somewhat darker than aromatic, tobacco-brown hay even though considerable amounts of the latter grade were included; a special effort was made to exclude dark, crisp hay in the central part of the stack. The black alfalfa varied from a dark-brown, crisp hay to a black, somewhat charred grade; it was selected as the least-severely damaged hay from a stack filled with chopped hay at

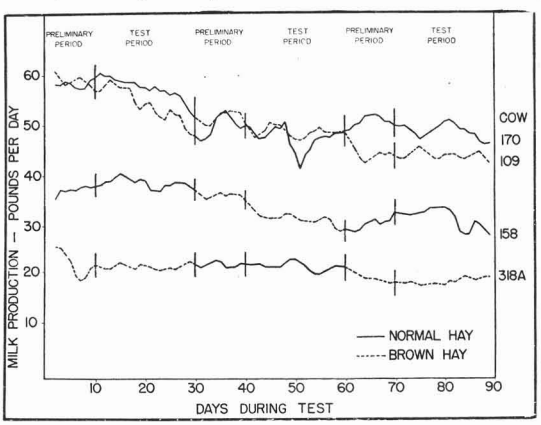

FIG. 2. Lactation graphs of cows during double-reversal feeding test in which normal and brown alfalfa hays were compared in rations containing Atlas sorgo silage and a grain mixture. Data plotted as three-day moving averages for milk as produced.

the time of harvest, and it was necessary to extinguish several small fires in the stack when hay was removed for the feeding tests.

No attempt was made in these experiments to determine the nature of the changes responsible for the altered digestibility of the various fractions in the excessively heated hays. However, in so far as protein is concerned, the reported ability of ruminants to synthesize proteins from simple nitrogenous substances probably by bacterial activity (13) has suggested either that feeding brown hay interfered in some manner with micro-floral activity in the cow's body or that excessive heating of the hay transformed crude protein in the hay to some complex, unavailable form of nitrogen.

Double-reversal feeding test. The lactation graphs (fig. 2) from the double-reversal feeding test in which the productive value of brown hay was compared with normal hay are presented only to supplement the digestion trials, as the limitations of data resulting from the use of only four cows are fully realized. Similar feeding tests with dairy cows by Cave and Fitch (6, 7, 8) of this station and others (5, 23) were more comprehensive. Also, the chemical composition of the brown hay used in this test indicated that it originated from hay which was superior to the normal hay with which it was compared.

Average daily milk production was somewhat greater during the normal hay feeding periods than when brown hay was fed (fig. 2); however, the number of experimental cows was insufficient to justify statistical treatment of the results. Unpublished data obtained from this test show that brown hay was eaten more readily than normal hay, as indicated by more than twice as much normal hay being refused. This was probably caused either by the greater leafiness of the brown hay or by the lower nutrient intake per pound of hay eaten, resulting from excessive heating as previously shown by the digestion trials. These results show that under some conditions brown hay may be more palatable than normal hay, particularly when the hays come from different sources and are of different original qualities. During this feeding test, hay represented slightly more than 50 per cent of the dry matter in the total ration.

SUMMARY AND CONCLUSIONS

Normal, long alfalfa hay was compared in chemical composition and in apparent digestibility with brown, excessively heated hay from the same source and with black excessively heated, chopped hay from a different source. The alfalfa hay was supplemented only with drinking water. Each type of hay was fed to two dairy cows in amounts equal to the animals' appetites. The data on digestibility were supplemented by comparisons with four cows in a double-reversal feeding test of the same type of brown hay with normal alfalfa hay from a different source. During most of this test, both hays were chopped before feeding; the hay supplied approximately

50 per cent of the total dry matter intake and was fed in conjunction with sorgo silage and a grain mixture.

The following interpretations of results seem justified:

1. Chemical analyses showed that brown hay contained more ash and more crude fiber than normal alfalfa hay from the same stack, and that excessive heating during storage resulted in decreased percentages of nitrogen-free extract and of ether extract. Similar, but larger, differences in percentage composition were noted between the normal hay and a black grade of alfalfa hay obtained from a different source.

2. Excessive heating during storage consistently lowered the apparent digestibility of all nutrients measured, except that of ether extract which was consistently greater in the heated hays. Protein was affected most, with average digestibility-coefficients of 67 for normal hay, 16 for brown hay, and 3 for black hay. Average coefficients for the other nutrients in the normal, brown, and black hays, respectively, were: Dry matter—60, 41, and 27; ether extract—25, 33, and 42; crude fiber—41, 36, and 14; and nitrogen-free extract—72, 59, and 53. The digestible protein and total digestible nutrients in these hays were calculated to be as follows: 14 and 56 for the normal hay, 3 and 38 for the brown hay, and 0.6 and 23 for the black hay.

3. When alfalfa hay was the sole source of dry matter in the ration, the daily intake of dry matter per 1,000 pounds of body weight was—20 for normal hay, 15 for brown hay, and 10 for the black hay. In terms of digestible nutrients, these daily intakes were equivalent to 2.9 pounds of digestible protein and 11.3 pounds of total digestible nutrients for the cows fed normal hay, 0.5 and 5.6 for the brown-hay cows, and 0.06 and 2.3 for the black-hay cows.

4. When dairy cows were limited to rations of alfalfa hay and tap water fed to the limits of the animals' appetites, nutrient intakes were adequate for the cows fed normal hay, but were decidedly inadequate for the cows fed the brown and black hays. Under these conditions, the total daily water intake minus the equivalent of that in the milk produced was: 4.9 pounds of water per pound of dry matter consumed in normal hay, 3.9 for brown hay, and 4.7 for the black hay. The cows fed heated hays lost considerable in body weight, developed a gaunt appearance, and—in the case of the black hay—also became uneasy and appeared to crave something in their rations. Feces voided by cows fed excessively heated hay took on a dark to black appearance, contained less moisture, and those resulting from the black hay were voided in the form of hard, dry, flattened pellets.

5. When fed in conjunction with sorgo silage and a grain mix and compared with normal hay from a different source, the brown alfalfa hay was consumed in greater amounts; but average daily milk production appeared to be somewhat in favor of the normal hay. However, these comparisons were limited to only four cows in a double-reversal feeding test, and the

chemical analyses indicated that the original quality of the brown hay was superior to that of the normal hay both in protein and in fiber content.

6. These results show that excessively heated alfalfa hay may be decidedly inferior in nutritive value to green alfalfa hay, and that this impairment in feeding value is more or less proportional to the intensity of brown to black color developed during excessive heating of the hay. Palatability observations—as measured by feed intakes and refusals—showed that brown alfalfa hay was less palatable than normal green hay from the same stack, but that this same type of brown hay was more palatable than normal green hay obtained from a different source and apparently containing less protein and more fiber than originally present in the alfalfa from which the brown hay was produced.

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BODY SIZE AND LACTATION RATE

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AGE AND WEIGHT CORRECTION OF LACTATION RECORDS

Not only standardization of environmental conditions, but age and weight "corrections," are means of making production records a reliable expression of inherent ability to produce milk.

Dickerson (2) has noted a significant increase in repeatability of records from age corrections. Age correction reduced the variance for the first 240-day butterfat yield to $\frac{3}{4}$ of the uncorrected variance. In contrast, Gaines, Rhode, and Cash (6) suggest substituting weight correction for the "biologically unsound principle of age correction."

Since cows, as a rule, continue to grow from their first lactation up to the age of 5 years, age and weight during this period are probably correlated. What is designated as an age correction may, then, to some extent be a hidden correction for weight.

To disentangle the combined effects of age and weight, the effect of one of these factors should be determined independently. The effect of weight as such can be derived on the basis of the theory that the productive capacity of animals is on the average proportional to their metabolic body size or the $\frac{3}{4}$ power of their body weight. This theory, suggested by Kleiber, was tested and confirmed by Brody and his co-workers. (On the literature see reference, 7.)

EMPIRICAL DETERMINATION OF WEIGHT EFFECT ON LACTATION RATE

Kleiber and Mead (7) showed why it is almost impossible to determine empirically the effect of size on lactation rate within one herd. The effect of size *per se* could be measured only if differences in inherent lactation ability among other variables could be eliminated. For such elimination, obviously, inherent lactation ability should at least be defined. Its definition for cows of different size, however, includes knowledge of the effect of size *per se* on production rate.

Undisturbed by these considerations, Gaines (5) claimed in a later publication that "within a dairy breed and within a herd (comparable environment) FCM (fat-corrected milk) *is* proportional to the 1.07th power of live weight." (The italics are ours.) This conclusion would support Gaines's (4) earlier notion that "potential lactation capacity (present or capable of development) is proportional to live weight."

Gaines's recent conclusion, however, is open to serious criticism:

Received for publication July 5, 1944.

1. The cows that he chose to prove his point were fed only alfalfa hay, to measure its nutritive value, and were 50 per cent below full production, as shown in the original publication by Dawson, Kopland, and Graves (1, table 10, p. 29). Cows whose production is reduced by feed restriction seem to be a rather unfortunately chosen source of information concerning the effect of size on lactation capacity.

2. More serious still is the selection of 1.07 as *the* representative exponent for body weight in its effect on lactation rate. This selection is made from exponents which range from 0.28 for body weights measured at the ninth month of lactation, to 1.49 if the body weight at the fourth month of lactation is chosen as the basis of body size. The correlation between 8 months' milk yield and body size is greatest (in the sample chosen by Gaines), to be sure, when body weight at the first month is used. (For the 10 months' milk yield it appears greatest for the fourth month's body weight.) None of the correlation coefficients, however, given in Gaines's table 1, which is based on 11 results, exceeds significance at the 1 per cent level of random probability as listed, for example, in table 7.2 of Snedecor (8). The difference between the correlation coefficients for the first month's weights and that for the fourth month's is insignificant.

3. Aside from these two criticisms Gaines's recent result, cited in support of his notion that lactation rate is proportional to body weight, is statistically very unreliable. The regression coefficient for the logarithm of yearly lactation rate and logarithm of body weight, calculated from the data in a U. S. Department of Agriculture bulletin, (1 table 10), amounts to 0.76 ± 0.59. The formula for calculating the standard deviation of the regression coefficient is found in Ezekiel's book on methods of correlation analysis (3, p. 252).

This is not a test for Gaines's calculations as such. The latter were based on milk energy, whereas the result here obtained is based simply on pounds of milk yield. Our calculation is used only to estimate the variability, which, judging from data in our own herd, is (within one herd) of the same order of magnitude for milk energy and for pounds of milk. With this variability, one would need the results from more than 500 cows to show a barely significant difference (random probability 5 per cent) between production rate per unit weight and per unit of metabolic body size, whereas Gaines based his conclusions on 11 records involving only 8 cows.

As reported earlier (7), in 24 Holsteins of the University of California herd the 10 months' milk energy yield did not, on the average, increase with increasing body weight; indeed, it decreased slightly. This trend appears to be somewhat enhanced when we use pounds of milk instead of milk energy, and the first month's weight instead of mean weight for a period, in order to compare our Holsteins with those of the U. S. Department of Agriculture herd. The difference between the best-fitting weight exponent

of the U. S. D. A. Holsteins (11 records), namely 0.76 ± 0.59, and that of the University of California Holsteins (23 cows), namely -0.32 ± 0.55, is statistically insignificant. Thus, in one case the lactation rate appears to increase in proportion to an increase of the $\frac{3}{4}$ power of body weight; in the other it appears even to decrease in proportion to the cube root of increasing body weight; yet there is no statistical significance in the difference between such extremes. This comparison best illustrates the reliability of Gaines's statement that the production rate is proportional to the 1.07th power of live weight.

Undoubtedly one can find many lactation records from which he can calculate that the lactation capacity of cows within one herd is proportional to body weight. Somebody else, however, might advance the opposing theory that within one herd the smaller cows produce more milk than the larger ones—and could also find records to prove his hypothesis. Again, some other investigator, using Gaines's method, might find "support" for a theory that lactation rate within a herd is proportional to the square of body weight.

HOW MANY COWS MIGHT PROVE THE POINT?

The explanation for this state of uncertainty is, of course, the variability of lactation rate aside from variations of body weight. The mean daily milk energy for 42 Jersey cows of the University of California herd amounted to 139 ± 5 kcal. per $kg.^{\frac{3}{4}}$; that of 24 Holsteins to 126 ± 3. The coefficient of variation of the lactation rate per unit of the metabolic body size was ± 22 per cent for the Jerseys and ± 11 per cent for the Hol-

TABLE 1

*Number of cows per herd necessary for significant difference between a herd with production rate proportional to body weight and a herd with production rate proportional to the $\frac{3}{4}$ power of body weight**

1	2	3	4	5
Mean body weight		Difference in mean body weight between heavy and light cows of each herd	Relative difference in body weight / mean weight (3)	Number of cows per herd necessary for significant difference in production rate as function of body size
of $\frac{n}{2}$ light cows	of $\frac{n}{2}$ heavy cows			
lb.	*lb.*	*lb.*		
400	1600	1200	1.2	11
600	1400	800	0.8	18
800	1200	400	0.4	66
900	1100	200	0.2	256
950	1050	100	0.1	1064
1000	1000	0	0.0	∞

* The calculation is based on the following assumptions:
1. Mean body weight for each herd is 1000 pounds.
2. Each herd has the same mean production rate.
3. Each herd has the same standard error of production rate amounting to $\frac{1}{10}$ of the mean production rate.

steins. This variability in production rate obviously overshadows the effect of size the more, the smaller the differences in body size are.

For the sake of argument we may assume that in a herd of cows the production rate is proportional to body weight, and that in another herd, with the same weights of cows and the same mean production, the production rate is proportional to the $\frac{3}{4}$ power of body weight. We may further assume that the standard error of production rate for each herd amounts to $\pm$ 10 per cent of the mean production rate—a rather optimistic assumption, as our records indicate. Table 1 shows how many cows per herd will be necessary to make the difference in production rate (as a function of body size) between the two herds statistically barely significant.

The calculation of this table is given in the appendix.

Within one herd, size differences are bound to be relatively small. This fact largely explains the hopelessness of attempting to calculate general size effects on lactation rates from data within one herd.

RELATIVE LACTATION CAPACITY

If production records are to be corrected for body size at all, it seems most advantageous to express production rates per unit of the metabolic body size and thus to make these rates directly comparable between animals that vary in size as much as rats and cows. Table 2 gives the metabolic size for weights varying from 600 to 1,600 pounds. The use of this table

TABLE 2

Metabolic body size vs. body weight for cows

Body weight	Metabolic size	Body weight	Metabolic size	Body weight	Metabolic size	Body weight	Metabolic size	Body weight	Metabolic size
lb.	$kg.^{\frac{3}{4}}$	*lb.*	$kg.^{\frac{3}{4}}$	*lb.*	$kg.^{\frac{3}{4}}$	*lb.*	$kg.^{\frac{3}{4}}$	*lb.*	$kg.^{\frac{3}{4}}$
600	67	800	83	1000	98	1200	113	1400	126
10	68	10	84	10	99	10	113	10	127
20	69	20	85	20	100	20	114	20	128
30	70	30	85	30	100	30	115	30	128
40	70	40	86	40	101	40	115	40	129
50	71	50	87	50	102	50	116	50	130
60	72	60	88	60	103	60	117	60	130
70	73	70	88	70	103	70	118	70	131
80	73	80	89	80	104	80	118	80	132
90	74	90	90	90	105	90	119	90	132
700	75	900	91	1100	106	1300	120	1500	133
10	76	10	92	10	106	10	120	10	134
20	77	20	92	20	107	20	121	20	134
30	78	30	93	30	108	30	121	30	135
40	79	40	94	40	108	40	122	40	136
50	79	50	94	50	109	50	123	50	136
60	80	60	95	60	110	60	124	60	137
70	81	70	96	70	111	70	124	70	138
80	82	80	97	80	111	80	125	80	138
90	82	90	97	90	112	90	126	90	139
800	83	1000	98	1200	113	1400	126	1600	140

makes the calculation of the relative lactation capacity just slightly more time-consuming than the calculation of the production rate per unit weight. This extra time is a small price to pay for a great increase in the range of application of results and for the establishment of a sound basis on which to calculate partial effects of age on lactation rate.

Relative lactation capacity defined as milk energy per day (the mean of 10 months' production) per unit of the mean metabolic body size (in kg.$^{\frac{3}{4}}$) should be used in summaries on lactation records. This relative lactation capacity would be valuable as a major criterion of selection for breeding dairy cattle.

SUMMARY

The influence of age on lactation rate can be determined when the effect of body size is derived independently. This derivation can be made on the basis of the theory that lactation capacity is proportional to the metabolic body size ($\frac{3}{4}$ power of body weight) of the cows.

There are two reasons why the effect of body size on lactation rate cannot be calculated from records within one herd: first, the variability of lactation rate, aside from the influence of size, ranges from ± 10 to ± 20 per cent of the mean rate, even in well-bred herds kept under uniform conditions; second, size differences within each of those herds are usually rather small.

The unreliability of results from a recent attempt to calculate size effects on lactation rates within a herd is demonstrated. A table is calculated showing the number of cows necessary to distinguish significantly between production rate per unit weight and production rate per unit of metabolic body size.

A table is supplied in which metabolic body size of cows can be read directly when body weight in pounds is given.

Lactation rate per unit of metabolic body size ($\frac{3}{4}$ power of body weight) is a sound basis for calculating the effects of age on lactation rate.

The average daily milk production during a 10-month period, expressed as milk energy or fat-corrected milk, and divided by the mean metabolic body size, is suggested as an important result in summaries of production records. When the cows have been kept under quasi-optimal conditions, such a result may be known as relative lactation capacity. This term, which expresses quantitatively the inherent ability of cows for milk production, would be useful as a major criterion for breeding dairy cattle.

APPENDIX

Number of cows necessary for significant difference between production rate per unit body weight and rate per unit of the $\frac{3}{4}$ power of body weight

In seeking to estimate how many cows are probably necessary to decide between body weight or its $\frac{3}{4}$ power as the more suitable base for comparing

production rates, we may consider two herds. One half of the cows of each herd shall all have the same weight W_l (light). The other half shall have the same weight W_h (heavy), so that the mean weight of all the cows of each herd shall be W_m. The mean rate of milk production for both herds shall also be the same, M.

The production rate in herd G, however, shall be proportional to body weight.

$$P_G = M \frac{W}{W_m} \quad \ldots\ldots (1)$$

The production rate in herd K shall be proportional to the metabolic body size, or the $\frac{3}{4}$ power of body weight.

$$P_K = M \frac{W^{\frac{3}{4}}}{W_m^{\frac{3}{4}}} \quad \ldots\ldots (2)$$

The heavier cows of herd G will have a greater mean production rate than the heavier cows of herd K, and the lighter cows of herd G will have a smaller mean production rate than the lighter cows of herd K.

We assume further that in both herds the standard error of a single production rate is $\pm$ 10 per cent of the mean rate of both herds, so that

$$\varepsilon = \pm 0.1\, M \quad \ldots\ldots (3)$$

Then our question is, how many cows (n) in each herd are necessary to make the difference in their production rate, as a function of body size, statistically barely significant—namely, with a random probability of 5 per cent?

The difference in production rate of the heavier cows of the two herds, namely

$$(P_G - P_K)_h = M \left(\frac{W_h}{W_m} - \frac{W_h^{\frac{3}{4}}}{W_m^{\frac{3}{4}}} \right)$$

measures the difference in size effect on production rate above the mean, M. Similarly, the difference in production rate of the lighter cows,

$$(P_K - P_G)_l = M \left(\frac{W_l^{\frac{3}{4}}}{W_m^{\frac{3}{4}}} - \frac{W_l}{W_m} \right)$$

measures the difference in size effect on production rate below the mean, M. The mean of the two differences, namely

$$\frac{(P_G - P_K)_h + (P_K - P_G)_l}{2} = \frac{M}{2} \left(\frac{W_h - W_l}{W_m} - \frac{W_h^{\frac{3}{4}} - W_l^{\frac{3}{4}}}{W_m^{\frac{3}{4}}} \right) \quad \ldots\ldots (4)$$

measures the mean difference in the effect of body size between that proportional to weight and that proportional to the $\frac{3}{4}$ power of weight.

The significance of this mean difference is measured by the quotient of the difference and its standard error.

With a standard error of $\pm \varepsilon$ of the production rate of one cow, each of the two differences between the heavier halves and between the lighter halves of the two herds, $\left(\frac{n}{2} \text{ cows}\right)$, is subject to an error of

$$\varepsilon_d = \sqrt{2}\,\frac{\varepsilon}{\sqrt{\frac{n}{2}}} = 2\,\frac{\varepsilon}{\sqrt{n}} \qquad (5)$$

The error of the mean of the two differences therefore is

$$\varepsilon_{\left(\frac{dl+dh}{2}\right)} = \sqrt{\frac{2\,(\varepsilon_d)^2}{2}} = \varepsilon_d = \frac{2\,\varepsilon}{\sqrt{n}} \qquad (6)$$

The significance ratio of the mean difference in the production rate as function of body size is consequently

$$t = \frac{\frac{M}{2}\left(\frac{W_h - W_l}{W_m} - \frac{W_h^{\frac{3}{4}} - W_l^{\frac{3}{4}}}{W_m^{\frac{3}{4}}}\right)}{\frac{2\,\varepsilon}{\sqrt{n}}} = \sqrt{n}\,\frac{M\left(\frac{W_h - W_l}{W_m} - \frac{W_h^{\frac{3}{4}} - W_l^{\frac{3}{4}}}{W_m^{\frac{3}{4}}}\right)}{4\,\varepsilon}$$

since, according to Kleiber and Mead (7), $\varepsilon = 0.1\,M$

$$t = \sqrt{n}\left(\frac{\frac{W_h - W_l}{W_m} - \frac{W_h^{\frac{3}{4}} - W_l^{\frac{3}{4}}}{W_m^{\frac{3}{4}}}}{0.4}\right) \qquad (7)$$

For significance with a random probability of 5 per cent, $t = 2$ when n is large. Introducing this value into 7 leads to

$$n = \frac{0.64}{\left(\frac{W_h - W_l}{W_m} - \frac{W_h^{\frac{3}{4}} - W_l^{\frac{3}{4}}}{W_m^{\frac{3}{4}}}\right)^2} \qquad (8)$$

The figures for the smaller numbers of n were multiplied by the factor $\frac{t^2}{4}$ because with small numbers a 5 per cent random probability requires t's greater than 2. The t's for the correction were taken from Snedecor (8, table 3.8, p. 55).

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EVAPORATED MILK AS RELATED TO GREENISH DISCOLORATION IN COFFEE

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Evaporated milk is often added to coffee as a means of modifying the color and flavor. Because of certain defects that may occur in the milk or the coffee, the desired results are not always obtained. One such defect encountered under certain conditions in the home is the occurrence of a dark-greenish or greenish-black discoloration as soon as the milk is added to coffee. The trouble is most likely to be encountered where only one member of a family is using evaporated milk. Under such conditions a partly empty can may remain in the refrigerator for several days—sometimes longer than a week.

Although this discoloration has been observed by one of the authors for several years, not until lately was it realized that the defect is common. The authors are unaware of any previous report dealing specifically with the problem.[1] A request from members of the evaporated-milk industry for an explanation of the trouble prompted the present study.

PROCEDURE

The coffee used in these tests was prepared by bringing 1000 ml. of water to a boil in a glass beaker, adding 48 grams of coffee tied loosely in cheesecloth, boiling for 5 minutes with frequent stirring, and then removing the cloth containing the coffee grounds. Occasionally larger or smaller batches were brewed; but the same proportion of water to coffee was used, and the same method of boiling maintained.

The coffee thus prepared was mixed with evaporated milk in test tubes in the proportions of 9 ml. of coffee to 1 ml. of evaporated milk. The tubes made it easy to observe and compare the resulting colors; furthermore, they required the use of less material than coffee cups would have done.

The discoloration intensity of the coffee and milk mixtures is reported in ppm. of iron. In arriving at these values, iron in the form of ferric chloride was added to a series of control tubes of coffee and freshly opened evaporated milk, and the degree of discoloration in the unknowns matched with that of the controls containing known concentrations of iron. The color intensity determined in this manner was shown by analysis to serve as a fair approximation of the iron content of evaporated-milk samples.

Received for publication July 11, 1944.

[1] Since this manuscript was prepared the following publication has appeared: Gould, I. A. Abnormal color production in coffe-evaporated milk mixtures with special reference to lactic acid. Michigan Sta. Quart. Bul. 26, No. 4: 335–340. 1944.

Certain of the samples were analyzed for iron. The procedure was the filtration method of Moir and Andrews (2), with suitable modifications for estimating the iron content of evaporated milk.

RESULTS

All samples of evaporated milk used in the study were satisfactory when freshly opened. None of them gave a noticeable greenish-black discoloration until the open cans had been allowed to stand.

The first comparisons made involved ten common brands of evaporated milk. The cans were numbered. In addition, two cans that had been stored unopened in the laboratory were included. One of these (can 8) had been kept a year; the other (can 12) over three years. The latter sample, though lumpy, produced no objectionable discoloration in coffee.

The twelve cans were opened by punching two small holes in the top of each. Before they were stored in a refrigerator at about 45° F., approximately one-fourth (100 ml.) of the milk was poured from each can into a sterile 125-ml. Erlenmeyer flask. The flasks were stoppered and placed in the refrigerator with the cans. The milk was tested at 3- to 5-day intervals for a month. When, after 8 days, none of the samples would produce discoloration in coffee, three-fourths of the milk from can 6 was removed in an attempt to hasten the development of the defect. The results for the twelve cans may be summarized as follows: can 2 produced discoloration on the 32nd day; can 1, 4, 5, 7, 9, and 12 produced none. Of the remaining cans, No. 10 showed the defect on the 24th day; No. 3 on the 17th; Nos. 8 and 11 on the 15th; and No. 6 (which contained the least milk) on the 12th day.

Clearly, when cans of evaporated milk are left two-thirds to three-fourths full, considerable time is required for the defect to appear. Some variation occurred with different brands treated in the same manner; but sample 6, where only one-fourth of the milk was left in the can, showed the defect most quickly. It is remarkable that 50 per cent of the samples did not cause the discoloration even after 32 days of storage. None of the control samples kept in glass containers developed the defect.

The following comparison was designed to show the influence of removing various amounts of evaporated milk from the cans before storage. Milk in four large (14½-oz.) and four small (6-oz.) cans was used. Each set included cans $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{10}$ full. All these samples were obtained from the same batch of evaporated milk.

Figures 1 and 2 illustrate the degree of discoloration that occurred in the samples of coffee and evaporated milk mixtures at 6 days and 21 days respectively. Clearly, the degree depends largely upon the amount of milk remaining in the can. Sample 9, the control, appears practically the same as Nos. 1 and 5. Samples 4 and 8, which contain the least milk, show the greatest discoloration.

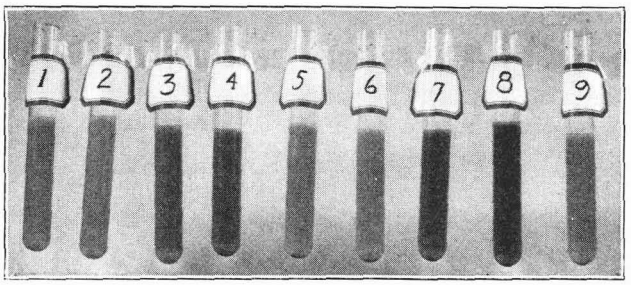

FIG. 1. Discoloration with samples held 6 days after cans were opened. Samples 1 to 4 inclusive from large cans (14½ oz.) ¾, ½, ¼ and 1/10 full respectively; samples 5 to 8 inclusive from small cans (6 oz.) ¾, ½, ¼ and 1/10 full respectively; sample 9 was the control from freshly opened can.

The degree of discoloration was definitely correlated with the iron content of the mixture of coffee and evaporated milk, as can be seen by figure 3.

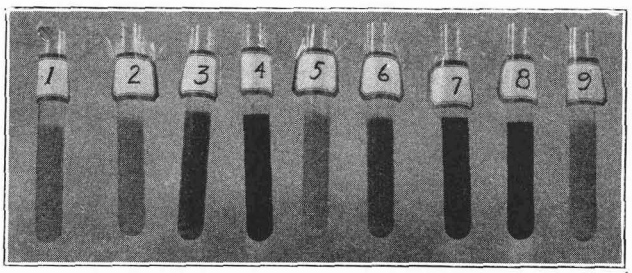

FIG. 2. Discoloration with same samples as in figure 1, held 21 days after cans were opened.

In this series of samples iron was added, in the form of ferric chloride, to give the following concentrations of iron: 0, 5, 10, 15, 30, 60, 100 and 200 ppm. in samples 1 to 8 respectively. As was expected, the same results in

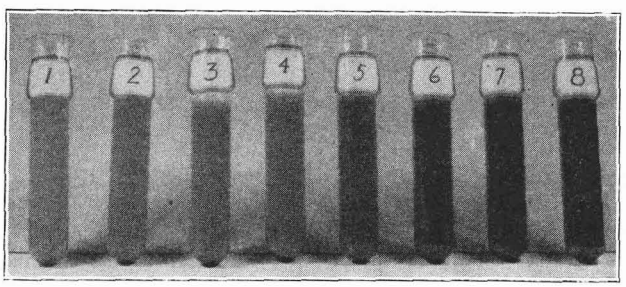

FIG. 3. Relation of iron content to extent of discoloration. Ferric chloride added to give concentrations of 0, 5, 10, 15, 30, 60, 100, and 200 ppm. of iron in samples 1 to 8 respectively.

discoloration were obtained whether the ferric chloride was added to the coffee or evaporated milk before mixing, or to the mixture itself. Since the color produced depends upon the iron content of the mixture, and since practically all the iron comes from the evaporated milk, the concentration of iron in the milk would be ten times its concentration in the mixture. With this as a basis for estimating the iron in evaporated milk, data were obtained on experimental samples. Figure 4 gives such data for four of the samples illustrated in figures 1 and 2.

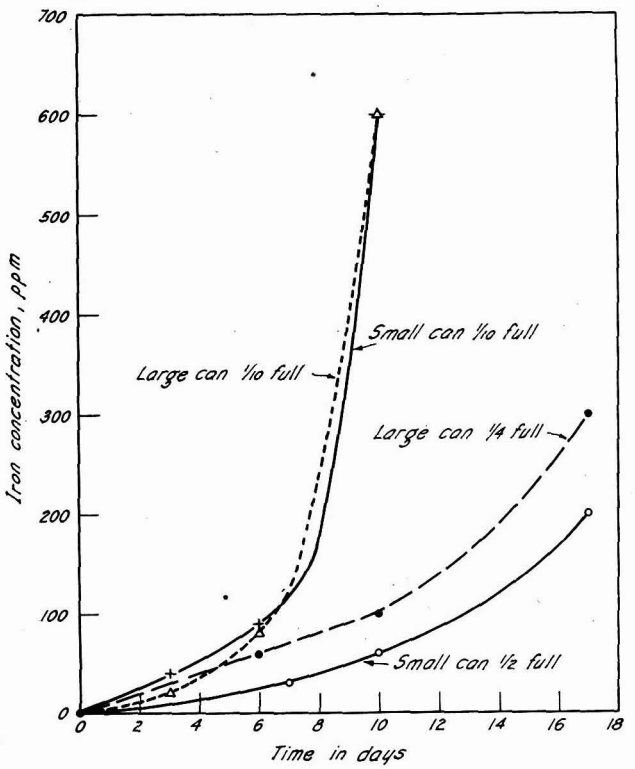

FIG. 4. Iron content of evaporated milk as related to fullness of can and length of holding after the can is opened.

As a further check, an adaptation of the method of Moir and Andrews (2) was used in analyzing samples of evaporated milk for iron. To establish the validity of this method, known amounts of iron were added to two freshly opened control samples, and these were then analyzed for iron by the adopted method. Results obtained showed a recovery of 99 per cent in both cases. Furthermore, these results agreed closely with those secured on the same samples by the ferric chloride discoloration test.

Table 1 compares the results obtained by this method of analysis with the results secured with the ferric chloride discoloration test. Clearly the ferric chloride test serves as a means of estimating the iron content. Although not intended as a substitute for analyses, this rapid test gives an indication of the increased iron content due to holding or aging the milk in cans that have been opened.

A number of other factors were considered in these experiments—for example, kind of coffee, source of water, salts added to evaporated milk and the pH of mixtures of coffee and evaporated milk. Accordingly, three different brands of coffee were used. None showed discoloration when mixed with freshly opened evaporated milk, although there was some variation in

TABLE 1

Determination of iron content of evaporated milk

Sample		Iron content of evaporated milk	
No.	Treatment	Determined by analysis*	Estimated by ferric chloride discoloration test
		ppm.	*ppm.*
1	Can opened just prior to analysis	0.4	0
2	Can opened just prior to analysis	0.3	0
3	Milk held in opened can	38.0	45
4	Milk held in opened can	135.0	145
5	Milk held in opened can	204.0	200
6	To 2.0544 grams of freshly opened evaporated milk ferrous ammonium sulfate was added to equal 0.4 mg. of iron	193.0	200
7	To 1.9204 grams of freshly opened evaporated milk ferrous ammonium sulfate was added to equal 0.4 mg. of iron	206.0	200

* Adaptation of filtration method of Moir and Andrews.

the color of the various coffees and coffee-and-milk mixtures. The greenish-black discoloration appeared at the same concentration of added iron in all samples of coffee. When 3 to 5 ppm. of iron was added, as ferric chloride, to mixtures of coffee and evaporated milk, there was an obvious discoloration; when the concentration was raised to 10 ppm., the discoloration was very definitely objectionable. From 30 to 50 ppm. of iron in evaporated milk would, then, be required to give a noticeable discoloration, provided no iron was supplied from other sources.

The iron content of the water is ordinarily low, but should not be neglected in certain cases. Other salts may also modify the result somewhat, especially if they materially change the pH of the coffee or coffee-and-milk mixture, since the final color is a function of pH. Salts added to evaporated milk during its processing may modify the color of coffee-and-milk mixtures; but, if used within the limits of commercial practice, they

do not significantly change the tendency for greenish-black discoloration to occur.

All samples tended to darken as the pH was increased. Evaporated milk which had been held too long after opening of the can sometimes caused a bluish-black discoloration when mixed with coffee. This color could not be duplicated by adding ferric chloride alone to control samples. If, however, the pH was increased by the addition of sodium hydroxide, and sufficient iron was also added, the color produced with the old evaporated milk could be duplicated. Checks on these samples showed that spoilage had occurred, resulting in sufficient increase in pH to account for the change in color.

The type of container used in brewing coffee was shown to modify the results, especially if the coffee was subsequently held in the container for relatively long periods. Chipped enamelware gave slight discoloration immediately after the coffee was brewed. When the coffee was in the container overnight, the discoloration was objectionable and was equal to that produced by adding 5 to 10 ppm. of iron, in the form of ferric chloride, to the control sample of coffee and evaporated milk. Coffee prepared in glass, stainless steel, and aluminum showed no such discoloration. Likewise, flavor and aroma were less pleasing in the discolored samples than in samples with normal color.

DISCUSSION

Tannins and certain other substances will produce a greenish or bluish coloration with dilute ferric chloride. Coffee is known to contain caffetannins or tannin-like substances (3). The greenish black in mixtures of coffee and evaporated milk occurs under conditions shown to result in increased iron content. Metallic ions other than those of iron, which are likely to occur in coffee or evaporated milk, do not give this same discoloration with tannins.

The presence of such ions as citrates and phosphates, because of their strong affinity for iron, may modify conditions so that for a given iron content one may not always obtain the same degree of discoloration. Iron likewise has been shown to combine with some proteins (casein, gelatin), hydroxy or dicarboxylic amino acids, and certain other substances in such a way as to acquire a low degree of dissociation (4). Tannins react with proteins and, under proper conditions, will cause their precipitation. It is not unlikely that protein-iron-tannates may be formed.

Dahlberg (1) has shown that a greenish-black color may be formed on the surface of ice cream next to metal cans not properly tinned. He attributes this color to the formation of ferric tannate. Similar reactions might be expected in other products.

The tannin content is higher in tea than in coffee; and the types of tannin in the two products are different. Ferric chloride imparts to tea a bluish black, not the greenish black that it normally produces with coffee. This difference cannot be explained on the basis of variations in pH alone.

The reaction of the system likewise influences the results, since the extent of discoloration, as well as the color itself, depends upon pH. Despite these possible variations in commercial samples, such factors are minor as compared with the iron content of the milk, which in turn is determined primarily by the amount of milk left in cans in storage and the time the cans are held after being opened.

Although information was not available concerning the plating of the cans studied, the type of tin plate would be expected to cause a difference in the life of the cans as well as in the tendency for iron to be dissolved by the milk after the cans were opened. Arrangements have been made to ascertain the importance of this factor.

SUMMARY

The iron content of evaporated milk has been shown to increase rapidly after the cans have been opened, especially if they are only a fourth or less full. Upon the addition of evaporated milk to coffee, the iron in the milk reacts with the tannins or tannin-like substances in coffee to give a dark-greenish or greenish-black discoloration.

A minimum of 3 to 5 ppm. of iron in a mixture of coffee and evaporated milk is required to give a noticeable discoloration. Iron concentration and pH of the system are the primary factors determining discoloration intensity in such mixtures.

Storing evaporated milk in glass or porcelain after the can is opened offers a means of preventing the occurrence of dark-greenish discoloration when the milk is used in coffee.

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THE FATTY-ACID COMPOSITION OF GLYCERIDE FRACTIONS SEPARATED FROM MILK FAT

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Milk fat has long been a subject of investigation. Detailed knowledge of its composition has been continuously increased by refinements of analytical technique. This paper will give information obtained by subjecting milk fat to fractionation, followed by analysis. The method of fractionation previously reported (15) separates fat into less complex portions for further analyses.

The fractionation was accomplished by dissolving milk fat in a low-boiling solvent, pentane, and precipitating successive portions at progressively lower temperatures. Fractions were removed by filtration at $-7°$ C., $-13°$ C., $-23°$ C. and $-53°$ C. These precipitates together with the filtrate remaining after the last precipitation ranged in physical properties from a white dry powder to a reddish oil. The chemical properties of each are given in the data tables.

REVIEW OF LITERATURE

Browne was among the first investigators who made a detailed analysis of milk fat by the methods available at the time (8). The fatty acids present in milk fat were freed and were separated into those soluble in water and those insoluble. The soluble acids were fractionally crystallized from water, and their neutralization numbers were determined. From these data the composition was calculated on the assumption that not more than two acids were present in any one fraction. The insoluble acids were analyzed in a similar way, being fractionally crystallized from alcohol. Composition data were calculated as before. Browne deplored the inability to get satisfactory separation of adjacent fractions by distillation.

Subsequent workers introduced ester distillation. Smedley obtained more satisfactory fractionation by distilling the methyl and ethyl esters rather than the acids themselves (32). She presented evidence for the existence of lower members of the oleic acid series. Crowther and Hynd also fractionally distilled the methyl esters (11). Although they observed unsaturated acids in the lower fractions, attempts to isolate decenoic acid were not convincing. Holland and his co-workers (25, 26) studied the effect of diet, breed, stage of lactation, and other factors on the composition of milk fat. Having fractionally distilled the ethyl esters of the fatty acids, they computed the composition from analysis of these fractions. Their fractionation seems to have been inadequate for satisfactory separation of the esters.

Received for publication July 17, 1944.

Channon, Drummond, and Golding, attempting to ascertain how oils in the diets of cows affect the composition of milk fat, criticized the method of ester fractionation (9). They stated that "as an exact quantitative method it is of little value."

As Hilditch and Jones have shown, however, as great a degree as possible of preliminary separation of the acids into groups of varying character should precede the ester distillation, and the distillation itself should be attended with all due precautions (18). To accomplish this separation, these workers first divided the original milk fat into three basic fractions: after removing those that are steam volatile they partitioned the nonvolatile into solid and liquid acids by lead-soap crystallization. The solid acids and the liquid acids were converted to the methyl esters. Each of the three groups was fractionated from a Willstätter bulb. The results thus obtained justified the use of methyl-ester distillation as an analytical tool. The data of Hilditch and Jones confirmed the presence of unsaturated acids below C_{18}. Hilditch and Sleightholme extended the method to cover additional samples of butter (22). In another paper these authors summarized and reviewed the data previously presented; they postulated possible glyceride configurations as a result of analyses and calculations (23). An advance in the methyl-ester fractionation procedure was made by Longenecker, who introduced the use of an electrically heated and packed column (29). He and Hilditch, having compared the results obtained by this equipment with those obtained with the simpler Willstätter bulb, concluded that the agreement was good; but the packed column gave more complete separation of the esters, particularly the unsaturated esters of low molecular weight.

Hilditch, Paul, and their co-workers introduced a technique for the preliminary separation of milk fat before fractional distillation (21): the milk fat was crystallized into three fractions from acetone at different temperatures; these fractions were subjected to examination by steam distillation and ester fractionation; and possible component glyceride combinations were calculated from the data.

Arup separated milk fat into 6 fractions by direct crystallization at different temperatures. He used Reichert-Meissl, Polenske, and Kirschner values to characterize the fractions (2). From these values he concluded that the acid groups are impartially distributed among the different glycerides.

Other studies have been made to determine how various factors affect the composition of milk fat, and whether certain fatty acids are present. Smith and Dastur studied the effect of inanition on the composition of milk fat (33). Their method resembled that used by Hilditch and his associates, and their results showed the composition of milk fat to be markedly affected. There was a decrease of about 80 per cent in the original content of lower acids up to and including C_{14}. The loss of these acids was almost entirely

compensated for by the increase in the oleic acid content. Recently Hilditch and Jasperson studied the effect of rations containing different oils upon the component acids of milk fat (17). The characteristics of the ration were reflected in the milk fat, but not quantitatively. High percentages of linoleic and of long-chain saturated acids in the diet did not appreciably alter the amount of these constituents in the milk fat. The oleic acid content of the feed, however, had a direct effect on this constituent.

The presence of other unsaturated fatty acids in the series with oleic, for which the first suggestions were made by Smedley (32) and by Crowther and Hynd (11), has since been amply confirmed. Grün and Wirth substantiated Smedley's postulation for the presence of decenoic acid in milk fat (14). Additional confirmation was later furnished by Grün, whose work also indicated the presence of C_{12}, C_{14}, and C_{16} unsaturated fatty acids (13). Bosworth and Brown have proved that C_{10} and C_{12} unsaturated fatty acids are present (4); and numerous other workers have since substantiated these findings (20, 24, 31).

The most convincing evidence for the presence of C_{10}, C_{12}, C_{14}, C_{16} unsaturated fatty acids is furnished by Hilditch and Longenecker (19). The unsaturated esters were oxidized with potassium permanganate in acetone. The oxidized products were removed, leaving only the saturated esters from the original esters. The saponification equivalents of the original esters and the residue-saturated esters were almost identical. This fact can be explained only if the unsaturated portion removed by oxidation had the same carbon-chain length as the residue.

Evidence for unsaturation greater than that of oleic acid has been somewhat confusing. Hilditch and Jones were probably the first to suggest that linoleic acid is a constituent of milk fat (18). Eckstein (12) detected linoleic and linolenic acids, but in quantities considerably smaller than those reported by Hilditch and his associates (18, 22). Bosworth and Brown, on the other hand, failed in all their attempts to verify the presence of linoleic acid (4). Bosworth and Sisson were likewise unable to identify this acid in milk fat (5). Later evidence, however, by Hilditch and his associates (19, 21) points strongly to its presence.

Several workers have noted unsaturated acids of longer carbon chain than C_{18} (4, 5, 7, 19, 21).

EXPERIMENTAL

The milk fat used in this experiment was collected at different seasons, and was separated into five fractions by freezing in a solvent, according to the scheme described previously (15). The fractions were obtained as precipitates at $-7°$ C., $-13°$ C., $-23°$ C., and $-53°$ C.; and the filtrate residue at $-53°$ C.

The fractions, as well as the original milk fat, were prepared for analysis as follows:

1. A 150-gram sample was saponified with alcoholic KOH. After removal of the alcohol and liberation of the fatty acids, the latter were steam-distilled 4 to 5 hours to remove the steam-volatile acids. A double trap was used ahead of the condenser. The distillate was extracted with ether; and the fatty acids, after solvent removal, were fractionated in a Willstätter bulb. The resulting fractions, along with the ether-extracted-distillate water

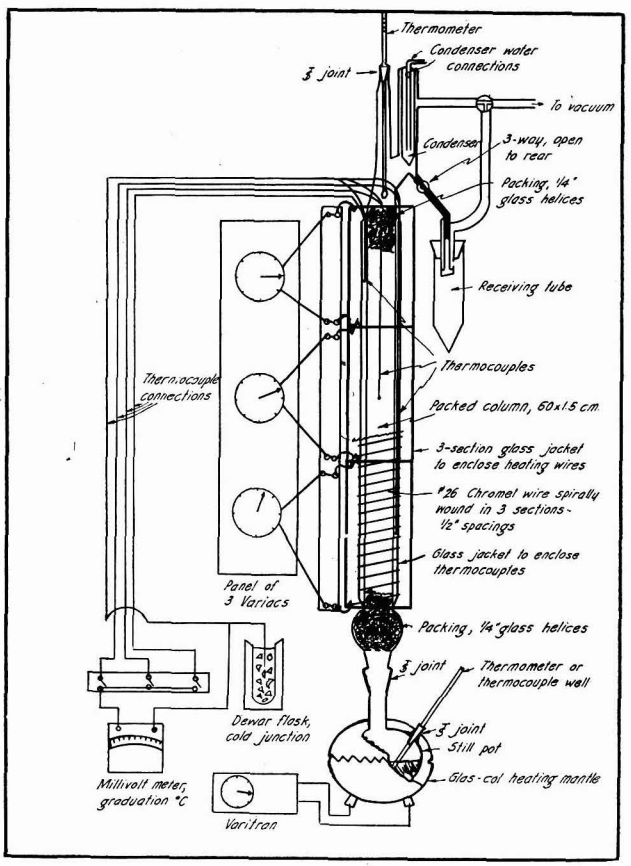

FIG. 1. Packed fractionating column electrically heated in three sections.

and the recovered ether, were titrated with standard alkali and calculated to the appropriate fatty acids.

2. The residues from the steam distillation, except the $-7°$ ppt. and the $-53°$ C. filtrate, were separated into "solid" and "liquid" acids by freezing from acetone at $-20°$ C (6). The $-7°$ ppt. was considered as containing only "solid" acids, and the $-53°$ filtrate only "liquid" acids. The recovered "solid" and "liquid" acids were esterified.

3. The resulting methyl esters were fractionally distilled in an electrically heated and packed column. Figure 1 shows details of the column.[1]

These details have been sufficiently described elsewhere (29), but certain modifications that were incorporated proved advantageous over previous models. The chief improvement lies in the use of three separate sections of heating wire on the column. The leads from these were taken off to a panel through notches in the ends of three abutting sections of an overall glass jacket. The heat supply to each section was controlled through a Variac. This arrangement permitted accurate control of the temperature gradient throughout the column. Thermocouples were inserted between the glass jacket supporting the heating wire and the external wall of the

TABLE 1

Fractionation of methyl esters of the −7° ppt. (Residue after steam distillation of acids)

Charge—50.9 gm.

Sample No.	Temperature °C.					Wt. grams	S.E.[a]	I.V.[a]
	Pot	Column			Vapor			
		Bottom	Middle	Top				
1	150	125	108	98	78–86	0.88	200.7	.....
2	163	134	121	114	90–97	0.78	216.7	.....
3	177	157	133	131	108–110	6.18	238.7	0.89
4	181	163	147	129	111–117	2.03	242.2	.....
5	180	162	148	136	119–126	1.23	272.0	.....
6	194	175	158	145	118.5–123	1.93	272.2	.....
7	198	176	163	150	123–129	8.03	273.0	1.28
8	203	182	166	146	127–129	9.38	272.7	1.03
9	210	189	174	158	129–138	2.88	275.5	0.69
10	224	195	185	170	137–147	3.68	294.0	28.03
11	254	196	182	170	147–falling	7.03	298.0	18.50
12	Hold up	.....	.....	.....	.....	3.80	304.0	15.33
13	Residue	.....	.....	.....	.....	2.40	331.5	17.27

[a] S.E. as used throughout the manuscript means *Saponification Equivalent.*
I.V. as used throughout the manuscript means *Iodine Value* (*Hanus*).

packed section; these, leading to a millivoltmeter, measured the temperature of the column. Another feature was the use of a Glas-Col mantle[2] for heating the still pot. Table 1 shows typical column-operating data.

4. The methyl esters of the 10 different lots were resolved into 135 fractions through the column. These lots included the esters of the "solid" acids and of the "liquid" acids separated from the milk fat, −13° C. ppt., −23° ppt., and −53° ppt., together with the esters of the −7° ppt. and the −53° C. filtrate. The 135 fractions were then analyzed for saponification and iodine numbers (3). The thiocyanogen value of the glyceride

[1] A sketch and dimensions of the still-head and column were furnished by Dr. Herbert E. Longenecker, University of Pittsburgh, Pittsburgh, Pa.

[2] Glas-Col heating mantle, manufactured by the Scientific Glass Apparatus Co., Bloomfield, N. J.

TABLE 2

Methyl-ester distribution of "solid" acids of −23° ppt.

Sample No.	Grams	S.E.	I.V.	Saturated						Unsaturated					
				C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}
24	0.74	215.5	.....	.....	0.65	0.09	.....	.....	.....	.....	.....	.....	.....	.....	.....
25	3.00	234.8	4.48	.....	0.72	2.15	.....	.....	.....	.....	.....	0.13	.....	.....	.....
26	1.71	249.0	3.06	.....	.....	1.27	0.39	.....	.....	.....	.....	0.05	.....	.....	.....
27	4.71	267.2	1.68	.....	.....	0.51	4.12	.....	.....	.....	.....	.....	0.08	.....	.....
28	4.08	269.0	1.52	.....	.....	0.02	3.99	.....	.....	.....	.....	.....	0.07	.....	.....
29	9.78	269.5	0.63	.....	.....	0.03	9.68	.....	.....	.....	.....	.....	0.07	.....	.....
30	7.98	271.0	0.63	.....	.....	.....	7.91	0.02	.....	.....	.....	.....	0.05	.....	.....
31	3.78	270.9	2.64	.....	.....	.....	3.67	.....	.....	.....	.....	.....	0.11	.....	.....
32	2.38	284.0	25.63	.....	.....	.....	1.12	0.55	.....	.....	.....	.....	.....	0.71	.....
33	6.98	292.8	22.53	.....	.....	.....	1.75	3.42	.....	.....	.....	.....	.....	1.81	.....
34–hold up	2.00	287.0	9.44	.....	.....	.....	0.77	1.01	.....	.....	.....	.....	.....	0.22	.....
35–residue	1.60	329.5	27.25*	.....	.....	.....	.....	.....	1.21	.....	.....	.....	.....	0.15	0.24
Totals	48.74	.....	.....	.....	1.37	4.07	33.40	5.00	1.21	.....	.....	0.18	0.38	2.89	0.24

* All linoleic acid was assigned to "liquid" acids on basis of comparison of thiocyanogen and iodine values of "solid" and "liquid" esters.

Typical calculation

Sample No. 33, 6.98 gm. S.E. 292.8, I.V. 22.53

$\frac{22.53}{85.50} \times 6.98 = 1.81$ gm. C_{18} unsaturated, assuming methyl oleate to be only unsaturated ester present.

(1) $\frac{X}{296.4} + \frac{Y}{298.4} + \frac{Z}{270.3} = \frac{6.98}{292.8}$

Where X = gm. me. oleate
Y = gm. me. stearate
Z = gm. me. palmitate

using reciprocals $x10^6$

(2) $3380 \times 1.81 + 3340Y + 3695Z = 6.98 \times 3430$

(3) $1.81 + Y + Z = 6.98$

Multiplying (3) by 3340

(4)

(5) Subtracting (2) − (4) $\frac{3340 \times 1.81 + 3340Y + 3340Z = 6.98 \times 3340}{40 \times 1.81 + 355Z = 6.98 \times 90}$

(6) $Z = 1.75$ gm. me. palmitate

(7) $Y = 6.98 - (1.81 + 1.75) = 3.42$ gm. me. stearate

fractions was used to calculate the linoleic acid content (1, 27). The saponification numbers in most instances were checked against the refractive index as proposed by Mattil and Longenecker for the analysis of methyl-ester fractions (30).

TABLE 3

Calculated composition of total original milk-fat acids
I.V. 32.42, Sap. No. 230.0

150 gm. fat yielded 138.12 gm. fatty acids 7.18 per cent steam-volatile acids. 47.20 per cent "solid" acids, 45.62 per cent "liquid" acids. Per cent of S.V. based on 138.12 gm.; per cent S.A. based on 50.54 gm. methyl esters equal to 47.41 gm. fatty acids; per cent L.A. based on 43.35 gm. methyl esters equal to 41.03 gm. fatty acids.[b]

Length of carbon chain	Steam-volatile acids		Solid acids		Liquid acids		Weight, per cent of total			Total weight	Mol
	gm.	*%*	*gm.*	*%*	*gm.*	*%*	*S.V.*	*S.A.*	*L.A.*	*%*	*%*
Saturated											
C_4	4.87	3.52	.....	.....	.....	.....	3.52	.....	.....	3.52	9.2
C_6	1.95	1.40	.....	.....	.....	.....	1.40	.....	.....	1.40	2.8
C_8	2.05	1.48	.....	.....	0.18	0.44	1.48	.....	0.20	1.68	2.7
C_{10}	1.05	0.78	0.14	0.30	1.57	3.83	0.78	0.14	1.75	2.67	3.5
C_{12}	.....	.....	1.07	2.25	3.13	7.63	.....	1.06	3.48	4.54	5.2
C_{14}	.....	.....	5.46	11.51	8.30	20.23	.....	5.43	9.22	14.65	14.8
C_{16}	.....	.....	26.41	55.73	3.36	8.19	.....	26.31	3.74	30.05	27.2
C_{18}	.....	.....	9.21	19.43	1.14	2.78	.....	9.18	1.27	10.45	8.5
C_{20}	.....	.....	1.20	2.53	0.44	1.07	.....	1.19	0.49	1.68	1.2
Unsaturated											
C_{10}	.....	.....	.....	.....	0.22	0.54	.....	.....	0.25	0.25	0.3
C_{12}	.....	.....	.....	.....	0.13	0.32	.....	.....	0.15	0.15	0.2
C_{14}	.....	.....	0.09	0.19	1.25	3.05	.....	0.09	1.39	1.48	1.5
C_{16}	.....	.....	0.41	0.86	4.75	11.57	.....	0.41	5.28	5.69	5.2
C_{18}	.....	.....	3.28	7.00	13.88	33.88	.....	3.25	15.44	18.69	15.3
C_{20}	.....	.....	0.14	0.30	0.76	1.85	.....	0.14	0.84	0.98	0.7
Linoleic	.....	.....	.....	.....	1.92	4.62	.....	.....	2.12	2.12	1.7

Non-saponifiable matter of milkfat = 0.28 per cent
Non-saponifiable matter of esters = 0.02 per cent

[b] For this analysis 150 gm. of fat was saponified and the acids liberated to yield 138.12 gm. fatty acids. These were steam-distilled, yielding 7.18 per cent steam-volatile acids. The 92.82 per cent non-volatile acids were separated into 47.20 per cent "solid" acids and 45.62 per cent "liquid" acids as described in the text. The entire yield of steam-volatile acids was fractionated, and weight percentages of the individual acids were calculated from the weights of each, based on 138.12 gm. total fatty acids. The "solid" and the "liquid" acids were converted to the methyl esters and then fractionated; 50.54 gm. of methyl esters derived from 47.41 gm. "solid" acids were fractionated, and the weight percentages of the individual fatty acids were calculated from the weights of each based on the starting weight (47.41 gm.). Similarly, the weight percentages of the "liquid" acids were calculated from the starting weight of "liquid" fatty acids (41.03 gm.). The same scheme applies to the other table headings in this series.

S.V. as used throughout the manuscript means *Steam-Volatile Acids.*
S.A. as used throughout the manuscript means *Solid Acids.*
L.A. as used throughout the manuscript means *Liquid Acids.*

5. Compositions of the different fractions were calculated from the analytical data according to the methods in general use (10, 16). The calcu-

lations are valid when the assumption is fulfilled that there are present not more than two adjacent saturated acids, and not more than two unsaturated acids of a homologous series. Data shown in table 1 indicate that this assumption is warranted. For example, sample 2 is very close to methyl laurate (S.E. 214.2); samples 3 and 4 are very close to methyl myristate (S.E. 242.2); the next five are predominantly methyl palmitate (S.E. 272.3); Nos. 10 and 11 are predominantly methyl oleate and methyl stearate (S.E. 296.4 and 298.4). The sharpness of fractionation is apparent. Such clean-

TABLE 4

Calculated composition of −7° ppt. fatty acids
I.V. 8.29, Sap. No. 211.0

150 gm. fat yielded 136.17 gm. fatty acids, 0.86 per cent steam-volatile acids, 99.14 per cent "solid" acids. Per cent of steam-volatile acids based on 136.17 gm.; per cent of solid acids based on 50.23 gm. methyl esters equal to 47.65 gm. fatty acids.

Length of carbon chain	Steam-volatile acids		Solid acids		Weight, per cent of total		Total weight	Mol
	gm.	*%*	*gm.*	*%*	*S.V.*	*S.A.*	*%*	*%*
Saturated								
C_4	1.17	0.86	.....	.....	0.86	.....	0.86	2.5
C_{10}	.....	.....	0.39	0.82	.....	0.82	0.82	1.2
C_{12}	.....	.....	1.82	3.82	.....	3.80	3.80	4.5
C_{14}	.....	.....	7.01	14.70	.....	14.58	14.58	16.6
C_{16}	.....	.....	20.30	42.60	.....	42.24	42.24	42.1
C_{18}	.....	.....	11.37	23.86	.....	23.66	23.66	21.1
C_{20}	.....	.....	2.25	4.74	.....	4.72	4.72	3.8
C_{22}	.....	.....	0.50	1.05	.....	1.01	1.01	0.8
Unsaturated								
C_{14}	.....	.....	0.05	0.10	.....	0.10	0.10	0.1
C_{16}	.....	.....	0.20	0.42	.....	0.42	0.42	0.4
C_{18}	.....	.....	3.25	6.82	.....	6.77	6.77	6.1
C_{20}	.....	.....	0.51	1.07	.....	1.02	1.02	0.8

Non-saponifiable matter of esters = 0

cut separation of adjacent homologues was not obtained, however, with the methyl esters of the "liquid" fractions, which were highly unsaturated; there were more fractions intermediate between adjacent homologues.

Table 2 shows the ester distribution of the "solid" acids from the −23° ppt. as calculated by the methods just described. A typical calculation (No. 33) illustrates the steps in determining the composition of all the fractions.

6. The calculated weights of methyl esters for each fraction and for the original fat were converted to the weights of the corresponding fatty acids. These weights and the derived weight percentages, together with the mol percentages of each acid, are summarized in the following tables (3–8).

From these values were calculated the percentages of each acid in the original fat, as based on the relative proportion of each fraction to the whole. Table 9 shows weight percentages on the original-fat basis for each fraction.

TABLE 5

Calculated composition of −13° ppt. fatty acids
I.V. 22.15, Sap. No. 230.2

150 gm. fat yielded 137.23 gm. fatty acids, 8.54 per cent steam-volatile acids, 57.35 per cent "solid" acids, 34.11 per cent "liquid" acids. Per cent of steam-volatile acids based on 137.23 gm.; per cent of solid acids on 50.89 gm. methyl esters equal to 48.28 gm. fatty acids; per cent of liquid acids based on 41.60 gm. methyl esters equal to 39.32 gm. fatty acids.

Length of carbon chain	Steam-volatile acids		Solid acids		Liquid acids		Weight, per cent of total			Total weight	Mol
	gm.	%	*gm.*	%	*gm.*	%	*S.V.*	*S.A.*	*L.A.*	%	%
Saturated											
C_4	2.39	1.74	.....	.....	.....	.....	1.74	.....	.....	1.74	4.6
C_6	2.02	1.47	.....	.....	.....	.....	1.47	.....	.....	1.47	2.9
C_8	5.21	3.80	.....	.....	0.30	0.76	3.80	.....	0.26	4.06	6.6
C_{10}	2.11	1.53	0.15	0.32	2.26	5.75	1.53	0.18	1.96	3.67	4.9
C_{12}	.....	.....	0.43	0.90	2.85	7.25	.....	0.51	2.47	2.98	3.5
C_{14}	.....	.....	3.08	6.21	8.60	21.87	.....	3.56	7.46	11.02	11.3
C_{16}	.....	.....	29.55	61.24	4.62	11.75	.....	35.13	4.01	39.14	36.2
C_{18}	.....	.....	8.78	18.22	1.12	2.85	.....	10.45	0.97	11.42	9.3
C_{20}	.....	.....	2.84	5.91	0.96	2.43	.....	3.39	0.83	4.22	3.1
Unsaturated											
C_{10}	.....	.....	.....	.....	0.19	0.48	.....	.....	0.16	0.16	0.2
C_{12}	.....	.....	.....	.....	0.20	0.51	.....	.....	0.17	0.17	0.2
C_{14}	.....	.....	.....	.....	0.95	2.42	.....	.....	0.82	0.82	0.9
C_{16}	.....	.....	0.67	1.40	3.97	10.10	.....	0.80	3.45	4.25	3.9
C_{18}	.....	.....	2.41	5.03	11.14	28.33	.....	2.89	10.73	12.62	10.6
C_{20}	.....	.....	0.37	0.77	1.57	3.99	.....	0.44	1.36	1.80	1.3
Linoleic	.....	.....	.....	.....	0.59	1.51	.....	.....	0.56	0.56	0.5

Non-saponifiable matter of esters = 0.03 per cent

TABLE 6

Calculated composition of −23° ppt. fatty acids
I.V. 23.46, Sap. No. 232.7

150 gm. fat yielded 111.68 gm. fatty acids, 6.88 per cent steam-volatile acids, 55.60 per cent "solid" acids, 37.52 per cent "liquid" acids. Per cent of steam-volatile acids based on 111.68 gm.; per cent of solid acids on 48.74 gm. methyl esters equal to 46.21 gm. fatty acids; per cent of liquid acids based on 38.90 gm. methyl esters equal to 36.77 gm. fatty acids.

Length of carbon chain	Steam-volatile acids		Solid acids		Liquid acids		Weight, per cent of total			Total weight	Mol
	gm.	%	*gm.*	%	*gm.*	%	*S.V.*	*S.A.*	*L.A.*	%	%
Saturated											
C_4	3.70	3.31	.....	.....	.....	.....	3.31	.....	.....	3.31	8.7
C_6	2.26	2.03	.....	.....	.....	.....	2.03	.....	.....	2.03	4.0
C_8	0.51	0.46	.....	.....	0.36	0.98	0.46	.....	0.37	0.83	1.3
C_{10}	1.21	1.08	.....	.....	1.94	5.28	1.08	.....	1.98	3.06	4.0
C_{12}	.....	.....	1.28	2.77	2.16	5.88	.....	1.53	2.21	3.74	4.2
C_{14}	.....	.....	3.83	8.28	9.56	26.03	.....	4.61	9.75	14.36	14.6
C_{16}	.....	.....	31.66	68.50	3.90	10.62	.....	38.09	3.98	42.07	38.1
C_{18}	.....	.....	4.76	10.32	0.81	2.20	.....	5.74	0.83	6.57	5.2
C_{20}	.....	.....	1.16	2.51	.....	.....	.....	1.39	.....	1.39	1.0
Unsaturated											
C_{10}	.....	.....	.....	.....	0.10	0.27	.....	.....	0.10	0.10	0.1
C_{12}	.....	.....	.....	.....	0.13	0.35	.....	.....	0.13	0.13	0.1
C_{14}	.....	.....	0.17	0.37	0.99	2.69	.....	0.21	1.01	1.22	1.2
C_{16}	.....	.....	0.36	0.78	4.52	12.30	.....	0.43	4.61	5.04	4.5
C_{18}	.....	.....	2.76	5.96	9.58	26.13	.....	3.32	9.80	13.12	10.7
C_{20}	.....	.....	0.23	0.50	2.21	6.01	.....	0.28	2.25	2.53	1.9
Linoleic	.....	.....	.....	.....	0.51	1.33	.....	.....	0.50	0.50	0.4

Non-saponifiable matter of esters = 0.01 per cent

TABLE 7

Calculated composition of −53° ppt. fatty acids
I.V. 31.18, Sap. No. 229.1

150 gm. fat yielded 136.14 gm. fatty acids, 7.12 per cent steam-volatile acids, 43.83 per cent "solid" acids, 49.05 per cent "liquid" acids. Per cent of steam-volatile acids based on 136.14 gm.; per cent of solid acids based on 52.46 gm. methyl esters equal to 49.64 gm. fatty acids; per cent of liquid acids based on 42.63 gm. methyl esters equal to 40.13 gm. fatty acids.

Length of carbon chain	Steam-volatile acids		Solid acids		Liquid acids		Weight, per cent of total			Total weight	Mol
	gm.	*%*	*gm.*	*%*	*gm.*	*%*	*S.V.*	*S.A.*	*L.A.*	*%*	*%*
Saturated											
C_4	4.09	3.01	.....	.....	.....	.....	3.01	.....	.....	3.01	7.9
C_6	3.96	2.91	.....	.....	.....	.....	2.91	.....	.....	2.91	5.8
C_8	0.94	0.69	.....	.....	0.33	0.82	0.69	.....	0.40	1.09	1.7
C_{10}	0.70	0.51	0.49	0.99	2.61	6.51	0.51	0.43	3.19	4.13	5.5
C_{12}	.....	.....	1.00	2.01	2.64	6.58	.....	0.88	3.23	4.11	4.7
C_{14}	.....	.....	5.65	11.38	9.47	23.61	.....	4.99	11.58	16.57	16.6
C_{16}	.....	.....	25.93	52.23	3.14	7.82	.....	22.90	3.83	26.73	24.0
C_{18}	.....	.....	8.57	17.26	2.45	6.11	.....	7.56	3.00	10.56	8.5
C_{20}	.....	.....	2.25	4.53	.....	.....	.....	1.98	.....	1.98	1.4
Unsaturated											
C_{10}	.....	.....	.....	.....	0.19	0.47	.....	.....	0.23	0.23	0.3
C_{12}	.....	.....	.....	.....	0.17	0.42	.....	.....	0.21	0.21	0.2
C_{14}	.....	.....	0.28	0.57	0.95	2.36	.....	0.25	1.16	1.41	1.4
C_{16}	.....	.....	0.46	0.93	2.11	5.26	.....	0.41	2.58	2.99	2.7
C_{18}	.....	.....	4.72	9.51	13.76	34.30	.....	4.17	16.75	20.92	17.2
C_{20}	.....	.....	0.29	0.59	1.86	4.60	.....	0.26	2.26	2.52	1.8
Linoleic	.....	.....	.....	.....	0.45	1.14	.....	.....	0.63	0.63	0.5

Non-saponifiable matter of esters = 0

TABLE 8

Calculated composition of −53° filtrate fatty acids
I.V. 58.37, Sap. No. 236.9 (Corrected for N.S.)

150 gm. of fat yielded 131.79 gm. fatty acids, 8.94 per cent steam-volatile acids, 91.06 per cent "liquid" acids. Per cent steam-volatile acids based on 131.79 gm. fatty acids; per cent of liquid acids based on 45.28 gm. methyl esters equal to 42.96 gm. fatty acids.

Length of carbon chain	Steam-volatile acids		Liquid acids		Weight, per cent of total		Total weight	Mol
	gm.	*%*	*gm.*	*%*	*S.V.*	*L.A.*	*%*	*%*
Saturated								
C_4	4.73	3.58	.....	.....	3.58	.....	3.58	9.3
C_6	4.61	3.50	.....	.....	3.50	.....	3.50	6.9
C_8	1.95	1.48	.....	.....	1.48	.....	1.48	2.4
C_{10}	0.50	0.38	1.37	3.19	0.38	2.91	3.29	4.5
C_{12}	.....	.....	1.53	3.56	.....	3.24	3.24	3.7
C_{14}	.....	.....	3.48	8.10	.....	7.38	7.38	7.4
C_{16}	.....	.....	7.39	17.21	.....	15.67	15.67	14.0
C_{18}	.....	.....	4.04	9.40	.....	8.56	8.56	6.8
Unsaturated								
C_{10}	.....	.....	0.49	1.14	.....	1.05	1.05	1.4
C_{12}	.....	.....	0.14	0.33	.....	0.30	0.30	0.4
C_{14}	.....	.....	0.87	2.02	.....	1.84	1.84	1.8
C_{16}	.....	.....	2.39	5.56	.....	5.06	5.06	4.6
C_{18}	.....	.....	17.29	40.25	.....	36.60	36.60	30.0
C_{20}	.....	.....	0.52	1.21	.....	1.10	1.10	0.8
Linoleic	.....	.....	3.45	8.03	.....	7.35	7.35	6.0

Non-saponifiable matter of esters = 0.08 per cent

For comparison, the weight percentages as determined on the original milk fat are included.

The values in the table for milk fat, reconstructed from data on the composition of each fraction, agree well with those obtained by analyzing the milk fat directly. The data for the individual fractions are believed to be more reliable for showing the presence of small quantities of the individual acids. For example, behenic acid (C_{22}) unquestionably occurs in the $-7°$ ppt. Yet the amount in the original milk fat is exceedingly small: it cannot influence the saponification value of the distillation residue sufficiently to make its presence certain.

TABLE 9

Weight per cent of original as based on average yield of each fraction

Weight per cent of $-7°$ ppt. $\times 11.5\%$, weight per cent $-13°$ ppt. $\times 20.5\%$, weight per cent $-23°$ ppt. $\times 20.5\%$, weight per cent $-53°$ ppt. $\times 23.9\%$, weight per cent $-53°$ Filt. $\times 23.6\%$.

Length of carbon chain	$-7°$ ppt.	$-13°$ ppt.	$-23°$ ppt.	$-53°$ ppt.	$-53°$ filt.	Total of fractions	Milk fat
Saturated							
C_4	0.10	0.36	0.68	0.72	0.84	2.70	3.52
C_6	.....	0.30	0.42	0.70	0.83	2.25	1.40
C_8	.....	0.83	0.17	0.26	0.35	1.61	1.68
C_{10}	0.09	0.75	0.63	0.99	0.78	3.24	2.67
C_{12}	0.44	0.61	0.77	0.98	0.76	3.56	4.54
C_{14}	1.69	2.26	2.95	3.96	1.74	12.60	14.65
C_{16}	4.87	8.03	8.61	6.39	3.70	31.60	30.05
C_{18}	2.69	2.34	1.35	2.53	2.02	10.93	10.45
C_{20}	0.54	0.87	0.28	0.47	.....	2.16	1.68
C_{22}	0.12	.....	.....	.....	.....	0.12	
					Saturated total	70.77	70.64
Unsaturated							
C_{10}	.....	0.03	0.02	0.05	0.25	0.35	0.25
C_{12}	.....	0.03	0.03	0.05	0.07	0.18	0.15
C_{14}	0.01	0.17	0.25	0.34	0.43	1.20	1.48
C_{16}	0.05	0.87	1.03	0.71	1.19	3.85	5.69
C_{18}	0.78	2.56	2.69	5.00	8.64	19.67	18.69
C_{20}	0.12	0.37	0.52	0.60	0.26	1.87	0.98
Linoleic	.....	0.12	0.10	0.15	1.74	2.11	2.12
					Unsaturated total	29.23	29.36
					Saturated total	70.77	70.64
						100.00	100.00

Figure 2 shows the molecular distribution of the fatty acids. The similarity of distribution in the original milk fat and in the $-53°$ ppt. is striking. Yet these fats are widely different in melting point. The milk fat melts at 32.8° C.; the $-53°$ ppt. at 11.4° C. Where the molar percentages are the same and the properties are so different, there is a difference in the distribution of the component acids in the glyceride molecule. Lea has cited a similar instance in sheep tallow and cocoa fat (28). These are almost identical in fatty-acid percentages, but differ in physical properties.

DISCUSSION

The composition of milk fat reported in this investigation closely resembles that previously reported from studies made with the same type of equipment.

Table 10 shows comparison with values obtained elsewhere. The values differ in that the present writers have reported the presence of a C_{22} satu-

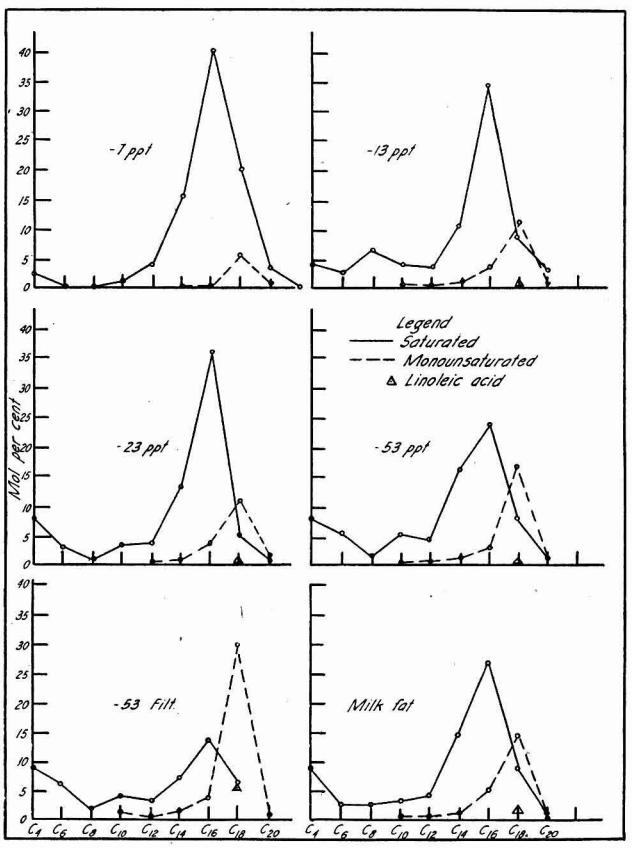

FIG. 2. Mol percentage of fatty acids in each fraction and in the original milk fat.

rated acid, whereas the others have combined the C_{20} and the C_{22} acids in a single listing. A second difference appears in the quantities of oleic acid; the values reported in this paper may seem low; but the possibility of error is minimized when one considers that the quantities are essentially the same whether determined directly on the original milk fat or from the increments in the fractions. Likewise, when the iodine values of the different fats are

compared with the total amount of unsaturation, this quantity appears teasonable.

The data obtained in this study, together with some additional values, may be used to calculate the fatty-acid composition of individual glycerides. Consideration will be given to this in a later publication.

TABLE 10

Comparison of fatty acids in mols per cent with values obtained in England using electrically-heated packed fractionating columns

Length of carbon chain	This investigation		Hilditch and Longenecker (19)*	Smith and Dastur (33)*	Hilditch and S. Paul (21)*
	Original milk fat LV. 32.42 (Hanus)	Composite from fractions I.V. 31.9 (calc.)	LV. 37.5	I.V.36.6	I.V.46.9
Saturated					
C_8	9.2	7.3	8.1	9.7	10.2
C_{10}	2.8	4.5	2.8	1.2	2.5
C_{12}	2.7	2.7	2.5	1.6	1.3
C_{14}	3.5	4.3	3.7	2.5	1.5
C_{16}	5.2	4.2	4.4	3.0	3.4
C_{18}	14.8	12.4	12.5	12.5	8.6
C_{18}	27.2	28.9	23.2	22.1	21.1
C_{18}	8.5	8.8	7.6	9.8	9.9
C_{20}	1.2	1.6	1.0	0.8	0.7
C_{22}		0.1			
Unsaturated					
C_{10}	0.3	0.5	0.4	0.3	0.2
C_{12}	0.2	0.3	0.9	0.3	0.2
C_{14}	1.5	1.3	1.7	1.0	0.9
C_{16}	5.2	3.6	3.7	3.0	2.8
C_{18}	15.3	16.4	24.8	30.5	31.4
C_{20}	0.7	1.4	0.2	0.6	0.5
Linoleic	1.7	1.7	2.9	1.0	4.9

* Refers to number cited in references.

SUMMARY

1. Milk fat, separated into five different fractions by precipitation from a solvent at low temperatures, was analyzed by the ester-fractionation method to determine the amount and distribution of the individual fatty-acids.

2. The amounts of each fatty acid present in each fraction and in the milk fat have been determined. These values, together with corresponding weight and mol percentages, are presented.

3. When the values for the composition of the original milk fat are compared with those obtained by reconstructing milk fat from the increments of the individual fatty acids contained in the fractions, an excellent agreement is observed. These data also compare favorably with those reported by others.

4. The occurrence of small quantities of individual fatty acids is detected with more reliability from the fractions than from the complex entire fat.

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THE DEGREE OF FAT DISPERSION IN CHEESE MILK AND ITS RELATION TO THE MECHANISM OF INCREASED LIPASE ACTION IN AGITATED MILK[1]

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Rancid and unclean flavor defects in raw milk cheddar cheese can readily be produced experimentally by vigorous agitation of warm cheese milk (4, 5). The underlying chemical reaction is lipolytic, involving enzymatic catalysis by endogenous milk lipase, which may be represented as,

$$\text{Butterfat glycerides} + \text{water} \xrightarrow[\text{lipase}]{\text{milk}} \text{glycerol or mono- and di-glycerides} + \text{free fatty acids.}$$

Only the lower fatty acids found in butterfat possess an objectionable flavor in the free state, which they impart to the cheese.

The mechanism of increased lipase action in milk induced by vigorous agitation has not been fully studied. Two alternate hypotheses may be advanced. According to the first, an alteration in the surface characteristics of fat globules is believed to be the factor responsible for the increase in lipase activity of agitated milk. This view is supported by Krukovsky and Sharp (6). In the second hypothesis primary importance in the increase of lipase action in agitated milk is attached to the dispersion of fat and the accompanying increase in the surface area of the fat globules, in an analogous manner to the increase in lipase activity in raw homogenized milk. Van Dam (9) showed that the degree of dispersion of fat is increased by shaking milk while fat is in the liquid state. The extent of fat dispersion is further brought out by Siegfeld (7), who found that churning at 50° C. for 2 hours completely homogenizes the fat in new milk.

In this communication further evidence on the mechanism of increased lipase action in agitated milk is presented. First, a new method of studying the degree of fat dispersion is described. Then, data are given on the extent of fat dispersion in milk agitated under conditions which were worked out for the experimental production of rancid and unclean flavors in cheddar cheese (4).

A METHOD OF STUDYING THE DEGREE OF FAT DISPERSION

It is well known from practical experience, and as a scientific fact, that small fat globules, as in homogenized milk, cannot be separated in a cream separator. The explanation given by Davies (1) is as follows: "Under the

Received for publication July 17, 1944.

[1] Contribution No. 192 (Journal Series).

influence of centrifugal force the time required for a globule of radius r to travel through a given distance is proportional to r^2 and as the number of gallons treated in a separator per hour is inversely proportional to this time, it follows that for any given radius of globule there is a critical value at which its velocity against the milk stream is equal to the velocity of the stream itself, so that the globules of smaller radius pass out with the separated milk." *For a given separator of uniform performance, therefore, the butterfat content of skim milk is an index of the degree of fat dispersion in milk.* It is thus analogous to the Farrall index (3).

Trout and Scheid (8) demonstrated this principle experimentally in their study of centrifugal separation of homogenized milk. While these workers were primarily interested in the recovery of fat from homogenized milk, it may be concluded from their work that the higher the percentage of homogenized milk, *i.e.*, the greater the number of small fat globules, the higher was the butterfat content of skim milk. In other words, the butterfat content of skim milk was an index of the degree of dispersion of butterfat in milk. It is important to get this principle clearly in mind because the interpretation of the work which follows largely depends upon it.

The performance of a De Laval cream separator No. 32, used at the Experimental Farm Creamery, although of larger capacity than was desired for experimental purposes, proved entirely satisfactory. The important requirement in the performance of a separator for this purpose is uniformity of skimming. Butterfat tests on skim milk, taken at intervals, must check closely during the entire period of separation of a given quantity of milk. From this point of view, a small De Laval No. 1 separator was found unsuitable. With respect to efficiency of skimming no adjustment other than that for general use in separating milk, in this case skimming to less than 0.04 per cent as determined by the normal butyl alcohol–Babcock test (10), was necessary. However, if a direct comparison of results obtained with two different separators were to be made, the efficiency of the separators used must be given.

The above method was applied to the study of the degree of fat dispersion in milk.

THE DEGREE OF FAT DISPERSION IN AGITATED MILK

Two 8-gallon cans of morning's milk from the Experimental Farm herd were used for each determination. Morning's milk was used because, in experiments where prolonged periods of agitation were necessary, there was less churning out of butterfat than was the case with night's milk. The milk was pooled in a small cheese vat and the temperature adjusted to 86° F. Four gallons of the pooled milk was then separated and two samples of skim milk taken at different intervals for butterfat determination. The remainder of the milk was next agitated in a Cherry Junior churn, Model 2B, for a definite period of time. The agitated milk was also separated and two sam-

ples of skim milk were taken as before. All butterfat determinations were done by the normal butyl alcohol–Babcock test. The procedure was repeated three times for each of 15-, 10-, 5- and 3-minute periods of agitation.

In addition to these experiments on raw milk, one trial each on pasteurized milk (30 min. at 143° F.) agitated for 5 and 10 minutes is included.

The results are summarized in table 1. It shows that the longer the period of agitation of milk, the higher is the fat percentage in the skim. In the preceding section, however, it has already been pointed out that the percentage of fat in the skim milk is an index of the degree of fat dispersion. The data presented in table 1, therefore, show that agitation of milk leads to a further dispersion of fat globules. In other words, large fat globules are broken up into smaller ones due to concussion and shearing action resulting from agitation.

There is no essential difference between the results obtained with raw and pasteurized milk. The higher butterfat content of skim obtained from agi-

TABLE 1

Per cent butterfat in skim from unagitated and agitated milk separated at 86° F.

Milk	Unagitated	Agitated at 86° F.	
	% BF	Min. agitated	% BF
Raw	0 03	15	0.41
"	0.04	10	0.31
"	0.04	5	0.18
"	0.02	3	0.12
Past.	0.03	10	0.32
"	0.06	5	0.21

tated milk is, therefore, not an artifact resulting from lipase action and the accumulation of free fatty acids in skim milk.

Fat dispersion is proportional to the time of agitation. There is almost a linear relationship between agitation and the degree of fat dispersion within the range studied.

To appreciate the extent of fat dispersion brought out in table 1, a comparison may be made with the work of Trout and Scheid (8) on fat percentages of skim milk obtained by separating various proportions of homogenized and non-homogenized milk. The extent of fat dispersion in milk agitated for 15 minutes at 86° F. in a churn may thus be deduced to be roughly equivalent to that in milk containing 20 per cent homogenized milk.

Assuming also that the area of the fat-aqueous interface increases thirty-fold when milk is homogenized (2), it may be calculated approximately that there is a seven-fold increase in the area of the fat globule surface in milk agitated for 15 minutes.

It should be borne in mind that the above comparisons are only approximate. It may be assumed that some dispersion takes place in the cream

fraction from agitated milk which would not be reflected by the skim milk fraction. This would not be the case in making up a mixture of 20 per cent homogenized and 80 per cent non-homogenized milk. This factor would tend to increase the total fat globule surface even more than shown by such a comparison.

DISCUSSION

By the application of the ordinary cream separator in conjunction with the normal butyl alcohol–Babcock test, it has been possible to extend our studies on rancid and unclean flavor defects in cheddar cheese. It has previously been shown that these flavor defects may be readily produced by increasing lipase action through vigorous agitation of warm cheese milk. It has now been further established that vigorous agitation leads to appreciable fat dispersion in milk. One is led to the conclusion that the increase in the area of fat-aqueous interface due to the creation of a large new surface may, in large measure, account for the increased lipase action in agitated milk.

The creation of new fat-aqueous surface in a medium such as milk would normally be attended by the adsorption of specific proteins (including milk lipase) in accordance with the well-known Gibbs' adsorption equation. An alteration in the surface characteristics of fat globule surfaces apart from fat dispersion, as suggested by Krukovsky and Sharp (6), may be an additional factor, but it is more likely to be of greater importance in less vigorously agitated milk and in cooling and warming of milk.

Parenthetically, it may be pointed out that it is preferable to speak of increase in lipase action instead of lipase activation where the change appears to be essentially in the substrate and not in the nature of lipase itself.

The method employed in the study of the degree of fat dispersion in this investigation has been found simple and reliable. Its application is being extended to other problems in dairy science.

SUMMARY

The cream separator in conjunction with the normal butyl alcohol-Babcock test has been found to be a simple and a reliable method of studying the degree of dispersion of fat in milk. By this method it has been shown that considerable fat dispersion takes place in vigorously agitated warm milk. Milk which was churned for 15 minutes at 86° F. showed fat dispersion roughly equivalent to that of 20 per cent homogenized milk. It is concluded that the increase in the fat-aqueous interface due to fat dispersion may explain, in large part, the mechanism of increased lipase action in agitated milk.

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To the Officers and Members of the American Dairy Science Association:

The Iowa State College joins with the Dairy organizations of Iowa in expressing great satisfaction that the American Dairy Science Association has decided to hold its 1945 annual meeting on our campus.

As one of the great dairy states of America, we welcome you. We recall with pleasure your meeting at the College in 1930; and we are looking forward with even greater anticipation to your visit next June, even though war conditions will probably compel us to temper somewhat our plans for your entertainment.

We are gratified that several of the presidents of the American Dairy Science Association have been alumni of the Iowa State College; and that an alumnus, Professor J. H. Frandsen of Massachusetts State College, established the Journal of Dairy Science, and for many years served as its editor-in-chief.

We hope that the 1945 meeting of the Association will contribute materially to the success of the present and the post-war plans of the dairy industry of the nation.

Sincerely yours,

Charles E. Friley
President

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Members who wish to give original papers at the annual meeting should send the titles to the Program Committee as promptly as convenient. All titles must reach the Program Committee before April 1, but early receipt of the titles is of real value in arranging the best program. All communications regarding general plans should be addressed to the chairman of the General Program Committee, but all titles of papers should be sent directly to the chairman of the program committee for the section before which the paper should be presented.

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VOLUME XXVIII JANUARY, 1945 NUMBER 1

JOURNAL OF DAIRY SCIENCE

Published by the
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ABSTRACTS OF LITERATURE

BACTERIOLOGY

1. **Problems in the Control of Thermoduric Bacteria from the Aspect of Maintenance of High Quality Production.** F. W. FABIAN, Mich. State Col., East Lansing, Mich. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 4: 43–50. May, 1944.

Thermoduric bacteria do not multiply during pasteurization so, when numbers are high, the farm is generally the source. It is a producer's problem. Micrococci from the udder have been found to be more than 40% thermoduric. They thrive in unclean utensils and are resistant to heat and chlorine, but can be effectively controlled by the use of acid cleaners. There is no evidence that thermodurics cause disease, but their presence is an indication of careless, unclean methods on the part of the producer.

Control measures listed are:

1. Clean farm utensils thoroughly.
2. Select suitable detergents.
3. Promptly cool milk to 50° F. or below.
4. Use air-space heaters and air-temperature recording thermometer to insure thorough pasteurization of foam.
5. Avoid pellicle formation.
6. Avoid repasteurization.
7. Shut down and clean pasteurizer every two or three hours.

Detection and enumeration of thermoduric bacteria depends upon some heat treatment to kill the non-thermodurics and then the use of a plate or microscopic count. The method of Mallman *et al.* is as follows:

After holding samples at 136.5° to 143° F. for 2 hours, bacteria surviving in milk are thermoduric and may be counted with the microscope. A smear of milk on a slide may be autoclaved for 10 minutes at 15 pounds' pressure. By either method, counts in excess of 40,000 are considered excessive.

E.F.G.

2. **Reproducible Data by Microscopic Method.** DAVID LEVOWITZ, N. J. Dairy Labs., New Brunswick, N. J. Internatl. Assoc. Milk Dealers, Assoc. Bul. *36,* No. 10: 139–150. June, 1944.

In the 15-thousand and 30-thousand-count brackets the results of the direct microscopic method, as described in the eighth edition of "Standard Methods for the Examination of Dairy Products," are so variable on the same sample by different operators that the method has not been used extensively. There are many reasons for this. Among these are: the small number of cells observed in the required minimum number of fields; the error due to "crest formation" as the film dries; and the fact that only

clumps actually within the field under observation are to be included and the number of fields must be kept count of. The proposal is made that more reproducible results would be obtained if a standard field 0.200 mm. in diameter were used and the microscope stage moved slowly away or toward the operator so that all clumps in a strip entirely across the stained film would be counted. If the first strip shows over 100 clumps, one strip is enough. If there are 50–100 clumps on the first strip, count two strips; if 20–50 clumps, count three strips; if 10–20 clumps, count four strips; and if there are less than 10 clumps in the first strip, count five strips. The 0.200 mm. lens gives a factor of 5,000 to be multiplied by the count of each strip to convert data to clumps per ml. of milk. The first strip is through the central part of the stained film, and each succeeding strip is approximately one field to the right of its predecessor until enough strips have been counted. It is claimed that data obtained by technicians with the strip method are fully reproducible. E.F.G.

3. **A Study of *Bacillus subtilis* and Related Organisms.** T. GIBSON, Bact. Dept., Col. of Agr., Edinburgh, Scot. Jour. Dairy Res., *13*, No. 3: 248–260. 1944.

The author has made a morphological and cultural study of 300 strains of *B. subtilis* and closely related organisms. He concludes that a group of species comprising *B. subtilis* and related organisms may be differentiated from other members of the genus Bacillus by (a) morphology and (b) the production of acid and acetylmethycarbinol from glucose, and that the group may be divided into three species:

(1) *B. subtilis* Cohn emend. Prazmowski, the synonyms of which include *B. aterrimus, B. globigii, B. mesentericus, B. niger, B. panis,* and *B. vulgatus.*

(2) *B. pumilus* Gottheil, which resembles strains of (1) that produce a smooth glossy growth. It differs in failing to secrete diastase and in other features, but its separation as a distinct species is still an open question. It is sometimes identified as *B. mesentericus.*

(3) *B. licheniformis* (Weigmann) Gibson, the synonyms of which include *B. subtilis* (as used by Ford), *B. globigii* and *B. mesentericus* ruber. This species may be differentiated from (1) and (2) by its cultural characters, slow proteolytic action, vigorous CO_2 production and ability to grow anaerobically. S.T.C.

CHEESE

4. **Some Problems in Cottage Cheese.** H. C. OLSON, A. and M. Col., Stillwater, Okla. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36*, No. 13: 175–183. July, 1944.

Good cottage cheese can be made by either the long- or short-set method, provided proper care for each technique is used. The flavor of cottage

cheese is so mild that off flavors in the original milk or any which might occur in the manufacturing operation are especially objectionable. Many advantages result from the use of high solids milk; these include the following: more rapid acid development, firmer curd, and easier cooking. A final whey acidity of 0.58–0.60 per cent is recommended for high-solids milk, whereas the final acidity for low-solids milk seldom exceeds 0.56 per cent. High-solids milk can be cooked at 5–10° F. below low-solids milk, and the cooking time is about half. Yields of 80 per cent moisture cheese were 19.2 per cent for high-solids milk and 14.6 per cent for low-solids milk, with 39.8 per cent and 33.6 per cent recovery of solids respectively. Low-solids skim milk may sometimes be reinforced with dry or condensed skim milk to advantage. Good starter, curd of right firmness, and careful cooking to proper degree of curd hardness are important points. Curd may be stored up to two weeks at 40° F. in a 3 per cent brine solution with little deterioration.

E.F.G.

5. **The Persistence and Recovery of Bacteriophage in Cheese.** Agnes A. Nichols and J. Z. Wolf, Natl. Inst. Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., *13*, No. 3: 302–307. 1944.

Several cheese in the manufacture of which bacteriophage activity had been noted were tested for bacteriophage activity. Extracts of the cheese were prepared by shaking samples in quarter strength Ringer's solution, which was afterwards passed through a Seitz filter. The presence and concentration of the phage were indicated by two methods: (1) inhibition of acid development when varying amounts of the cheese extract were added to milk inoculated with susceptible starter strains; and (2) clearing of the turbidity due to growth using yeast dextrose broth as a medium.

Phage activity in the cheese extracts in dilutions as high as 10^{-8} and 10^{-9} was detected. There was very little change in the titre of the phage during cheese storage up to one year.

S.T.C.

6. **The Influence of Abnormal Milk upon the Yield and Quality of Cheddar Cheese.** C. K. Johns and C. A. Gibson, Dept. of Agr., Ottawa, Canada. Jour. Dairy Res., *13*, No. 3: 287–294. 1944.

An attempt was made to segregate abnormal milk in a commercial cheese factory. Abnormality was judged by catalase content, cell count, initial acidity, pH, chloride, solids-not-fat and casein contents. These various tests often failed to agree. Cheese was made from both abnormal and normal milk, and the yield and quality compared.

In some of the trials a lower yield of cheese was secured from the abnormal milk, but this was not consistently true. There was good agreement

between the solids-not-fat content of the milk and the yield of cheese. In general, the cheese from the abnormal milk was inferior in quality, particularly after ripening. S.T.C.

7. **The Influence of Bacteriophage on the Cheese-Making Process.** G. J. E. HUNTER, Dairy Res. Inst. (D.S.I.R.), Palmerston North, New Zeal. Jour. Dairy Res., *13,* No. 3: 294–301. 1944.

Varying amounts of a stock solution of bacteriophage active against the organisms of the single strain cultures used as starter were added to pasteurized milk to be used for cheesemaking. The extent to which the presence of the phage is made evident during cheesemaking was shown to depend largely upon the amount of initial infection. There was sufficient multiplication of the phage with a heavy infection to cause lysis of the organisms and consequent cessation of acid development before the manufacturing process was complete. A light infection produced no noticeable influence upon the rate of acid development. S.T.C.

CHEMISTRY

8. **Measurement of the Consistency of Plastic Vegetable Fats. A Standard Micropenetration Technique.** R. O. FENGE AND A. E. BAILEY, South Region. Lab., New Orleans, La. Oil and Soap, *21,* No. 3: 78. March, 1944.

The theory of the plasticity of fats is discussed and related to factors that influence the consistency of solidified fats. It is pointed out that factors such as solidifying and tempering conditions must be rigidly controlled in order to measure the consistency of fats with a high degree of precision. A quick micropenetration method is described. J.L.H.

9. **Improvement Produced in the Stability of Lard by the Addition of Vegetable Oils.** R. W. RIEMENSCHNEIDER, J. TURER AND W. C. AULT, East. Region. Res. Lab., Philadelphia, Pa. Oil and Soap, *21,* No. 4: 98. April, 1944.

The stability of lard was increased by the addition of one to ten per cent of various vegetable oils. The increase in stability was largely attributed to the tocopherol content of the added oils. The tocopherol of the added oils appeared to act synergistically with certain other known antioxidants such as d-isoascorbyl palmitate and commercial lecithin. J.L.H.

10. **The Component Fatty Acids of Soybean Lecithin.** M. H. THORTON, C. S. JOHNSON AND M. A. EWAN, Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. Oil and Soap, *21,* No. 3: 85. March, 1944.

A preparation of lecithin from crude soybean phosphotides (Pangborn modification of the cadmium chloride method) contained 97 per cent lecithin

and 3 per cent cephalin. The per cent fatty acid composition of the preparation was as follows: Palmitic 15.77; stearic 6.30; oleic 12.98; linoleic 62.92; linolenic 2.02. J.L.H.

11. The Fractionation of Milk Fat from a Solvent at Low Temperatures. J. L. HENDERSON AND E. L. JACK, Univ. of Calif., Davis, Calif. Oil and Soap, *21,* No. 3: 90. March, 1944.

A procedure and apparatus for fractionating milk fat is described. The procedure consists of progressively freezing out, at lower temperatures, fractions of the fat from solvent (Skelly Solve A). Fractions were removed at −7, −13, −23 and −53° C. with the remaining filtrate taken as a final fraction. In physical appearance the fractions varied from a dry white powder to a reddish yellow oil; in melting point from 53° C. to −10.6° C.; in iodine number from 8.29 to 58.37; and in saponification equivalent from 262.8 to 235.2.

The fat fractions concentrate the glycerides of similar properties in separate fractions and makes them available for detailed study of composition, configuration and other properties. J.L.H.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

12. Scientific Advances Which Will Present New Competitive Products after the War. P. H. TRACY, Univ. of Ill., Urbana, Ill. Bul. *36,* No. 11: 155–161. June 20, 1944.

Use will be made in the post-war period of some of the advances in processing evaporated milk and powdered whole milk, which will render these products more acceptable to the consumer than in the past. Fresh condensed milk has certain advantages, and the frozen milk product is good although inconvenient and expensive to distribute. These new products and others should stimulate the fluid milk industry to improve its processing and marketing efficiency and will suggest new approaches to the customer. Quality of product and efficiency on the farm, in the plant and in distribution—with emphasis on the nutritional value of milk—will help the fluid milk industry to go forward in the post-war period without fear of other competitive foods. E.F.G.

13. The Effect of Storage on the Plate Counts of Milk and Whey Powders. CONSTANCE HIGGINBOTTOM, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scot. Jour. Dairy Res., *13,* No. 3: 324–328. 1944.

Plate counts were made on roller- and spray-process milk and whey powders after storage for periods of up to 12 months. The count was de-

termined in milk agar plates incubated for 3 days at 37° C. (98.6° F.) and 2 days at 55° C. (131° F.). Most, but not all, samples showed a decrease in count, but this decrease varied materially. The author suggests that all powders should normally be examined within 14 days of manufacture.

S.T.C.

14. Bacteriological Studies of Roller-Dried Milk Powders, Roller-Dried Buttermilk and of Roller- and Spray-Dried Whey. CONSTANCE HIGGINBOTTOM, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scot. Jour. Dairy Res., *13,* No. 3: 308–323. 1944.

Bacteriological studies were made of some 400 samples of dried milk products, usually within 1 to 3 days of manufacture. All samples were secured from plants in Great Britain. The mean values for the plate counts at 37° and 55° C. (98.6° and 131° F.) of the roller-dried products were all below 1,000 per gram as against ½ to 2½ million at 37° C. (98.6° F.) and 10,000 to 30,000 at 55° C. (131° F.) for the spray-dried products. Marked differences were observed in the plate counts of samples derived from different plants, some plants giving consistently low counts and others higher and more variable figures.

Cultures from plates made from the roller-dried products after incubation at 37° C. (98.6° F.) consisted chiefly of micrococci, sarcinae and spore-forming bacilli. The flora were markedly heat resistant. Cultures from plates made from the spray-dried whey after incubation at 37° C. (98.6° F.) consisted almost entirely of *Str. thermophilus* and *Str. faecalis.* Spore-forming bacilli were the principal flora on plates incubated at 55° C. (131° F.).

S.T.C.

DISEASE

15. Sulfonamide Preparations in Eliminating Mastitis. W. W. SWETT, R. R. GRAVES, P. C. UNDERWOOD, C. A. MATTHEWS, J. FRANK CONE, Bur. Dairy Indus., U. S. Dept. Agr., Milk Plant Monthly, *33,* No. 8: 42–50. Aug., 1944.

Experiments with the Beltsville, Maryland, dairy herd indicated that treatment of mastitis with sulfonamide preparations was very effective. By this treatment the infecting organisms were cleared from 80% of the quarters containing streptococci, 90% of the quarters containing staphylococci, 55% of the quarters containing *Pseudomonas aeruginosa,* and 83% of the quarters containing coliform bacteria. Approximately 94% of the quarters that responded to the treatment were cleared as a result of the first, second or third treatments. Unfavorable reactions to sulfonamide injections were almost non-existent.

G.M.T.

16. Reviews of the Progress of Dairy Science. Section E. Diseases of Dairy Cattle. P. S. WATTS, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scot. Jour. Dairy Res., *13,* No. 3: 340–370. 1944.

This is an excellent review of recent literature dealing with mastitis, contagious abortion and tuberculosis in dairy cattle. 239 references.

S.T.C.

FEEDS AND FEEDING

17. The Excretion of Borate by the Dairy Cow. E. C. OWEN, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scot. Jour. Dairy Res., *13,* No. 3: 243–248. 1944.

Two Ayrshire cows in mid-lactation were fed for 42 days on rations containing 18 to 20 grams of added borax. The consumption of borate in the control rations was about 2 grams per day. The daily excretion of borate during the experimental feeding period was 13 to 16 grams in the urine, 5 to 7 grams in the feces and 0.3 to 0.6 grams in the milk compared with figures of 1.5, 1.0 and 0.1 for the control period. There was no detectable retention of borate in the body, and the levels of borate excretion rapidly returned to normal after cessation of the experimental feeding. No adverse effects of the borate feeding were observed. The live weight of the cows remained normal, there was no diuretic effect, and the milk yield was well maintained. The borate content of the milk increased from 0.7 to over 3.0 ppm.

About 98 per cent of the ingested borate was excreted in the feces and urine. The borate content of the combined excreta was found to be about 500 ppm. Thus 5 tons of such excreta would contain about 5 pounds of borax. The author draws attention to the fact that there may be some risk of building up the boron content of soils to toxic levels for crops by the application of manure made from the extreta of animals fed on boronated rations.

S.T.C.

18. Protein Supplements in Dry Calf Starters for Calves on Limited Quantities of Milk. G. W. TRIMBERGER AND H. P. DAVIS, Nebr. Agr. Expt. Sta. Res. Bul. 134. 24 pages. Aug., 1944.

Fifty-one grade Holstein calves which had received whole milk for three weeks were fed to six months of age. Each group was allowed fifty pounds of skim milk, after which they were expected to obtain their nutrients from the protein supplement provided in liquid form, a dry calf starter, and green alfalfa hay (U.S. No. 1). The source of supplemental protein in the dry calf starter for each group was: Group 1, soybean meal; Group 2, tankage; Group 3, ground soybeans; Group 4, dried whey and blood meal; Group 5, dried skim milk powder; and Group 6, blood meal. A vitamin A and D concentrate first was fed to each calf in the liquid, and later it was thoroughly

mixed with the calf starter. The several calf starters were fed to four months of age, after which all the groups received the same basal grain mix.

The calves on the soybean meal (Group 1) and the ground soybeans (Group 3) lost weight and became very emaciated, but were allowed to remain on experiment as long as possible with the hope that they might recover from the temporary setback. However, when these calves became too weak, they were switched to a more desirable ration. Recovery was gradual, and the rate was apparently dependent upon the degree to which growth had been checked and also on the quantity of skim milk or whole milk supplied. When fed the additional milk, they eventually gained sufficiently to be normal in weight for their age. These two groups had the highest requirement for total digestible protein and total digestible nutrients per hundred pounds of gain, which indicates that it is poor economy to feed improper rations to young calves. It may be concluded that, under the conditions of this experiment, soybean meal and ground soybeans were not satisfactory as the principal source of protein in a dry calf starter for calves of approximately one month of age.

The four groups of calves receiving the animal protein in the dry calf starter, namely, tankage, dried whey and blood meal, dried skim milk, and blood meal, made normal growth. Average weights at six months of age per calf and the average daily gain per calf were as follows: Group 2 (tankage) 349.0 pounds, 1.40 pounds; Group 4 (whey and blood meal) 338.1 pounds, 1.34 pounds; Group 5 (dried skim milk) 361.4 pounds, 1.49 pounds; and Group 6 (blood meal) 352.5 pounds, 1.44 pounds.

The results were tested statistically by analysis of variance; and the differences in the gains between the calves on the soybean meal and soybeans, when compared to the gains in weight of the four groups receiving animal protein, were highly significant at eight weeks of age. The statistical test was also applied at the age of four months, when the feeding of the dry calf starter was discontinued, and again at six months at the completion of the experiment; and no significant differences were obtained among the six groups. This indicated that the two groups which originally were stunted on the poor rations had recovered on the more desirable rations. Except for the tankage group at eight weeks, there were no significant differences for the gains among the four groups of calves receiving the animal protein in their rations at any period of the experiment. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

19. **The Effects of Commercial Processing and of Storage on Some Nutritive Properties of Milk. Comparison of Full-Cream Sweetened Condensed Milk and of Evaporated Milk with the Original Raw Milk.** K. M. Henry, J. Houston, S. K. Kon and S. Y. Thomp-

SON, Natl. Inst. Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., *13*, No. 3: 329–339. 1944.

Evaporated milk and sweetened condensed milk were prepared simultaneously on several occasions, each time from a common bulk of raw milk. Biological value and digestibility of the proteins of the milks were measured on rats. Vitamin A and carotene, vitamin B_1, riboflavin and vitamin C were measured by chemical or physical methods.

The data show no loss of vitamin A, carotene or riboflavin in either of the processes. The mean loss of vitamin C was 10 per cent for the condensed and 60 per cent for the evaporated milk. The vitamin B_1 losses were 3.5 and 27 per cent respectively. The biological values and true digestibilities of the proteins of the raw, condensed and evaporated milks were respectively 85.6 and 94.2, 84.6 and 98.8, 84.1 and 93.7. The differences in biological value were not statistically significant, but the digestibility of the condensed milk was significantly higher than that of the other two milks.

The condensed milk was stored for one year at 15° C. (59° F.). There was no loss during that period of either vitamin A, carotene, or riboflavin. The vitamin C loss amounted to 42 per cent and that of vitamin B_1, 30 per cent. At 37° C. (98.6° F.) 64 per cent of the vitamin B_1 and 35 per cent of the riboflavin were destroyed in 6 months. The vitamin A and carotene were unchanged. S.T.C.

HERD MANAGEMENT

20. The Prospective Requirements for Dairy Cattle Replacements in Europe as a Result of the War. KARL BRANDT, Food Res. Inst., Stanford Univ., Calif. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36*, No. 15: 99–204. Sept., 1944.

The author's estimate is that in the summer of 1944 the European dairy herd was probably less than 10 per cent below the prewar level. Although there will need to be some replacement of dairy stock after the war, it is the opinion of this writer that such replacement requirements will be met almost exclusively with European stock and that shipments of breeding stock from the United States will not occur in substantial volume, if at all. Among the reasons for the above conclusions are:

1. Since the main reduction of herds in Europe has been due to lack of imported concentrates, an increase in these supplies will result in rapid build-up of herds, with perhaps prewar numbers reached in 2 or 3 years.

2. Many of the breeds of dairy cattle needed in Europe are not to be found in the United States.

3. Farmer organizations in the various dairy cow deficient countries will want to keep the profitable replacement business for their own people.

4. Good dairy cattle are not readily transported without considerable injury, especially during a long, rough sea voyage.

5. American dairy cattle today are probably the highest-priced cattle in the world.

It is suggested that a practical substitute for cattle importation into Europe would be the sending of grain and oil seeds to these countries. In this way they could quickly replenish their own herds. In the meantime, the United States could continue shipping milk products to Europe as long as needed. E.F.G.

21. Milk Cow Numbers and Herd Replacements by 1950. HOWARD R. TOLLEY, U. S. Dept. Agr., Washington, D. C. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 8: 99–113. June, 1944.

Two forecasts are made on the basis of assumption of two different conditions in 1950: The first is an expanding economy arising from conditions of full employment and economic collaboration, which would require 126.2 billion pounds of milk. The second is a depressed economy arising from conditions of relatively widespread unemployment as a result of failure to promote economic peace either at home or abroad, which would require 116.5 billion pounds of milk. Estimates of milk cow numbers and replacements may well be based upon an annual milk production ranging from 116 to 126 billion pounds. Over a considerable period of time, rate of decrease of milk cows on farms has been at about the same rate as increase of human population, namely, 1%. Ordinarily, each year about 19 out of every 100 milk cows 2 years or more of age are culled out and about 20 heifers 1 to 2 years of age retained for replacements. This and other normal practices followed till 1950 would result in 8% less heifers on farms in 1950 than on January 1, 1944.

At the 1% rate of increase, there would be 27.5 million producing milk cows by 1950, and these at the 1943 average production per cow would yield approximately 126.7 billion pounds of milk. This is about the amount which would be consumed under conditions of full employment.

Perhaps nutritional needs should be considered in this problem. If the family groups with incomes below $1,500 per year were brought to an adequate milk consumption basis, a production of 140 billion pounds would be required. If this goal is wholly impractical, then through a series of programs such as the school milk program, skim milk enrichment of bread, and some broad program for low-income groups, the amount of milk representing the difference between depression and full employment consumption could be utilized and the industry thereby stabilized in both good and bad times. There is considerable room for increasing milk production per cow so that this added production could be obtained with no great increase in milk cow numbers. E.F.G.

ICE CREAM

22. Maintaining Ice Cream Quality During Wartime. J. HOFFMAN ERB, Borden Co., Columbus, Ohio. Ice Cream Field, *44,* No. 4: 32. Oct., 1944.

According to the author, solids in one form or another have always been considered important in securing the desired body and texture. He claims that present experience shows that the manner in which the dispersed particles are suspended in ice cream is also important.

Effective homogenization is essential to assure proper fat dispersion, and it is stated that one or two per cent fresh egg yolk aids materially in securing small air cells that are important to good body. Proper freezing and hardening are stressed and the fact pointed out that low-solids mixes develop coarseness in the dealer's cabinet much faster than higher-solids ice cream. The view is expressed that the "ideal" stabilizer should have both good water-binding capacity and good emulsifying powers.

The opinion of the author is that corn sweeteners perform a worthwhile function and their use is likely to be continued, but he states that cereal products are of doubtful advantage as possible replacement agents for milk-solids-not-fat. He encourages attractive and pleasing flavor combinations and stresses the need for sanitation. W.C.C.

23. Studies on Certain Varieties of Fruit in Ice Cream. G. H. WILSTER, E. H. WEIGAND AND THOMAS ONSDORFF, Oreg. State Agr. Expt. Sta., Corvallis, Oreg. Ice Cream Field, *44,* No. 4: 30. Oct., 1944.

The authors list the following reasons for fruit being used in ice cream: (1) It adds to the flavor of ice cream. (2) It gives pleasing color to the ice cream. (3) It greatly increases varieties that can be made. (4) It adds important nutritional constituents.

They stress the advantages of using pure fruits in liberal amounts in ice cream and give the results of studies carried out at the Oregon Agricultural Experiment Station, pointing out that certain parts of the work were in cooperation with the Commodity Processing Division of the Western Regional Research Laboratory, United States Department of Agriculture, Albany, California.

The authors considered the following fruits in their studies: raspberry, black raspberry, blackberry, strawberry, Blue Damson plum, and apricot. The mixes to which the fruit was added were approximately 12.5 per cent fat and 37 per cent total solids. Sucrose, dextrose and corn sirup were used as sweeteners, and fresh whole egg was also used in the mix. The ice cream was frozen in a fifty-quart batch freezer.

The following conclusions are drawn as a result of this work:

(1) Berry purees, when used in ice cream, resulted in excellent flavor and attractive color. (2) The addition of 15 per cent strawberry and plum

purees gave satisfactory results, whereas 15–20 per cent raspberry puree and 20 per cent black raspberry puree were required. (3) For marbled ice cream the addition of 15 per cent apricot puree was very satisfactory.

W.C.C.

24. Frozen Foods and the Locker Plant. DONALD K. TRESSLER, Mgr., General Electric Consumers Institute, Bridgeport, Conn. Ice Cream Field, *44,* No. 4: 38. Oct., 1944.

Although the importance of frozen foods was known before the end of the 19th century, according to the author it was not until about 1930, when low storage temperatures became generally recognized, that frozen foods became popular. Their use has increased markedly since that time due largely to improved quality and especially to increased facilities for storage and distribution. It is stated that many companies are now considering the house-to-house distribution of frozen foods.

It is predicted that the locker plant should play an important rôle in the future frozen-food industry since it will supply increased storage facilities and enable the plant to sell desired items to its patrons. Also, if conditions are right, local products may be processed and frozen. The author warns against overoptimism in the case of the last-mentioned function because considerable technical knowledge is necessary to process successfully and freeze such foods. W.C.C.

25. How To Merchandise Frosted Foods. CARL SEABERGH, Pres., Frostar Frozen Food Centers, Inc., New York. Ice Cream Field, *44,* No. 4: 52. Oct., 1944.

In order for a dealer successfully to sell frozen foods, "he must induce his sales people to talk about them to the customers," it is stated. Using posters and display materials helps, but frozen foods are sealed in packages and stored where it is difficult to display them to advantage.

The author points out in some detail things the sales people should say to customers about frozen foods. He stresses the importance of being familiar with the products for sale, the manner in which they are processed, the locality in which they are grown, and best ways of cooking and serving them.

W.C.C.

MILK

26. Advantages and Disadvantages of Two- and Four-Quart Containers. W. A. JIMISON, Borden Co., Chicago Milk Div., Chicago, Ill. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 12: 167–172. June, 1944.

A brief history of multiple-quart containers in the Chicago market is given. The 4-quart container has been handled by outlying stores. It has

been less acceptable to the consumer than the 2-quart bottle, however, because it is too large for the present-day refrigerator. Also, with every-other-day delivery, the necessity for storage of two 1-gallon containers creates a further problem. There was not enough difference in the price of the 2-quart and 4-quart containers to make that a factor in consumer preference. The 2-quart bottle was 32¢ in both Chicago and suburban areas, and the gallon 62¢ in the former and 59¢ in the latter. However, if there were a substantial decrease in family income, the larger container might become more popular. Both milk dealers and container manufacturers should work closely with refrigerator manufacturers in the development of more satisfactory post-war milk containers. E.F.G.

27. **Our Experience with EOD Delivery.** ROBERT A. BURNS, Whiting Milk Co., Boston, Mass.; E. K. SCHWARTZ, Abbotts Dairies, Inc., Philadelphia, Pa.; T. KLINE HAMILTON, Diamond Milk Products, Columbus, Ohio. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36*, No. 9: 119–133. June, 1944.

Experience with EOD delivery indicates advantages in efficient and economical operation, with few serious disadvantages.

The main disadvantages were: occasional wholesale accounts on retail routes, such as offices or apartment houses; and lessened sales initiative because of high earnings due to route consolidation. However, the advantages so greatly outweigh the objections to EOD delivery that it will be a "must" in post-war planning.

The experience of the Diamond Milk Products Company in combining 6-day with the later EOD delivery, resulting in regular 3-day-per-week deliveries, indicated that the following advantages of both systems could be obtained: mileage and gasoline cut in two; man hours cut by more than half; number of trucks and mileage per vehicle cut almost in two; units delivered per man hour, more than 2½ times; daily units delivered per mile, more than 3 times; hours worked per man reduced one-fifth. The question is, "Can we afford not to retain wartime efficiencies in the post-war period?" The best competitive weapon that any business can have is low operating cost. The slogan for the post-war period might be: "More milk to more people at less cost." E.F.G.

28. **The Connecticut Three-Point Laboratory Program as an Aid to Control of Pasteurized Milk.** FRIEND LEE MICKLE, Conn. State Dept. of Health, Hartford, Conn. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36*, No. 14: 187–193. July, 1944.

See Abstract 48, JOUR. DAIRY SCI., *27*, No. 2: A23. 1944. E.F.G.

29. Recommendations for the Standardization of the Method of Determining the U.S.P.H.S. Index of Homogenization of Milk. RANDALL WHITAKER AND LUTHER D. HILLIER, Natl. Dairy Prod. Corp., Baltimore, Md. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 6: 67–81. May, 1944.

The 1943 U. S. Public Health definition of homogenized milk is reasonable. This specifies that there shall be not over 10 per cent difference in butterfat between the upper 100 ml. and the remainder of a quart bottle after 48-hour storage. A number of variables possible under the U.S.P.H. definition have been studied, and a laboratory technique which gives good results is outlined as follows:

Duplicate cooled standard quart bottles from the line are held at 40–45° F. without agitation for 48 hours. Eighty per cent of the fat which collects at the top is in the upper 10 ml. and all in the upper 30 ml. A method for pouring the top 100 ml. of milk is suggested. This is to place the thumb at about the 80-ml. mark on a 100-ml. cylinder and pour the 100 ml. quickly in 2 to 3 seconds. This method is somewhat less accurate and gives a higher result than the suction method but is much more practical. Average homogenized market milk will have an average U.S.P.H. index of 6–9 per cent with this sampling method, using a good modified Babcock method for fat determination. E.F.G.

30. Human Relations Is Management's Job. R. B. STOLTZ, Ohio State Univ., Columbus, Ohio. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 5: 55–64. May, 1944.

Twelve qualifications for the successful manager are listed. Each is explained or illustrated.

1. Like people and be sympathetic to their problems. 2. Create a democratic organization. 3. Do not take anything for granted. 4. Do not underestimate the individual's mental capacity. 5. Do not overestimate the individual's mental capacity. 6. Keep each worker loaded to his mental and physical capacity. 7. Use group pressure (influence of the majority in aligning the uncooperative minority). 8. Create respect. 9. Inform employee in advance regarding changes. 10. Use incentives (there are many others than money). 11. Recognize accomplishments. 12. Consider publications (information, recognition or employee self-expression by means of house-organ or other means). E.F.G.

31. Train Salesmen Now for Post-War Selling. E. R. QUACKENBUSH, Dairyman's Ohio Farmers Milk Co., Cleveland, Ohio. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 3: 28–38. May, 1944.

Customer good will is a factor which will be more important than ever

after the war. Courtesy in explaining the present unavoidable inconvenience will tend to build up good will.

Home delivery of milk will always call for some basic training in the fundamentals of accurate, courteous and dependable service. For new men taken on under war conditions, this training should be started at the earliest possible moment, using the "Balanced Job Manual" supplemented with specific information about your own company's products and policies. E.F.G.

32. Future Operations in the Milk Industry. P. H. TRACY, Univ. of Ill., Urbana, Ill. Milk Plant Monthly, *33,* No. 7: 36–40. July, 1944.

The author emphasizes that in our present complicated society the philosophy of "the greatest good for the greatest number" involves the regulation of business for the best interest of the public. In the transition from war to peace, depleted supplies and commodities must be made available. Rationing may be necessary for some items. Due to the extensive use that will be made of concentrated forms of dairy products such as cheese, butter, powder and evaporated milk by military and lend-lease agencies, an appreciable surplus of these products will not be realized for some time. If purchasing power is maintained, the consumer will respond to the advice of nutritionists to increase milk consumption.

As purchasing power shrinks and competition of other basic foods becomes keener, those in the fluid milk industry will find it increasingly difficult to maintain their sales. Greater efficiency must be established if the milk plant is to survive. Nine factors contributing to high plant costs are listed. The author presents an excellent list consisting of 31 conditions which will contribute to greater efficiency of the milk plant. This list is in itself a challenge to any milk plant operator. G.M.T.

33. What Is the Future of Retail Milk Distribution? A. R. STEVENS, Sheffield Farms Co., Inc., New York, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 1: 3–12. May, 1943.

Prior to the war a certain amount of change from home to store delivery —retail to wholesale—had taken place in recent years. War conditions have at least temporarily reversed this trend.

Trends of population indicate that adult milk consumption will be more important in the future. The route man, his ability and qualifications, will call for more careful scrutiny in the post-war period since the successful retailing of milk depends mainly upon the route personnel. Service and customer relations will be of vital importance.

Milk has too long been associated with infants. Only 8.6% of the white population is under 5 years of age while 63% is over 20 years, and the latter group should receive more attention. E.F.G.

34. Practical Suggestions to the Industry to Meet Quality Problems on the Farm and in the Plant under War Conditions. WALTER D. TIEDEMAN, N. Y. State Dept. of Health, Albany, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36*, No. 2: 15–21. May, 1944.

Four points are listed which cannot be safely slighted even under war conditions. They are as follows:

1. Skilled inspection of milk in the receiving room. 2. Proper operation of the pasteurizing equipment. 3. Efficient washing and sterilization of equipment and containers. 4. Inspection of filled bottles.

Also, it would be of great value if competent field service were available. However, if the four operations above are performed satisfactorily, safety and quality will be under control. E.F.G.

35. Fieldman's Share in Producing Quality Milk. X. Straining Milk—and Filter Discs. N. C. ANGEVINE. Milk Plant Monthly, *33*, No. 7: 22, 23, 31. July, 1944.

Foreign material, whether soluble or insoluble, has no place in clean milk production. Much of the soluble matter can be removed at once through immediate and proper straining of milk on the farm. Examination of spent filter discs after each milking will show the producer the amount and kind of dirt getting into the milk. Proper methods, which include care of animals, barn, milk house, milking equipment, and handling of milk, are necessary to keep foreign material in milk to a minimum. In filtering milk, the disc must fit the strainer. A slow filtering disc usually produces unsatisfactory results. Hence, the producer is forced to clean and replace the filter disc or to jostle the strainer on top of the can, thus destroying the filter disc and permitting the milk and accumulated sediment to flow into the milk. A good filter disc should have efficiency, speed, wash-resistance, and capacity. G.M.T.

36. Fieldmen's Share in Producing Quality Milk. XI. The Sediment Test. RALPH E. IRWIN, Dir., Bur. Milk Sanit., Pa. Dept. Health. Milk Plant Monthly, *33*, No. 8: 58. Aug., 1944.

The author describes briefly sediment testing of milk and raises the question as to who should remove sediment from milk—the producer or the distributor. Properly strained milk may appear clean upon subsequent sediment tests. While the sediment test was based on a pint sample of milk, the "off-the-bottom" tester was effective in showing the producer just how much foreign material there was in the milk. If straining is necessary, a single service strainer should be used. Probably municipal or state officials should promote the production of milk that may be delivered to the distributor without straining. G.M.T.

37. Fieldman's Share in Producing Quality Milk. XII. Aspects of Filtering Milk on Farms. K. G. WECKEL, Dept. Dairy Indus., Univ. of Wis. Milk Plant Monthly, *33,* No. 9: 42, 43. Sept., 1944.

Since milk first elaborated from the udder is entirely free of extraneous material, there are only two plausible reasons for the use of the strainer at the farm, namely, to remove promptly any extraneous material, and to provide the milk producer with a first-hand sediment test of the milk. Four important sources of sediment are noted: (1) dirty flanks and udders, (2) wet and dirty hand milking, (3) dirt in the utensils and cans, (4) dirt and dust gathered from the air during handling, either in the barn, milk house or truck. Unless the purpose and function of milk strainers is properly understood by field men and producers alike, emphasis may be placed on taking extraneous material out of milk rather than producing milk under conditions which will keep the milk free from extraneous material. The strainer, at best, must be considered only an adjunct to, and not the principal part of, the process of producing clean milk. G.M.T.

38. Fieldman's Share in Producing Quality Milk. XIII. Is Aeration of Milk Necessary? G. M. TROUT, Mich. Agr. Expt. Sta. Milk Plant Monthly, *33,* No. 10: 22–25. Oct., 1944.

Early reports concerning the aeration of milk indicated that this process was very beneficial in overcoming flavor defects of milk. However, research studies showed that aeration was not 100 per cent effective, although the off-flavor was less objectionable after the process.

The value of aeration is often over emphasized. Aeration of milk in the milk plant has been detrimental to stability of flavor of the bottled product. The process, at its best, is an attempt to remove the odor which got into the milk through faulty milk production rather than avoiding the odor in the first place.

Improvements in milk production during the past score of years have minimized the importance of aeration of milk. These improvements have resulted, in a large part, from: (1) investigations of the causes and prevention of off-flavors in milk due primarily to feeds; (2) construction of better ventilated dairy stables; (3) changed practices of feeding in respect to time of milking; (4) development of milk-cooling units; (5) universal tendency toward prompt handling and cooling of milk; (6) introduction of new cleaning and sterilizing compounds; and (7) closer inspection of milk supplies. G.M.T.

39. Fieldman's Share in Producing Quality Milk. XIV. The Operation and Interpretation of the Sediment Test. JOHN TAYLOR, Chief, Bur. Dairy Prod., Ind. State Board of Health. Milk Plant Monthly, *33,* No. 8: 58–59. Aug., 1944.

The sediment test for milk was believed to be of little value unless

used with other tests in a complete quality program. Its use should accompany a bacteria test such as the methylene blue or the Breed microscopic count, together with a study of the dairy production facilities on the farm. Nine principles set forth in the Indiana program for the improvement of milk in plants manufacturing cheese, evaporated milk and milk powder are given. The program consisted only of flavor and sediment tests. The cans of milk rejected over a five-year period because of sediment test alone indicate that the quality of the milk was not improved. A satisfactory program of milk sanitation must begin on the farm. G.M.T.

40. The Freezing Point of Milk. II. The Influence of Various Factors and Their Bearing on the Detection of Added Water. R. ASCHAFFENBURG AND B. C. VEINOGLOU, Natl. Inst. Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., *13,* No. 3: 267–280. 1944.

The freezing point depression of the milk from all the Shorthorn cows in the Institute herd (30 to 40 cows) was determined at fortnightly intervals over a period of 16 months (17 April, 1941, to 19 August, 1942). The milk from a smaller number of animals was sampled at about 3-day intervals.

The general mean for the freezing point depression was found to be 0.548° C. (0.986° F.), and only two values deviated by more than 4.5 per cent from this mean. Significant seasonal trends were observed, which indicate that milk with somewhat smaller freezing point depression may be produced during the spring.

The freezing point depression of evening milk was generally higher than that of morning milk except during the winter period when the cows were stabled at night, when the depression values of the morning milk were the higher. Environmental temperatures may thus be a factor.

No evidence was found of any influence of age or stage of lactation of a cow on the depression values. No relation was detected between milk yield and only a low positive correlation between the solids-not-fat content of milk and its freezing point depression.

The data show that the limiting freezing point depression value of 0.530° C. (0.954° F.), commonly used for the differentiation of genuine milk from adulterated milk, is well chosen. S.T.C.

41. The Freezing Point of Milk. III. A Survey of Producer's Milk as Delivered to Creameries in England and Wales. R. ASCHAFFENBURG, P. L. TEMPLE AND B. C. VEINOGLOU, Natl. Inst. Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., *13,* No. 3: 281–286. 1944.

The freezing point depression was determined on some 4,000 samples of milk as delivered by individual producers to creameries in England and

Wales. About 6 out of every 100 samples were found to contain over 10 per cent or more of extraneous water. S.T.C.

42. The Occurrence in Winter of Milk with a Low Content of Solids-Not-Fat. S. J. ROWLAND, Natl. Inst. Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., *13,* No. 3: 261–266. 1944.

Data are presented which show that much of the milk produced in certain areas during the winters of 1942 and 1943 was below the legal minimum of 8.50 per cent solids-not-fat. Samples of milk from 36 representative producers at one creamery and 72 at another were analyzed at semi-monthly intervals from January through June of 1943. The solids-not-fat content of the milk samples was persistently low until about the middle of April, but rose to normal levels in May. The solids-not-fat content of the milk of each producer was without exception greater in the May–June period than in the winter period, the mean increase in solids-not-fat percentage being 0.45. The freezing point depression of the milk was normal.

The author suggests that the low level of solids-not-fat was due to a low plane of nutrition of the cow. S.T.C.

43. Producing Clean Milk. G. H. WILSTER AND H. P. EWALT. Oreg. State Col. Ext. Bul. 630. 24 pages, illus. Jan., 1944.

A detailed, popular treatment of the subject, profusely and attractively illustrated. A summary of 24 important points in the production of clean milk is appended. J.G.A.

MISCELLANEOUS

44. Probable Trends in Post-War Dairy Equipment. ROBERTS EVERETT, Dairy Industries Supply Assoc., Inc. Internatl. Assoc. Milk Dealers, Assoc. Bul., *36,* No. 7: 87–96. June, 1944.

It is thought by dairy machinery and supply manufacturers that in the initial post-war period machinery will be very similar to the pre-war models. It will take time to assemble engineering talent and get new models past the experimental stage.

Later, the skill which the worker has acquired in war work will be utilized in making better machines, built to closer tolerances, with resultant better performance. Also, experience in mass production of war items will result in greater workman efficiency and greater value to the customer. In the user's hands the machine will show low-cost operation, maximum efficiency and minimum labor requirements. Prospective high labor and tax costs make this very important, and only the best equipment will be satisfactory. Better utilization of stainless steel will prevail, and the finished

product will have more "eye-appeal." On outside surfaces, where stainless steel is not used, metallic and plastic coatings of various kinds will to a large extent replace paint.

Large plants will be interested in synchronizing equipment for continuous operation with automatic control. High-temperature-short-time pasteurization, with its regeneration savings, fits into this picture. Post-war boilers with 10 per cent higher efficiencies than pre-war will be available. In the receiving room improved mixing for fat sampling, and perhaps automatic dumping of milk from cans to weigh tank, will be available. The continuous manufacture of butter will open up possibilities of greater uniformity.

Looking farther ahead, it will likely be necessary that some type of "raw material" control be practiced to aid the farmer in reducing costs. Also, there should be some way of reducing the price of fluid milk as compared with the cost of milk in some other forms, and country to city transportation cost should be cut. Improved plant efficiency may be attained with application of electronics to measurements of tests, detection of dirt in bottles, etc. Maintenance of product quality will be especially important.

Better methods of maintaining and routing dairy vehicles have been learned. These vehicles will be lighter due to use of materials such as magnesium and aluminum molded plywood. Automatic and semi-automatic controls will be another factor in better performance of post-war vehicles. There will be no streamlining because this would not allow as efficient use of space.

Changes wanted and needed by the dairy industry will influence the manufacturer to do his best to search for practical ways to fill the requirements. E.F.G.

45. Norelac—A Proposed New Synthetic Coating Material. J. C. COWAN, A. J. LEWIS AND L. B. FALKENBRING, North. Region. Res. Lab., Peoria, Ill. Oil and Soap, *21,* No. 4: 101. April, 1944.

A new synthetic coating material, Norelac (the ethylene diamine polymer of polymeric fat acids) is described and some of its properties indicated. It appears to be an effective protective coating for wood, metal and other materials and an adhesive for heat-sealing and laminating of paper, cellophane, glassine and other packaging materials. J.L.H.

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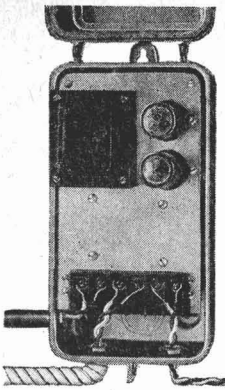

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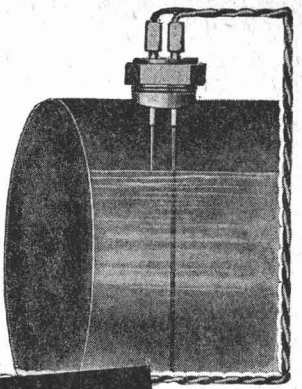

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