The Determination of Sorbitan Monostearate in Cake Mixes and Baked Cakes[1]

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Abstract

A method has been developed for the determination of sorbitan monostearate (SPAN 60®, Atlas Chemical Industries, Inc.) in cake and cake mixes. The method involves extraction of sorbitan monostearate from the sample, partial purification of the extract by silica gel chromatography, recovery of the extract polyol and analysis by either gas or paper chromatographic procedures. The isosorbide moiety of the emulsifier is measured by gas chromatography. In the paper chromatographic procedure, the 1,4-sorbitan fraction is isolated, oxidized with periodic acid and the absorbance of the formaldehyde-chromotropic acid color is measured. The sorbitan monostearate content of the sample is calculated using the appropriate conversion factor obtained by analyzing known solutions of polyols from the product.

The method was applied satisfactorily to several samples of baked white cake, yellow and chocolate cake mixes. Analyses of blank cakes showed that there are no interferences from other constituents of cake and that a blank cake is unnecessary in the application of the method.

Introduction

SORBITAN MONOSTEARATE (SMS) is used in the preparation of cake mixes and baked cakes. Its use imparts improved texture, cake volume and improves the retention of "as baked" qualities. A satisfactory analytical method for the determination of the emulsifier concn at the level of use was needed. This presented the problem of developing a technique for the determination of low emulsifier contents, usually ranging from 0.1–0.6%, with meaningful accuracy and precision.

A method was reported in the literature for the identification of sorbitan fatty acid monoesters in mixtures of fats and sugars in some baking preparations by Schrepfer and Egle (1). Their procedure was a qualitative one and apparently was not applied to the significantly more complex mixture found in cake. Recently, Gatewood and Graham (2) reported a method for the assay of sorbitan monoesters in pharmaceutical preparations. This method is based on the periodate oxidation of the polyol and the measurement of the formaldehyde formed by means of a colorimetric procedure employing chromotropic acid. While apparently directly applicable to certain pharmaceutical formulations, this method could not be applied to cake mixes and cakes in which there are various components such as sugars, glycerine, etc., which react with periodic acid to form formaldehyde.

The crux of the cake analysis problem obviously was to isolate quantitatively the SMS or a component fraction thereof which could be measured without interference.

[1] Presented at the AOCS Meeting, Toronto, 1962.

Nominally, SMS is sorbitan monostearate; however, the product actually consists of esters of a complex but reproducible mixture of polyols, i.e., sorbitol, monoanhydrides derived from sorbitol and isosorbide. For developing a method, the investigation was directed to the polyols, for it would be impossible to distinguish the fatty acid moiety of SMS from that of the shortening used in the preparation of cake mixes and cakes.

The isosorbide which is present in SMS is detectable by gas chromatography and this technique offers a rapid means of separation and estimation. The monoanhydrides from sorbitol are satisfactorily separated from the other components of SMS into two fractions by paper chromatography. The faster moving zone which includes 1,4-sorbitan may be determined by periodate oxidation and spectrophotometric measurement of the formaldehyde-chromotropic acid color complex.

Thus, two alternative techniques appeared feasible for the estimation of SMS in cake. Both would depend on the complete recovery of SMS polyol in a suitable concn and state of purity for successful application.

Experimental

SMS is extracted from the sample with ethanol. Much of the extracted sugars and some of the extracted triglycerides precipitate in the ethanol solution and are removed by filtration. Ethanol in the filtered solution is replaced with *n*-heptane by azeotropic distillation. The resulting mixture is subjected to partition chromatography on silica gel to remove the remaining triglyceride and a part of the other extracted material.

The recovered SMS fraction is saponified with KOH. This is followed by deionization of the saponification mixture by means of mixed bed ion exchange.

The deionized solution containing the SMS polyol is concd and adjusted to a standard volume. The polyol solution is then analyzed either by gas or paper chromatography.

Recovery of SMS Polyol from Cake

Apparatus. Extraction Apparatus, Soxhlet, 50 mm i.d., Capacity of flask, 250 ml.

Fritted glass filter funnel, capacity 60 ml. Porosity, coarse.

Erlenmeyer flasks, alkali-resistant, 24/40 standard taper joint, 300 ml capacity.

Stirrer-hotplate (Gyratherm, Will Corp.).

Chromatograph column tube, 28 mm i.d., length 300 mm, Teflon stopcock, coarse fritted disc adsorbent support.

Ion exchange column tube, 11 mm i.d., length 600 mm, glass wool resin support.

Evaporator, Rinco rotating vacuum type, small.

Reagents. Silica gel, Davison grade 923, 100–200 mesh. Adjust the water content to $5 \pm 0.1\%$. The

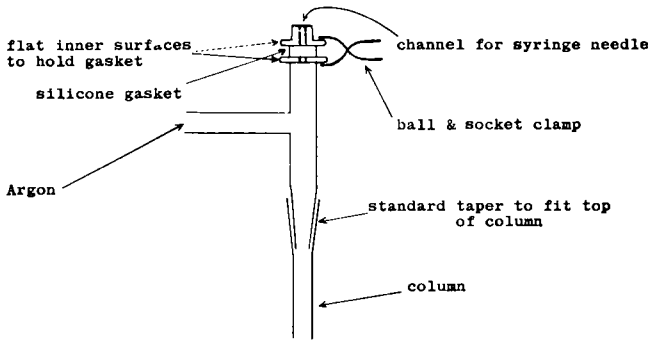

FIG. 1. Sample injection port.

water content should be determined by oven drying a 10-g sample at 200C for at least 4 hr.

Illco-Way research grade mixed ion exchange resins (Illinois Water Treatment Co., Rockford, Ill.).

Procedure. Crumble ca. 100 g of baked cake into a weighed crystallizing dish. Dry in a vacuum oven at 50C to constant weight and calculate the yield of dried cake. Cake mixes do not require any preliminary drying.

Weigh 25-g samples of dried cake or cake mix to the nearest cg into Soxhlet extraction thimbles. Extract with absolute ethanol for 24 hr. Filter the contents of the extraction flask through a coarse fritted glass funnel. Wash the extraction flask and the precipitate on the funnel several times with ethanol.

Assemble suitable apparatus for azeotropic distillation of the filtered ethanol extract solution. The apparatus should include a 300-ml alkali-resistant Erlenmeyer flask to be used as the distillation flask, a magnetic stirrer-heater, a Teflon covered stirring bar for agitation, an addition funnel and a thermometer. Conc the solution to ca. 100 ml and add 50 ml of hot *n*-heptane. Precipitation occurs as the heptane is added. Continue to distill, adding hot heptane as required, until the ethanol-heptane azeotrope is completely displaced by heptane. The binary mixture consisting of 49% ethanol and 51% heptane boils at 71C and is readily separated from the heptane which boils at 98C.

Slurry 50 g of silica gel (adjusted to 5% of water) with heptane and pour the mixture into a chromatograph tube. Transfer the contents of the distillation flask as completely as possible to the silica gel column. Retain the flask for the later collection of the ethanol eluate. Adjust the stopcock for a flow of ca. 5 ml/min. When the level of heptane in the column just reaches the top of the silica gel, eluate with 300 ml of reagent grade benzene. Discard both the heptane and benzene effluents. Finally elute with 200 ml of absolute ethanol, collecting the eluate in the 300 ml alkali-resistant flask.

Place a boiling rod in the flask containing the ethanol eluate and evaporate in the steam bath to a volume of ca. 100 ml. Add 1 g of KOH and reflux on a hotplate for 0.5 hr. Add 50 ml of water and continue to reflux the mixture for an additional 0.5 hr. Cool the mixture and add 50 ml of water.

Prepare an ion exchange column using 25 g of cation and anion exchange resins slurried with a 1:1 mixture of ethanol and water. Ion exchange the saponification mixture using a flow rate of approx 3 ml/min. Collect the effluent in a 1000-ml round-bottom flask. Wash the column with 300 ml of 1:1 ethanol-water.

Evaporate the combined effluent and washings in the 1000-ml flask to dryness using a Rinco evaporator. Use an oil bath maintained at 70–80C and a water aspirator. Wash the sides of the flask with absolute ethanol and heat on the steam bath for a few minutes to dissolve the polyols. Conc the ethanol solution and adjust the volume to exactly 5 ml.

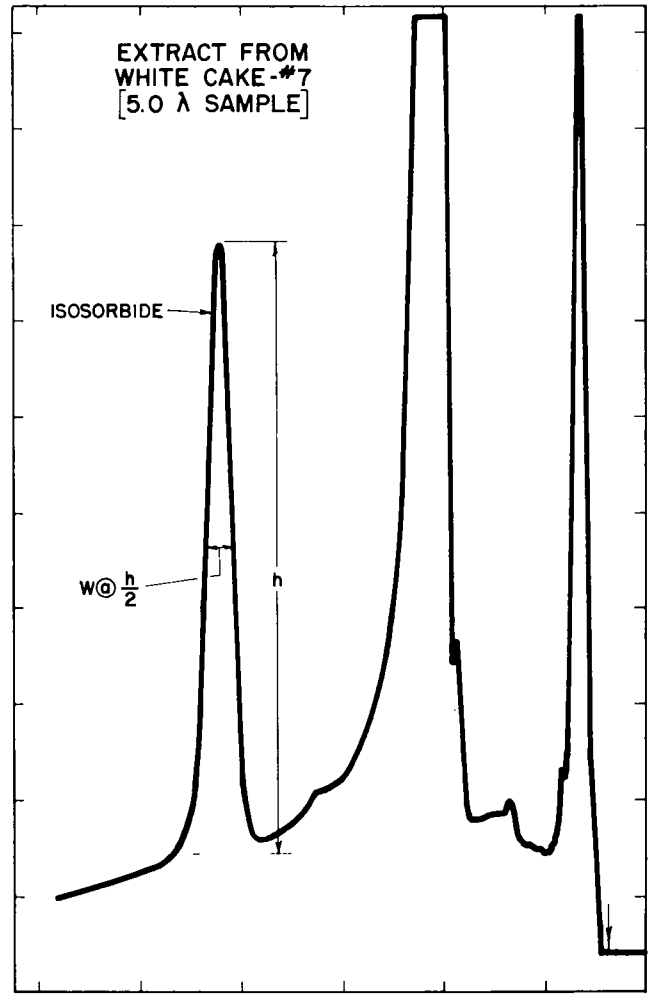

FIG. 2. Typical isosorbide peak for white cake.

Standard SMS polyol solutions are used for calibration purposes. These should be prepared in each laboratory by subjecting 100-mg quantities of SMS to the analytical procedure, starting with the saponification step.

Analysis by Gas Chromatography

A sufficiently sensitive GLC such as the Pye Argon Instrument, fitted with strontium-90 detector may be employed. The column employed for these analyses was the standard glass column provided with the Pye instrument, but modified to permit the use of a shorter column length of packing. The column as supplied is a glass tube 54-in. long and 7 mm o.d. (5 mm i.d.). The lower 30 in. were replaced with capillary tubing of 0.5 mm i.d. and of the same approx o.d. as the original tubing. This modification permits packing 18 in. of column with stationary phase, the entire 18 in. being inside the aluminum heat sink used to maintain constant temp.

The liquid phase employed to separate isosorbide from the other components of the sample is Carbowax 20M. The material is used as received in a 15% concentration on Chromosorb W.

In order to permit introduction of the sample without interruption of carrier gas flow, a modified sample introduction system was designed for use in these analyses. The "L" tube which is supplied with the instrument is replaced with the sample injection port shown in Figure 1. A flash heater was designed to fit over the upper 5 in. of glass column which project above the constant temp zone. This consists of a piece of glass tubing ¾ in. i.d. wrapped with a spiral of resistance wire, the ends of which are wired to a Powerstat. The entire asembly is wrapped in fiberglass and fitted into a metal container for protective purposes. The openings in the container are of sufficient size to allow the assembly to fit over the top of the column. The top of the flash heater is closed with a piece of fiberglass insulation cut to fit snugly around the top of the column. In this manner, it is possible to heat the upper zone of the column to temp far in excess of column temp without damage to the stationary phase.

Five μl of sample are injected directly into the carrier gas stream. This is accomplished by passing the needle through the silicone gasket into the zone surrounded by the flash heater.

The experimental operating variables employed for the isosorbide analyses are not critical. The conditions listed below are typical. Minor fluctuations in temp and Argon flow rate do not affect resolution or analytical results.

INSTRUMENT: Pye Argon Chromatograph.
COLUMN: 18 in. Carbowax 20M—15% on acid washed Chromosorb W—80/100 mesh.
TEMPERATURE: 195 ± 3C.
ARGON FLOW: 115 ± 5 ml/min dried with Linde Molecular Sieves 5A.
DETECTOR VOLTAGE: setting = 1000 (nominal value—varies among instruments for this setting).
SENSITIVITY: x3 (or x10 as required).
RECORDER: 5 mv full scale.
SAMPLE VOLUME: 5.0 μl—Use a 10-μl syringe with fixed needle (Hamilton type 701N). A suitable preheater operated at a temp ~ 100C higher than column temp should be employed around the injection site.

Calibration curves may be prepared by injecting into the instrument known amt of isosorbide in ethanol. Peak areas may be measured by the conventional technique of peak height times width at one-half peak height. A linear calibration curve is obtained by plotting peak area vs. μg of isosorbide. The isosorbide peak obtained from a typical cake extract is shown in Figure 2.

The isosorbide content of the 5.0 μl aliquot of recovered cake polyol solution as well as standard SMS polyol solution is determined directly from the calibration curve or by multiplying the observed peak area by the slope of the curve (μg of isosorbide/unit area).

$$\%\ \text{SMS in dried cake (or cake mix)} = \frac{I \times 5000 \times 100}{10^6 \times 5 \times f \times W}$$

where I = μg of isosorbide found in 5 μl aliquot of recovered polyol solution by gas chromatography

W = g of sample take for extraction

5000 = total μl of recovered polyol solution

5 = μl of recovered polyol solution taken for gas chromatography

TABLE I

Comparison of Production Batches of SMS

SMS batches	Conversion factors	
	% Isosorbide yield	μg SMS/unit absorbance
A	4.84	2881
B	4.81	2813
C	4.79	2845
D	5.30	2797
E	4.95	2761
F	4.93	.....
G	4.92	.....
H	4.47	.....
Mean	4.88	2819
% Avg dev. from mean	±3.1	±1.3

f = fractional isosorbide yield for SMS

10^6 = to convert μg to g

% SMS in baked cake = % SMS in dried cake × fraction of dried cake from baked cake

Analysis by Paper Chromatography

Apparatus. Chromatographic jar (12 x 12 x 24 in.) and accessories for descending solvent paper chromatography, apparatus for capillary elution of paper chromatograms, Beckman spectrophotometer, Model B.

Reagents. Silver nitrate spray reagent. Prepare the following two solutions: a) Dissolve 50 g of silver nitrate in 450 ml of distilled water. Store in an amber bottle; b) dilute ammonium hydroxide solution; add 120 ml of concd ammonium hydroxide to 330 ml of water. When required, combine equal volumes of solutions "a" and "b" for use as a spray reagent.

Periodic acid reagent (0.03M). Dissolve 3.42 g of periodic acid (H_5IO_6) in 100 ml of 0.25M H_2SO_4 and dilute to 500 ml with 0.25M H_2SO_4.

Stannous Chloride Reagent. Dissolve 3.2 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in 40 ml of 0.3N HCl. Transfer the solution to a 100 ml volumetric flask and dilute to volume with 0.3N HCl. Prepare fresh daily. The stannous chloride solution should be titrated with the periodic acid reagent before use and so adjusted that 10.0 ml of the stannous chloride reagent will titrate 10.2 ml of the periodic acid reagent. For the titration 5 ml of concd HCl are added to 10 ml of stannous chloride plus 1 ml of starch indicator (a blue color indicates the endpoint).

Chromotropic Acid Reagent. Dissolve 2.000 g of chromotropic acid (4,5-dihydroxy-2,7-napthalene- disulfonic acid, disodium salt) in 40 ml of water in a 1000-ml volumetric flask. Dilute to the mark with 15M H_2SO_4.

Procedure. Using a razor blade, cut a sheet of Whatman No. 4 chromatographic paper (22 x 18.25 in.), against the machine direction, into two pieces

TABLE II

Recovery of SMS in Baked White Cake (Dry Basis)

Cake No.	Sample No.	% SMS calc.	Gas chromatography		Paper chromatography	
			% SMS found	% Recovery	% SMS found	% Recovery
B	1	0.444	0.426	96	.....	.....
C	1	0.558	0.391	70	0.559	100
C	2	0.558	0.511	91	0.611	110
C	3	0.558	0.480	86	0.564	101
C	4	0.558	0.434	78	0.530	95
C	5	0.558	0.541	97	0.541	97
C	6	0.558	0.467	84	0.547	98
C	7	0.558	0.467	84	0.575	103
Mean value (Cake C)				84		100
% Avg dev. from mean				±6		±3

TABLE III

Recovery of SMS in Yellow Cake Mix

% SMS—0.424

Sample No.	Gas chromatography		Paper chromatography	
	% SMS found	% Recovery	% SMS found	% Recovery
1	0.481	113	0.454	107
2	0.502	118	0.455	107
3	0.507	120	0.536	126
4	0.505	119	0.417	98
5	0.453	107	0.369	87
6	0.508	120	0.437	103
7	0.425	100	0.431	102
Mean value		114		104
% Avg dev. from mean		±6		±8

TABLE IV

Recovery of SMS in Chocolate Cake Mix

% SMS—0.592

Sample No.	Gas chromatography		Paper chromatography	
	% SMS found	% Recovery	% SMS found	% Recovery
1	0.576	97	0.633	107
2	0.577	97	0.600	101
3	0.528	89	0.550	93
4	0.574	97	0.564	95
5	0.515	92	0.644	108
6	0.527	89	0.592	100
Mean value		94		100
% Avg dev. from mean		±4		±5

measuring 9 x 18.25 in. Divide each 9-in piece into four strips approx 2.25 in. wide by cutting out 1/16-in. slots starting at a point 1 in. from the top and extending to a point 0.5 in. from the bottom of the paper. One 9-in. sheet is required for each sample, i.e., two strips for duplicate sample application, one strip for pilot and one strip for paper blank. Label the strips consecutively as sample, pilot, sample and blank. Mark all strips suitably for descending solvent chromatography by drawing the fold line at 2.25 in. from the top and the sample origin points at 3 in. from the top of the paper. Cut the bottoms of the strips with pinking shears.

Apply approx 50 μg of known 1,4-sorbitan to the pilot strips at the origin by spotting 1 μl of an aqueous solution containing 50 mg of 1,4-sorbitan/ml. This is done to insure that the spot containing 1,4-sorbitan may be detected after spraying. Apply 20 μl of the cake polyol solution to both the sample and pilot strips.

At least 24 hr before chromatography, place a small beaker of water and a small beaker of *n*-butanol in the chromatographic jar. Cover the jar and allow equilibrium to take place.

Place the solvent assemblies for descending chromatography on the rack in the jar. Mount all of the strips in the jar and replace the lid, making sure that it fits tightly. Condition the paper for at least one hr. Then remove the corks from the openings in the lid of the jar and add *n*-butanol saturated with water to the solvent troughs. Replace the corks and allow the chromatograms to develop for 22 hr.

Remove the strips from the jar, blot the extremities with paper towels to remove excess solvent, and hang up to air dry for at least one hr. Cut apart the pilot, sample and blank paper strips. Spray the pilot strips evenly using a glass atomizer containing freshly prepared silver nitrate spray reagent. Place the sprayed pilot strips in an oven at 100C for 5–10 min.

The various polyhydric substances in the polyol appear as brown-black spots on a relatively light brown background. The spot containing 1,4-sorbitan lies immediately above the glycerine spot which is a relatively large area located near the bottom of the strip.

Outline the desired spot on the pilot strip with a pencil. Then draw a line square across the strip immediately below the bottom of the spot and another, ca. 1 cm above the spot. Place the pilot strip next to the corresponding sample strip so that the origins coincide and clamp the two strips in position. Mark off the desired zone on the sample strips. Mark off the corresponding area on the paper blank strips. Cut out the marked areas using tweezers to handle the paper tabs.

Fold back each paper tab, sample and blank, 1/4 in. from the top and cut the bottom to form a point starting about 1/8 in. up on each side of paper. Insert the tops of the paper tabs between two glass plates up to the fold and put the plates in the apparatus for capillary elution. Elute with distilled water, collecting 0.5 ml of eluate in a 1-ml volumetric flask (the flask is roughly calibrated and marked at a volume of 0.5 ml). Transfer the eluate quantitatively to a 25 x 200 mm test tube, using three 0.5 ml washings of distilled water. The total volume should be ca. 2 ml.

Pipette 1 ml of 0.03M periodic acid reagent into each test tube. Swirl gently and allow to stand at room temp for exactly 15 min to oxidize the separated polyol. Pipette 1 ml of stannous chloride reagent into the test tube and swirl to mix the contents. Add 5 ml of chromotropic acid reagent and again mix by swirling. Place the test tubes in a boiling water bath for 30 min to develop the formaldehyde-chromotropic acid color. Cool the test tubes to room temp and transfer the contents to 25-ml volumetric flasks. Use 8M H_2SO_4 to rinse each test tube and adjust the volume to the mark.

Using a Beckman Model B Spectrophotometer, measure the absorbance values of colored solutions in a 1-cm cell at 570 mμ against air as a reference. Correct the observed absorbance of the samples by subtracting the absorbance of the paper blank using the same cell. If the net absorbance exceeds 0.2, it will be necessary to repeat the chromatography using a smaller aliquot of the polyol solution. The amt of SMS equivalent to the net absorbance is obtained by using a conversion factor expressing weight of SMS/unit absorbance. This conversion factor was obtained by analyzing standard SMS polyol solutions.

$$\% \text{ SMS} = \frac{A \times f \times 5000 \times 100}{10^6 \times V \times W}$$

where A = net absorbance of sample

f = mean conversion factor, μg of SMS per unit absorbance

5000 = total μl of recovered polyol solution

V = μl of aliquot taken for analysis

W = g of sample taken for extraction

10^6 = to convert μg to g

Results and Discussion

The SMS polyols from several production batches of the product were subjected to analysis by the gas and paper chromatographic techniques. Table 1 lists the percentage isosorbide yield by gas chromatography and the number of μg of SMS/unit absorbance by paper chromatography. The data listed indicate that the product is reproducible from batch to batch.

Several samples of baked white cake containing SMS were analyzed. The results obtained are listed in Table II.

Tables III and IV list the results obtained by the analysis of yellow and chocolate cake mixes, respectively.

The analysis of blank white cake and blank yellow and chocolate cake mixes showed the absence of apparent SMS, indicating that there was no interference by the other constituents in the cake.

The time required for the preparation of the polyol solution is approx 3–4 days. Assay by gas chromatography requires only a few hours additional time. Paper chromatography, on the other hand, requires 2–3 additional days of elapsed time for completion. In addition, while the paper chromatographic technique appears straight forward, one has to develop the skill and patience to perform the analyses. After this phase of familiarization is passed, an operator may perform satisfactory analyses using the paper chromatographic technique.

ACKNOWLEDGMENT

Gas chromatographic analyses by Aaron Cooper; paper chromatographic analyses by Clarence Hall.

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[Received July 25, 1963—Accepted August 17, 1964]

A Study of Methods for Evaluation of the Stability of Fats and Shortenings

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Abstract

The data from several laboratory tests [Active Oxygen Method (AOM), modified ASTM bomb, modified ASTM bomb with catalyst added to the fat, a manometric method and an accelerated oven test] were compared with data from a storage test at 85F evaluated organoleptically at selected intervals. Tests were carried out on lard, hydrogenated vegetable oil and tallow, with and without added monoglyceride, and with and without selected antioxidants. A comparison of the data from the laboratory tests with that from the storage tests indicated: 1) that the different types of fats behave differently, 2) the laboratory tests cannot be used as an index of shelf stability except for a given type of formulation of fat for which the relationship between the laboratory test and the shelf stability is known, 3) the modified ASTM bomb method is the most reliable for estimating shelf life, and 4) the modified ASTM bomb method with catalyst is not as precise as the bomb method, but may be used for control purposes where some accuracy may be sacrificed in the interest of shorter time.

Introduction

ALMOST ALL SHORTENINGS are tested for keeping qualities as a part of quality control programs, or to be certain they meet a specification under which they are sold. The purpose of these tests is to assure the producer and the customer that the product has a satisfactory shelf life and will not develop an off flavor during the period that it will remain in the plant, store or home before it is ultimately used. The relationship between the shelf life and the laboratory keeping tests is often questionable, but little or no sound data have been available to throw light on this situation.

The purpose of this investigation was to obtain more detailed information on the relationship between shelf stability of various fats and the laboratory tests customarily used to predict stability. The time a product can remain in storage, in a store or a home, without the development of objectionable flavors is the property that storage tests attempts to duplicate and laboratory tests to predict. The time required for a sample in storage to develop a selected degree of off flavor will be taken as an index of the stability of the sample. This will be the value with which the results by other methods will be compared in order to select the laboratory test or tests that most accurately indicate the stability of fat or shortening.

[1] Presented at the AOCS Meeting in Minneapolis, 1963.

Experiment and Results

The samples prepared for this investigation were made using three types of fat, (lard, hydrogenated vegetable oil and tallow) with and without added monoglyceride and various antioxidants as indicated below:

Code	Type	Monoglycerides	0.01% BHA, 0.01% BHT	0.01% BHA, 0.01% propyl gallate	0.01% BHA, 0.0025% NDGA	0.01% BHA
L-O-O	Lard	None				
L-O-A	Lard	None	X			
L-O-B	Lard	None		X		
L-O-C	Lard	None			X	
L-O-D	Lard	None				X
L-M-O	Lard	5%				
L-M-A	Lard	5%	X			
L-M-B	Lard	5%		X		
L-M-C	Lard	5%			X	
L-M-D	Lard	5%				X

Samples with the same combinations of monoglycerides and antioxidants were prepared using hydrogenated vegetable oil (V-O-O) and tallow (T-O-O).

These samples were subjected to the following laboratory tests for evaluating stability: active oxygen method (1), a monometric method (2), modified ASTM bomb (3), a modified ASTM bomb with catalyst added to the fat (4) and an oven test at 140F in which the 8-oz samples were held at 140F in a jar