Methods for the Determination of Cyclopropenoid Fatty Acids. IV. Application of the Step-wise HBr Titration Method to the Analysis of Refined and Crude Cottonseed Oils[1]

J. A. HARRIS, F. C. MAGNE, and E. L. SKAU, Southern Regional Research Laboratory,[2] New Orleans, Louisiana

Abstract

A method is described for the determination of cyclopropenoid fatty acids in refined and crude cottonseed oils to within 0.01%. It is based upon a stepwise hydrogen bromide titration at 3C and 55C after removal of interfering substances by adsorption on activated alumina. Highly oxidized cottonseed oils first must be converted to methyl esters.

Introduction

IN A PREVIOUS publication (1) it was shown that in the absence of interfering substances cyclopropenoid fatty acid derivatives can be precisely determined by a stepwise titration with hydrogen bromide in acetic acid at 3C and 55C. The preliminary titration at 3C overcomes the interference of those non-cyclopropenoid substances, such as epoxides, which can be titrated selectively at that temp. Experiments on a number of non-cyclopropenoid vegetable oils, however, indicated that all glyceridic oils, including cottonseed oil, contain traces of interfering substances resulting in small titrations not only at 3C but also at 55C. The accuracy of the stepwise titration method as originally reported (1) is therefore inadequate for the analyses of cottonseed and other oils having low cyclopropenoid moiety content since the 55C titration error involved, though equivalent to only a few tenths of a per cent, is of the same order of magnitude as the cyclopropenoid concentration being measured.

The present report deals with the development of a method for eliminating these interfering substances, based upon their selective adsorption on activated alumina, so that the 55C titration values for refined and crude cottonseed oils will be an accurate measure of the cyclopropenoid moiety content.

Experimental

Materials. The *Sterculia foetida* oil was obtained by extraction of the meats of *Sterculia foetida* seeds with petroleum ether following the procedure previously reported (1). F-20 grade, 80–200 mesh Alorco activated alumina was obtained from the Aluminum Co. of America. Standard 0.1N hydrogen bromide solution (Durbetaki reagent) was prepared by adding the appropriate amount of glacial acetic acid to the concd solution of anhydrous hydrogen bromide in glacial acetic acid purchased from Eastman Kodak Co. This reagent was standardized daily against anhydrous sodium carbonate. All titrations were performed in a closed system as in a Durbetaki oxirane titration (2) using a 10-ml automatic reservoir semimicro burette graduated to 0.05 ml.

Alumina Treatment. One hundred g of the activated alumina are poured in several small portions into a chromatograph tube (16 mm inside diam) containing a sufficient volume of the appropriate solvent to completely cover the entire charge of adsorbent. The packing density is that attained by gravitational settling without tamping or tapping. A 25-g sample of oil dissolved in an equal volume of solvent is poured onto the column when the solvent has drained to within ca. 0.5 cm of the top of the packing. It is allowed to percolate by gravity and eluted by controlled addition of about 225 ml solvent from an automatic syphoning separatory funnel. The top of the packing is never exposed to the atmosphere during the percolation or washing operation. The combined percolate is then filtered and the solvent removed on a rotary evaporator under reduced pressure at a temp below 60C. Proportionally smaller amounts of alumina are used for smaller samples, maintaining about the same ratio of packing height to column diam as with the standard 25-g sample.

Titration. Accurately weighted 7-g specimens of the solvent-free alumina-treated sample are titrated with the standard hydrogen bromide solution using the 3C–55C stepwise titration technique previously described (1). Appropriate correction is made for the solvent blank. The titration value in ml at 55C, v, is a measure of the cyclopropenoid moiety present. The titration value at 3C should be negligible. If not, it can be assumed, on the basis of similar experiments with refined non-cyclopropenoid oils, that the sample still contains interfering substances contributing to the titration at 55C and an additional alumina treatment will be required. The cyclopropenoid moiety concn is calculated in terms of either sterculic acid or malvalic acid, the predominant cyclopropenoid moiety in *Sterculia foetida* oil and cottonseed oil, respectively.

$$\% \text{ Sterculic Acid} = 29.45 \frac{Nv}{w}$$

$$\% \text{ Malvalic Acid} = 28.04 \frac{Nv}{w}$$

Where v is the number of ml of HBr solution of normality N required for w g of sample.

Refined Cottonseed Oils

For refined oils the solvent used in the alumina treatment is petroleum ether (30–60C). Effectiveness of this treatment for removing the interfering substances is shown by the results obtained for a number of non-cyclopropenoid salad oils and for a sample of methyl oleate (Table I), all of which originally had appreciable hydrogen bromide titration values at both 3C and 55C. The interfering substances involved are probably oxidation products. This follows from the fact that the alumina-treated oils and even freshly vacuum-distilled methyl oleate, which contain no in-

[1] Presented at AOCS Meeting in Minneapolis, 1963.
[2] So. Utiliz. Res. and Dev. Div., ARS, USDA.

TABLE I

HBr Titration Values for a Number of Non-Cyclopropenoid Salad Oils and Methyl Oleate Before and After Al_2O_3 Treatment

Sample	HBr equivalent as % sterculic acid			
	Original		After Al_2O_3 treatment	
	at 3C	at 55C	at 3C	at 55C
Corn oil	0.09	0.36	0.00	0.02
Peanut oil	0.10	0.09	0.00	0.00
Peanut oil	0.10	0.09	0.00	0.01
Soybean oil	0.14	0.07	0.01	0.01
Safflower oil	0.01	0.03	0.00	0.01
Methyl oleate	0.26	0.80	0.00	0.01

terfering substances, gradually develop positive titrations at both 3C and 55C on exposure to air.

The stepwise titration technique was then applied to a series of *Sterculia foetida* oil-refined peanut oil mixtures of known cyclopropenoid acid content in the range of concn anticipated for cottonseed oils. The peanut oil used gave titration values corresponding to 0.10 and 0.09% sterculic acid at 3C and 55C, respectively. Therefore, the results reported in Table II prove that the alumina treatment is effective in removing the interfering substances without affecting the cyclopropenoid constituents at these concentration levels and that the analyses are usually accurate to within 0.01%.

Table III shows the cyclopropenoid moiety content, calculated as malvalic acid, of a number of commercial, "brand-name" cottonseed salad oils purchased at retail outlets and includes the results for two samples (No. 12,13) of refined, unbleached, and undeodorized cottonseed oils. Originally, all except one of the salad oil samples gave a titration at 3C, ranging from 0.06–0.64% calculated as malvalic acid, and all contained substances which interfered at 55C, in amounts ranging from 0.03–0.18%. The true cyclopropenoid content of the eleven salad oils fell in the range from 0.04–0.42%.

Identical results were obtained on quadruplicate specimens of oil sample No. 3. The alumina-treated Sample No. 11 was subjected to three successive additional alumina treatments. Analysis after each treatment gave 0.31%. These results indicate that a precision of ±0.01% is attainable by this procedure and confirm the conclusion that the alumina treatment does not affect the cyclopropenoid moiety at these concn. Since there is a certain amount of hold-up of oil occurring in each column treatment, these results also imply that it is not necessary, at these cyclopropenoid concn, to recover 100% of the oil to insure accuracy. This was further verified by deliberately varying the oil recovery to 65% and 85% with no observable change in the analysis.

The refined but undeodorized oils, Samples 12 and 13, exhibit higher cyclopropenoid moiety contents than the commercial (deodorized) salad oils. On the basis

TABLE II

Analysis of Mixtures of *Sterculia foetida* Oil with Refined Peanut Oil[a] After Al_2O_3 Treatment

% *Sterculia foetida* oil in mixture	HBr equivalent at 55C as % sterculic acid[b]		
	Observed	Calculated[c]	Deviation
100.00	51.06	51.06	.....
3.99	2.05	2.03	+0.02
2.06	1.04	1.05	−0.01
1.00	0.52	0.51	+0.01
0.61	0.32	0.31	+0.01
0.21	0.11	0.11	0.00
0.00	0.00	0.00	0.00

[a] HBr equivalent of original refined peanut oil (as sterculic acid) was 0.10% at 3C and 0.09% at 55C.
[b] HBr equivalent at 3C was zero.
[c] Based on value for *Sterculia foetida* oil, 51.06%.

TABLE III

Analysis of Cottonseed Salad Oils

Sample No.	HBr equivalent as % malvalic acid			
	Original		After alumina treatment	
	3C	55C	3C	55C[a]
1	0.00	0.09	0.00	0.04
2	0.15	0.30	0.00	0.19
3	0.15	0.47	0.00	0.30
4	0.13	0.36	0.00	0.33
5	0.23	0.14	0.00	0.11
6	0.14	0.28	0.00	0.22
7	0.15	0.27	0.01	0.23
8	0.06	0.48	0.00	0.42
9	0.33	0.43	0.00	0.40
10	0.40	0.25	0.00	0.21
11	0.64	0.44	0.00	0.32
12[b]	0.12	0.66	0.00	0.63
13[b]	0.10	0.78	0.00	0.60

[a] True cyclopropenoid acid content calculated as malvalic acid.
[b] Refined, unbleached, and undeodorized cottonseed oils.

of this and other unpublished information there is reason to believe that in the processing of cottonseed oil, the greatest loss in cyclopropenoid content occurs during the deodorization step.

Crude Cottonseed Oils

Crude cottonseed oils require special treatment for the removal of interfering substances since they contain phosphatidyl constituents and are often highly colored. The phosphatides give a hydrogen bromide titration and the color bodies sometimes interfere with the endpoint. The procedure for the analysis of crude oils therefore involves two different alumina treatments before the stepwise hydrogen bromide titration. The oil is first passed through an alumina column using a diethyl ether-methanol (39:1) solvent system as in the official AOCS method for neutral oil (3). After removal of the solvent, the oil is subjected to the procedure for refined oils. As before, the results cannot be considered reliable unless the 3C titration is zero.

The effectiveness of this treatment was tested on a refined cottonseed oil of known "malvalic acid" content (0.44%) to which 1.5% of a phosphatide, soybean lecithin, had been added. After the dual alumina column treatment the HBr equivalent at 3C was zero and the 55C titration corresponded to 0.44% malvalic acid.

A number of crude oils were analyzed by this procedure (Table IV), including two screw pressed oils, a number of hexane extracted oils, and a mixed-solvent (hexane-acetone-water) extracted oil (4). The cyclopropenoid acid contents, ranging from 0.52–0.90% calculated as malvalic acid, averaged 0.64%. There was no apparent correlation with the iodine value or the free fatty acid content.

Old or Rancid Oils

Old and badly oxidized oils present a special problem since they usually exhibit rather high titration values at both 3C and 55C which cannot be completely eliminated even by a number of successive alumina treatments. It was found that this difficulty can be overcome by methanolysis of the glycerides, subjection of the methyl esters to the alumina treatment for crude oils, and stepwise titration.

Methanolysis: Fifty g glyceride are added to 150 ml absolute methanol in which 0.15 g metallic sodium in excess of the free fatty acid equivalent of the glyceride has been dissolved. The mixture is gradually brought to the bp and maintained at reflux for 15 min beyond the attainment of homogeneity. The cooled mixture is stirred into a slight excess of chilled dilute

aqueous HCl and the methyl esters are taken up in petroleum ether, washed free of mineral acid, dried over anhydrous sodium sulfate, filtered, and stripped on a rotary evaporator under reduced pressure at temp not in excess of 60C.

The results obtained for a highly oxidized refined peanut oil proved that all the interfering substances are eliminated by this procedure. This oil, after three successive alumina treatments, still gave HBr titrations equivalent to 1.97% at 3C and 0.35% at 55C calculated as malvalic acid. After conversion to the methyl esters and treatment by the dual column procedure used for crude oils, these values were reduced to 0.00% and 0.01% respectively.

A 9-year-old refined cottonseed oil after petroleum ether-alumina treatment had an HBr equivalent of 1.16% at 3C and the endpoint at 55C could not be determined with precision because of the development of a reddish-brown color, a common occurrence with such oils. The esters formed by methanolysis gave a zero titration at 3C after the dual column treatment and exhibited normal behavior at the 55C endpoint corresponding to 0.42% malvalic acid.

The cyclopropenoid moiety is not affected by the methanolysis procedure. For example, the malvalic acid contents of the refined cottonseed oil samples (No. 8, 12 in Table II) as determined on the methyl esters were 0.42% and 0.63%, respectively; i.e., identical with the results obtained by direct analysis of the oils. Good agreement was also obtained between the analyses of a number of crude cottonseed oils before and after methanolysis (Table IV).

TABLE IV

Analyses of Crude Cottonseed Oils

Type of oil[a]	% Free fatty acid	Iodine value (Wijs)	HBr equivalent at 55C[b] (as % malvalic acid)	
			Oil	Methyl esters
H	1.55	109.0	0.63	.....
H	2.16	102.7	0.56	0.57
H	3.89	.....	0.59	.....
H	4.69	102.3	0.59	0.56
H	4.86	107.0	0.71	0.72
H	5.18	108.9	0.90	0.90
H[c]	0.70	110.6	0.66	0.66
H[c]	0.48	109.8	0.62	.....
E	6.49	107.4	0.62	0.60
E	1.14	104.0	0.52	.....
MS	3.96	108.0	0.66	.....
MS	4.76	103.4	0.60	.....

[a] H = hexane extracted; E = expeller; MS = mixed (hexane-acetone-water) solvent extracted.
[b] HBr equivalent at 3C was zero.
[c] Glandless cottonseed.

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Caking Test for Dried Detergents[1]

HENRY WATANABE and W. L. GROVES, Continental Oil Company, Ponca City Oklahoma

Abstract

A test to measure the caking tendency of dried detergents is described. This test has good reproducibility and should be useful in screening new products, studying formulation changes and examing anticaking agents.

The test measures the force necessary to break cylinders formed from test detergents. Forming and breaking forces are extremely critical and are provided by an Instron Universal Tester. The sensitivity of the test demands careful control of the environment; a number of critical factors are discussed. These include test procedure variables such as cylinder length, forming force, forming rate and the time to form the cylinder and product variables such as moisture content and particle size. An increase in the forming force and time, particle size within certain limits and moisture, increase the breaking force.

Results on drum-dried products were found to correlate poorly with spray-dried products. Heavy duty products show a lower caking tendency than do light duty products. Anticaking additives cause a very marked decrease in breaking force.

Introduction

IT IS OFTEN DESIRABLE to obtain quantitative comparisons of the caking tendency of different dry detergents, especially in evaluating new surfactants or anticaking additives. Visual observations are often useful but frequently inadequate. Results are qualitative and rather large differences are required for sample difference to be seen.

Previous attempts to measure cakiness have been made. Tests are described in patents claiming additives to decrease cakiness. One test described in a patent (1), claiming the use of aluminum silicate as an anticaking additive, consists of making cylinders of the detergent and measuring its compressive strength along its axis. Our test is based on this line of thinking, but with an improved method for forming the cylinder and for measuring its compressive strength. Other tests described in patents (2,3) consist of placing the detergent in cartons similar to those used in retail trade and exposing them to a humid atmosphere for an extended period of time. The cartons are then carefully opened and the contents poured through a coarse screen. The amount remaining on the screen is considered caked. This test provides a good practical evaluation but does not satisfy the need for early screening.

Our early work indicated that these tests lack control, giving poor reproducibility and sensitivity. Subsequent work led to our current test which offers the following advantages:

1) the reduction of human factors to the very minimum
2) the exact duplication of all mechanical manipulations involved
3) a high precision in reading results

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