TABLE VII
Comparable Acceptability Tests for Global Spreads

Spread	Acceptability by ranking			"Getaway" score
	1st place	2nd place	3rd place	
Army margarine	0	2	2	3.1
Global spread	1	12	13	1.4
Margarine	14	1	0	0.7
	Acceptability by scoring			
Army margarine		3.3	....	
Global spread A		1.2	1.3	
Global spread B		1.4	1.5	
Margarine		....	1.1	
Significance		high	none	

difference is shown between the Army margarine and the global spread, but a significant difference does not exist between global spreads and regular margarine. The acceptability shown by ranking and the getaway score are indicative of the quality of the product.

Extensive work on flavor and flavor stability was not undertaken until compositional studies and processing conditions had reached a favorable position in the course of developmental work. Synthetic flavors have not been entirely satisfactory, and present compositions include 0.04% of Butr-trate 2XW, a product described as a special-distilled, butter-flavor concentrate. Taste-panel studies indicate that a very minimum of flavor is desirable. This agrees with the trend that less and less flavor is desired in the staple food items which are consumed in large quantities in the daily diet. Flavor, flavor stability, and oxidative stability of the global spreads under various storage conditions are the current problems under investigation.

Summary

Global spread of improved plasticity characteristics has now been produced on a pilot-plant scale. Chilling equipment similar to that used in margarine production was slightly modified; and because of the scale of operation, throughput was reduced to one-fifth the rated capacity.

Global spread produced by the formulation previously described suffered the defect of becoming progressively firmer when stored, consequently both the "mouth feel" and general acceptability were impaired. This deficiency was largely overcome by tempering at 112°F.(44°C.) for 24 hrs. in the presence of 0.2% oil-free soybean phosphatides which softens the spread and slows the hardening process which occurs during prolonged storage at elevated temperatures. Spreads currently being produced compare favorably with commercial margarines in general acceptability when evaluated at room temperature and are superior in plasticity characteristics when compared at low and high temperatures.

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Measurement of Oxidation in Dried Milk Products With Thiobarbituric Acid[1,2]

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THE need for objective tests for measuring flavor deterioration in food products and the use of 2-thiobarbituric acid as a reagent for such methods were discussed in a previous paper (8).

The chemical test used most often for measuring oxidative changes in fat-containing food products is the determination of peroxides. The absolute peroxide value may sometimes have no significance in relation to flavor score because of the simultaneous formation and decomposition of the peroxides (2). A peroxide value of approximately 2.0 m.e./kg. of fat is normally considered to be low for most fats, and they are generally free of oxidative off-flavors at this level of peroxide value. The reverted flavor of soybean oil is detectable at a peroxide value of 2.5 (4). Milk powder also undergoes serious flavor changes of an oxidized nature by the time the fat reaches a peroxide value of 2.0 (6).

Patton and Kurtz (7) and Dunkley and Jennings (3) have ably reviewed the literature leading to the application of 2-thiobarbituric acid as an agent for the detection of oxidized fat in dairy products. Procedures have been described for the application of the thiobarbituric acid (TBA) test to fluid milk (3), butterfat (7, 8), and to cheese, butter, and milk powder (1).

The procedure to be described offers certain advantages over previously published methods. Freedom from the physical interference of proteins as well as freedom from turbidity in the test solution is accomplished by steam distillation of an acidified slurry of milk powder. Less complicated apparatus than that of the only other published procedure for milk powder (1) is required. Furthermore no organic solvent, such as isoamyl alcohol or pyridine, is neces-

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[1] Presented at the 27th Fall Meeting of the American Oil Chemists' Society, Chicago, Ill., Nov. 2-5, 1953.

[2] This paper reports research undertaken by the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 452 in the series of papers approved for application. The views and conclusions contained in this paper are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

sary for the separation of the developed color from the proteins prior to measurement (3).

Experimental

The reagents, apparatus, and details of the procedure are as follows:

Reagents

a) Hydrochloric acid, C. P. grade.
b) Glacial acetic acid, C. P. grade.
c) 2-Thiobarbituric acid (TBA), reagent grade. Material should be a white powder.
d) TBA reagent. Prepared by adding 0.67 g. of TBA to approximately 75 ml. of glacial acetic acid in a 100-ml. beaker. Heat on a steam bath for several minutes and add 2 ml. of concentrated hydrochloric acid to facilitate solution. Transfer solution to a 100-ml. volumetric flask, cool, and dilute to the mark with glacial acetic acid.
e) 3 N HCl. Prepared by diluting 1 vol. concentrated hydrochloric acid (12 N) with 3 vol. distilled water.

Apparatus

a) *Steam distillation assembly. Made up of 800-ml. Kjeldahl flasks. Iowa State distilling heads and condensers. May be operated in multiple unit setups by passing steam from steam line through trap and manifold.*
b) Boiling water bath.
c) Spectrophotometer. For evaluation of stored and fresh samples, the same spectrophotometer and cuvettes obviously should be used.

Procedure

Weigh 15.0 g. milk powder into a clean, dry Kjeldahl flask, add 75 ml. of distilled water and swirl to wet and disperse the powder. Add 7.0 ml. of 3 N HCl and swirl the flask to mix the contents.[3] Daub a small quantity of Antifoam A (Dow Corning Corporation)[4] onto the lower end of the steam inlet tube to prevent foaming. Assemble apparatus and pass steam through at such a rate that 10 min. are required to collect 50 ml. of distillate in a 50-ml. graduated cylinder. The start of timing shall begin at the moment the steam comes into contact with the milk powder.

Mix the distillate, pipette 20 ml. into a 25- x 200-mm. test tube, and add, by pipette, 2.0 ml. of TBA reagent. Mix the contents of test tube and immerse it in the boiling water bath *for exactly 35 minutes. Cool to room temperature and transfer* a portion to a cuvette. Read the absorbancy of the pink color against distilled water at a wave-length of 530 mμ. Correct *the reading* by subtracting a reagent blank. The results are reported as absorbancy, and duplicates should agree within 0.01 absorbancy unit.

Procedure Notes

Acidification of the Reconstituted Powder. Without acidification of the milk powder slurry, no TBA reactive material was liberated by steam distillation. The pH range for maximum liberation was found to be 1.0 to 1.5 for both a badly oxidized powder and a powder rated borderline in acceptability by an expert panel. Seven milliliters of 3 N hydrochloric acid produced a pH within this range for 15.0 g. of whole milk powder. Figure 1 shows the effect of variation in the pH of the milk powder slurry upon the yield of TBA reactive material.

Volume of Distillate. In common with previously published TBA procedures (1, 3, 7), this procedure is empirical. In a check upon the rate of distillation it was found that TBA reactive material decreased progressively in the second and third 50-ml. fractions of distillate from a badly oxidized sample. Since reproducibility was found to be good with a 50-ml. fraction, the procedure was standardized with this volume.

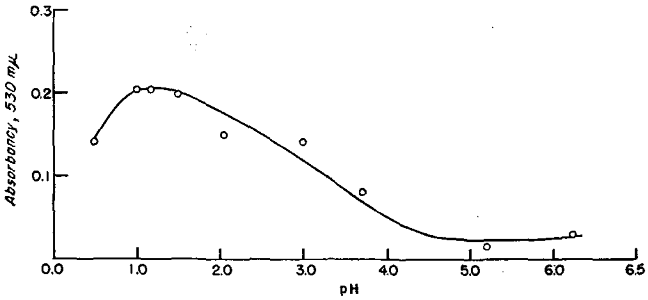

FIG. 1. Effect of pH of milk powder slurry on intensity of TBA color.

Distillation Time. Distillation time is counted from the moment the steam comes into contact with the powder. The variation in TBA values of a badly oxidized sample when distillation periods of 9, 16, and 21 minutes were used was not considered significant. For this apparatus a 10-min. interval was found to be satisfactory.

Effect of Variation in pH of the Combined Distillate-TBA Solution Upon Color Development. Variation of the pH of the combined distillate-TBA solution between the limits of 0.6 and 2.2 had little effect upon absorbancy values.

Ratio of Volume of TBA Reagent to Volume of Distillate. The TBA value increased as the volume of reagent added to 20 ml. of distillate was increased from 0.5 to 2.0 ml. Additional reagent up to 5.0 ml. caused no further change. Inasmuch as this experiment was performed with an extremely oxidized sample, 2.0 ml. of reagent are sufficient to insure an excess for any sample in a lower stage of oxidation.

Heating Time for Color Development of the Combined Distillate-TBA Solution. The curve of the TBA values *versus* heating time of the combined distillate-TBA solution is given in Figure 2. A heating period of 35 min. was adopted.

Color Measurement. The absorption spectrum of the color produced from an oxidized sample of whole milk powder is shown in Figure 3. The single maximum at 530 mμ (Beckman DU Spectrophotometer) agrees closely with that shown for oxidized milk fat (7) and oxidized milk (3). A second sample of badly oxidized milk powder showed an identical absorption.

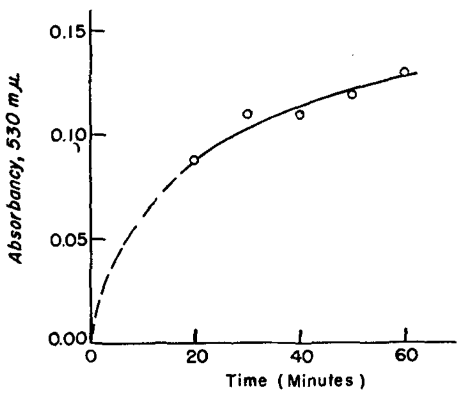

FIG. 2. Effect of time of heating distillate with TBA at 100°C. upon intensity of color.

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[3] If desired, the quantities of sample, water, and acid may be increased as much as threefold. The amount of volatile material recovered increased as the weight of sample was increased, but it was not always proportional to the sample size.

[4] The mention of this product does not imply that it is endorsed or recommended by the Department of Defense over other similar products not mentioned.

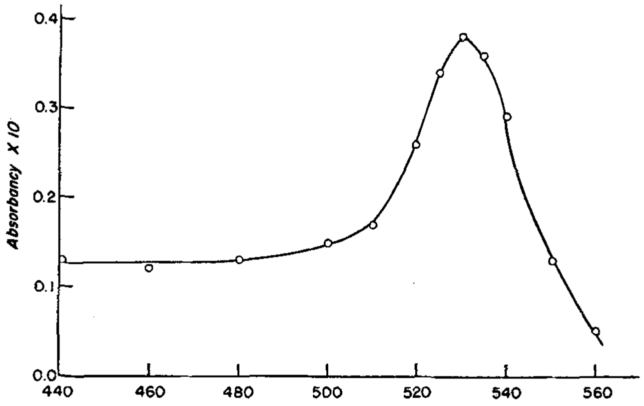

FIG. 3. Absorption spectrum of the TBA color of the steam-distillate obtained from oxidized dry whole milk.

Precision. Ten replicate determinations were made upon a sample of whole milk powder having a TBA value of 0.05. The arithmetic mean was found to be 0.052, the standard deviation 0.006, and the standard error of the mean 0.002. The 95% confidence limits of the mean, calculated from the standard error of the mean, were 0.048 to 0.056, a range of 0.008. It appears advisable to report the result of a single determination only to the second decimal place.

Results

Association of TBA Values With Sensory Evaluation of Milk Powders. The procedure was developed with and applied almost entirely to dry whole milk samples containing approximately 26% fat. Table I contains data from samples in low and high stages of oxidation. These samples were evaluated sensorily by an expert panel selected from laboratory personnel. No numerical scores were assigned.

TABLE I

Comparison of TBA Values and Sensory Evaluation of Milk Powders

Sample	TBA values[a, b]	Sensory evaluation
Whole milk		
1	0.003	Good
2	0.021	Old, stale, but not rancid
3	0.024	No. 3, was rated superior to No. 4
4	0.036	
5	0.188	Badly oxidized
6	0.256	Badly oxidized
Modified sweetened milk[c]		
7	0.032	No. 7 was rated superior to No. 8
8	0.090	

[a] All values corrected by subtracting reagent blank of 0.014.
[b] Average values.
[c] Fat content, 12.5%.

Mixture of Badly Oxidized Sample With Non-Oxidized Sample. A non-oxidized sample (No. 1 of Table I) was diluted with increasing quantities of a badly oxidized sample, and the reconstituted milk samples were then evaluated by 21 judges. Table II shows the samples used in this experiment.

Samples 1, 3, and 5 were presented to each subject with the statement that they have minimum, moderate, and maximum amounts, respectively, of off-flavor. The series of five samples was then coded and presented to the panel twice in a random order with instructions to judge the samples from 1 to 5 on intensity of off-flavor. It appeared from the observations of the judges that the more oxidized samples were sufficiently strong to introduce some confusion due especially to a lingering after-taste. Examination of the data in Table II disclosed that they fitted the phi-log-gamma hypothesis (5), thus suggesting that the TBA measurement has validity in terms of flavor.

TABLE II

TBA Values and Sensory Scores of Whole Milk Powder Samples (Prepared by mixing non-oxidized powder with oxidized powder)

Sample	Oxidized powder in sample	TBA values[a]	Intensity of off-flavor
	%		
1	0.0	0.012	1.26
2	12.5	0.042	2.50
3	25.0	0.071	2.83
4	37.5	0.089	2.83
5	50.0	0.116	3.81

[a] Average of 3 determinations. All valeus corrected by subtracting reagent blank of 0.014.

Evaluation of Whole Milk Powder in a Low Stage of Oxidation. Whole milk powders from three manufacturers, nitrogen-packed at time of processing, were stored at 100°F.(38°C.) for 6 months in order to evaluate their stability. They were scored initially and at the end of the storage period by a 4-member panel, which rated them by means of a scale ranging from 1 to 9, with 5 representing the lower limit of acceptability. The TBA values and flavor scores for these samples are shown in Table III. The sample of borderline acceptability (No. 4) had the highest TBA value.

Effect of Oxygen Concentration and Temperature of Storage on TBA Values of Dry Whole Milk. Table IV shows the absorbancy values which were obtained on samples of dry whole milk stored for 3 months at 72° and 100°F.(22° and 38°C.).

TABLE III

Correlation of TBA Values and Flavor Scores of Whole Milk Powders (Nitrogen-packed and stored 6 months at 100°F.(38°C.)

Sample	TBA values[a, b]	Flavor score[c]
1	0.023	6.7
2	0.023	6.6
3	0.033	6.5
4	0.048	5.5

[a] All TBA values were corrected by subtracting a reagent blank of 0.014.
[b] The TBA value of good milk powder was 0.014.
[c] The flavor score of good milk powder was 7.2.

TABLE IV

Effect of Oxygen Concentration and Temperature of Storage on TBA Values of Dry Whole Milk

Sample[a]	TBA values[b]		
	Initial	Three months	
		72°F. (22°C.)	100°F. (38°C.)
Air-packed	0.011	0.042	0.058
Nitrogen-packed	0.011	0.020	0.026

[a] In sealed tin cans.
[b] All values corrected by subtracting a reagent blank of 0.014.

Inasmuch as the values are directly related to the time and temperature of storage and to the concentration of oxygen, the TBA reaction is a measure of the degree of oxidation of whole milk powder.

Summary

Steam-distillation of dry whole milk was found to be an effective means of separating the products of fat oxidation preparatory to reacting them with 2-thiobarbituric acid (TBA). The milk powder samples were reconstituted and acidified prior to steam distillation. A fraction of the distillate was reacted with the reagent to produce the red-colored product, which was then estimated spectrophotometrically at 530 $m\mu$. Factors which influence the sensitivity and reliabillity of the method were investigated. The method is rapid and reproducible, and the test solutions are free of turbidity. The reaction is a measure of the oxidation of dry whole milk, and when it was applied to samples from storage, the results had validity in terms of flavor.

Acknowledgment

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Some Investigations with Long Chain Compounds

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THE Organic Chemistry Division of the National Chemical Research Laboratory has devoted a large proportion of its effort during the past five years to the study of a variety of natural and manufactured products containing long chain carbon compounds. This choice of subject has followed from the importance which these substances have in relation to South Africa's economy. Fortunately, during these years, a growing interest in long chain compounds has emerged in almost every country of the world so that there has been the constant stimulus of important new publications.

It is the author's privilege to present this report on behalf of those colleagues who, at various times, have been concerned with the program: A. L. Clingman, J. P. de Villiers, H. H. Hahn, D. A. Harris, C. A. J. Hoeve, D. H. S. Horn, F. W. Hougen, D. Ilse, F. J. Joubert, S. P. Ligthelm, M. Matic, J. R. Nunn, D. E. A. Rivett, S. D. Rossouw, H. M. Schwartz, H. H. Sephton, M. H. Silk, M. M. von Holdt, and E. M. von Rudloff.

In the following paragraphs this laboratory's research on the individual topics studied is reviewed. In order to keep this review reasonably short, the background of literature given in each case is brief.

Wool Wax

Wool wax, which is likely to become increasingly important in South Africa, is one of the most complex natural mixtures known. It is the sebaceous secretion of the sheep, which is recovered as crude "wool grease" from commercial wool-washing processes. The pure wax is essentially a mixture of esters together with a small proportion of hydrocarbons (19). The percentage of free uncombined carboxyl and hydroxyl groups is small (77). Separation of some individual esters by laborious repetitive crystallization has been claimed recently by Tiedt and Truter (71) in England. These are cholesteryl 24-methylhexacosanoate, cholesteryl 26-methyloctacosanoate, cholesteryl 28-methyltriacontanoate, and a less pure ester of a hydroxy-acid. In view of the large number of individual acids and alcohols present, it is to be expected that the percentage of any given ester will be small if anything approaching a random distribution obtains, but there is very little information available on this point (74). Nevertheless crude fractionation of wool grease to yield a series of materials with graded physical properties is likely to become commercially important. The hydrocarbons of wool wax are of unknown constitution although their physical properties suggest that they are not solely normal unbranched paraffins.

The major contribution to our knowledge of wool wax acids was made by Weitkamp (79), who showed that members of four homologous series were present, *viz.*: a) normal (even-numbered) acids from C_{10} through C_{26}; b) *iso*-branched

$$[(CH_3)_2CH(CH_2)_nCOOH]$$

(even-numbered) acids from C_{10} through C_{28}; c) optically active (dextrorotatory) *anteiso*-branched

$$[CH_3CH_2CH(CH_3)(CH_2)_nCOOH]$$

(odd-numbered) acids from C_9 through C_{27}, and C_{31}; d) normal optically active α-hydroxy-(even numbered) acids of C_{14} and C_{16} chain length. The percentage of d) was small, but this was contrary to the later publication of Bertram (8), who presented analytical values for the hydroxyl content of wool wax derivatives. It seemed probable that the preliminary chromatography and subsequent lengthy precision distillation used by Weitkamp may have resulted in some loss or decomposition of hydroxy-acid components. These components were therefore isolated from total wool wax acids, using the mild Craig countercurrent solvent distribution procedure. It was found that nearly 30% of α-hydroxy-acids were present, including those isolated by Weitkamp together with the normal C_{12} and C_{18} homologues and 2-hydroxy-16-methylheptadecanoic acid (36). An additional method of study-