Sesame Oil. IV. Determination of Free and Bound Sesamol[1]

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THE unusual resistance of hydrogenated sesame oil to oxidative rancidity has been referred to in previous reports of this series (1, 2). Sesamin, sesamolin, and a phytosterol which are present in the unsaponifiable fraction of sesame oil do not exhibit any appreciable antioxidant activity when tested by the active oxygen method with lard as a substrate (3). However sesamol, a component of sesamolin, was found to be effective as an antioxidant in lard and a number of vegetable oils (3). Free sesamol has not been isolated from sesame oil, but Honig (4) has demonstrated its presence in oils which have been bleached with "acid type" bleaching earths.

Sesamol is known to be responsible for the characteristic Villavecchia test, which has been adopted by the American Oil Chemists' Society (5) for the qualitative detection of sesame oil.

The role of sesamol in the stability of sesame oil has never been investigated, presumably because no method has heretofore been available for quantitatively estimating the amount of free and bound sesamol in this oil. A spectrophotometric method, based on the Villavecchia test, for the determination of free and bound sesamol is described in the present report.

According to the Villavecchia test (5) for the detection of sesame oil, the sample of oil is shaken with concentrated hydrochloric acid and a small amount of an alcoholic solution of furfural. If the test is positive, a crimson color is formed in the acid layer and persists after dilution with water. Although the test is predicated on the presence of sesamol in the sample, sesamolin will also produce the same color reaction because sesamol is liberated from the former by concentrated hydrochloric acid. It is therefore not possible to distinguish between free sesamol and sesamolin (subsequently referred to as "bound sesamol") by the Villavecchia test.

The absorption spectra of the red color systems produced by furfural-sulfuric acid and (A) sesamol, (B) sesame oil, and (C) sesamolin measured against furfural-sulfuric acid are shown in Figure 1. The sesamol was prepared by synthesis (3) and the sesamolin was obtained from sesame oil. All of the spectral curves shown in Figure 1 exhibit characteristic maxima in the wave length regions of 414 and 518 mμ, respectively.

Although the direct application of the Villavecchia test to an oil produces a color intensity corresponding to the total sesamol content of the oil, it is possible to distinguish between free and bound sesamol in the following manner. The oil, dissolved in an inert solvent, such as iso-octane, is extracted with a dilute solution of aqueous alcoholic alkali in which the free, but not the bound sesamol, is soluble. The Villavecchia test can then be applied to the original oil solution, the alkali-extracted oil solution, and the alkaline extract. The intensities of the colors produced with the three solutions correspond to the total, bound, and free sesamol, respectively, which were present in the original oil.

The measurement of the color intensity produced by sesame oil with furfural and concentrated hydrochloric acid presents certain difficulties of which the most serious is the persistent turbidity present in the acid layer. This turbidity also has a tendency to reappear after filtration and seems to be connected with the presence of sesamin. The same turbidity was observed in soybean oil to which 1% of sesamin had been added when it was shaken with concentrated hydrochloric acid. Under the same conditions, soybean oil alone does not produce turbidity.

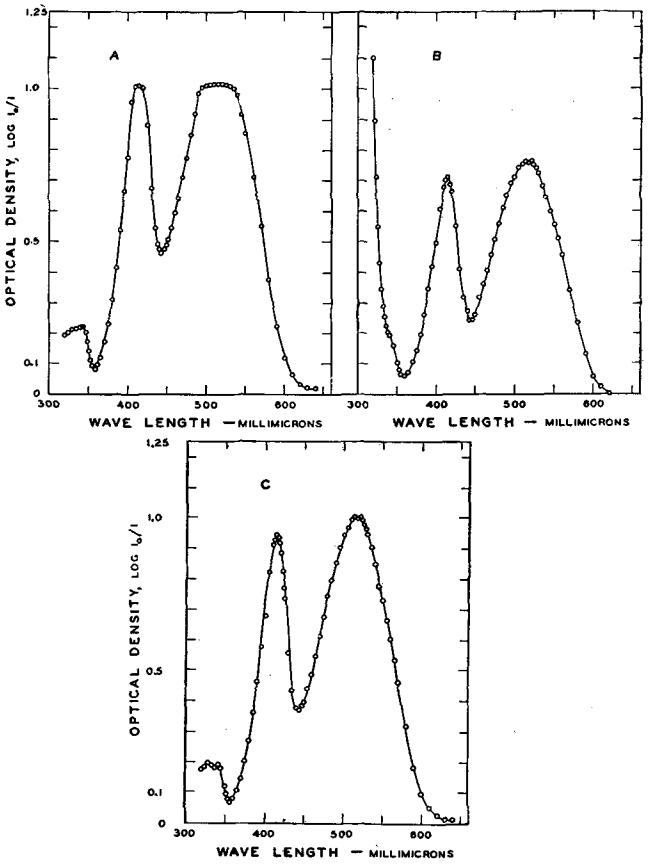

FIG. 1. Absorption spectra of the red color system, furfural-sulfuric acid and (A) sesamol, (B) sesame oil, and (C) sesamolin.

Another difficulty with the use of hydrochloric acid in this reaction is the production of extraneous color formation in some sesame oils. Also considerable destruction of sesamol may occur in the presence of concentrated hydrochloric acid prior to the formation of the red pigment. This was strikingly evident by the reduction or complete absence of color when sesame oil was shaken with hydrochloric acid for some time prior to the addition of furfural.

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TABLE I

Spectral Characteristics of the Color Formed by Sesamol and Various Aromatic Aldehydes in the Presence of Hydrochloric and Sulfuric Acids

Aldehyde plus synthetic sesamol [a]	Hydrochloric acid		Sulfuric acid	
	Wavelength (mμ)	Ext. coef. (after 30 min.)	Wavelength (mμ)	Ext. coef. (after 75 ± 10 min.)
Vanillin	552	244	542-544	32
Isovanillin	550-552	241	538	44
Furfural	518	167	516-520	127
Protocatechuic	546-554	248	542	54
Cinnamaldehyde	538-544	121[b]	.....	.....
Anisaldehyde	528-532	239	518	42
Piperonal	550-558	246	542	35
Salicylaldehyde	520	128	498-502	7
p-Hydroxybenzaldehyde	528-532	215	516-520	50
Furfural plus sesame oil	520-522	86	518-520	126

[a] The synthetic sesamol used in these tests was not highly purified. Extinction coefficients obtained with highly purified sesamol are approximately 10% higher.
[b] Turbid solution.

The use of aqueous sulfuric acid, approximately 1:2 by volume, was found to obviate the above-mentioned difficulties although with this acid the color develops more slowly than in the presence of concentrated hydrochloric acid.

A series of aromatic aldehydes which, like furfural, react with sesamol to form color bodies in the presence of strong mineral acids were tested with the results shown in Table I. In the presence of hydrochloric acid most of the aldehydes produced more intense colors than did furfural, but for the aforementioned reasons hydrochloric acid could not be used for the quantitative estimation of the resultant color. On the other hand, when sulfuric acid was used, furfural produced a stronger coloration than any of the other aldehydes which were tested. On the basis of the data in Table I it is apparent that the furfural-sulfuric acid reagent is the most suitable for applying the Villavecchia test as a quantitative method.

The color development as a function of time for synthetic sesamol in an ethanol solution, for sesamol in a soybean oil solution, and for sesame oil is shown in Figure 2. The maximum color intensity is reached after about one hour from the time the sample is treated with the furfural-sulfuric acid reagent. Between 50 and 75 minutes after the start of the reaction the color intensity remains practically constant, and optical measurements can be made during this period. The color produced by the reaction of sesame oil after successive dilutions with the sulfuric acid medium was found to follow Beer's law, *i.e.*, the optical density was inversely proportional to the dilution factor.

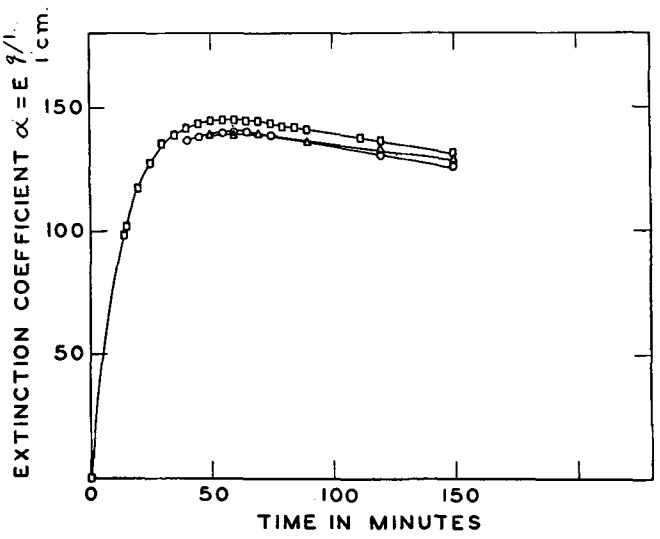

FIG. 2. Rate of color development in the system sesamol-furfural-sulfuric acid. Sesamol in soybean oil ○, sesame oil △, sesamol in ethanol □.

The calibration curve (optical density *vs.* amount sesamol) shown in Figure 3 was obtained by using two different procedures, namely, (a) synthetic sesamol was dissolved at different concentrations in refined soybean oil and the corresponding optical densities determined as for "total sesamol," and (b) synthetic sesamol was dissolved at different concentrations in ethanol and the optical densities determined as for "free sesamol." The resultant values obtained by the two procedures fall on a single straight line and show that the transfer of sesamol from the oil phase into the aqueous phase is entirely quantitative.

A Beckman Model DU spectrophotometer was used to determine the optical densities. The calibration curve is a straight line, the slope of which corresponds to an extinction coefficient of 140. Therefore if the optical density is known a simple calculation will give the concentration of sesamol in the sample. However, if another instrument is used it usually will be necessary to construct a calibration curve.

Quantitative Method

Reagents. The following reagents are required for the quantitative determination of free and bound sesamol: (a) Optically pure iso-octane, (b) optically pure ethanol (99%), (c) sulfuric acid, sp. gr. 1.37 at 15°C., (d) 2% solution of furfural prepared by dissolving 2 g. of freshly redistilled furfural in 100 ml. of ethanol, (e) potassium hydroxide solution prepared by dissolving 10 g. of reagent grade pellets in 80 ml. of distilled water and adding 20 ml. of ethanol (99%).

A slightly yellow color in the furfural solution is not objectionable. The solution is stable if it is stored in an ice box.

Apparatus. The following apparatus is required: a) Beckman spectrophotometer and 1-cm. absorption cells, b) 100-ml. separatory funnels, c) 125-ml., glass-stoppered Erlenmeyer flasks, d) 1-, 2-, and 50-ml. volumetric pipettes, e) 1-ml. pipettes graduated in 1/100 ml., and f) mechanical shaker.

Procedure. Dissolve 10 g. of the oil to be tested in iso-octane and make up to 100 ml. with the same solvent. This solution serves for the determination of total, free, and bound sesamol.

A. Determination of Total Sesamol. A 50-ml. portion of the sulfuric acid solution is pipetted into a 125-ml., glass-stoppered, Erlenmeyer flask. A 1-ml. portion of furfural solution is added, followed by the addition of 2 ml. of the oil solution. The flask is tightly stoppered and shaken on a mechanical shaker for 30 minutes. The contents of the flask are then poured into a 100-ml. separatory funnel, and the layers allowed to separate. The acid layer may present a slight initial turbidity which will clear up gradually. At the time the color is measured no significant turbidity should be observed. A 1-cm. absorption cell is filled with a portion of the colored

acid layer, and its optical density at 518 mμ is read between 50 to 75 minutes from the moment shaking was started, against a blank obtained under similar conditions but without added furfural.

A reagent blank consisting of sulfuric acid and furfural only need not be used as it does not present any appreciable absorption against pure ethanol or water at 518 mμ.

Under the above-described conditions the precentage of total sesamol in the sample is calculated as follows:

$$\% \text{ sesamol} = \text{optical density} \times 0.183$$

where the factor 0.183 = vol. aqueous soln./2 × ext. coef. This relation may not hold if an instrument other than the Beckman spectrophotometer is used, in which case a calibration curve must be drawn.

B. Determination of Free Sesamol. A 50-ml. portion of the original oil solution is pipetted into a 100-ml. separatory funnel and either 5 or 10 ml. of potassium hydroxide solution are added. The 10-ml. quantity is used if the free sesamol content is above 0.05%.

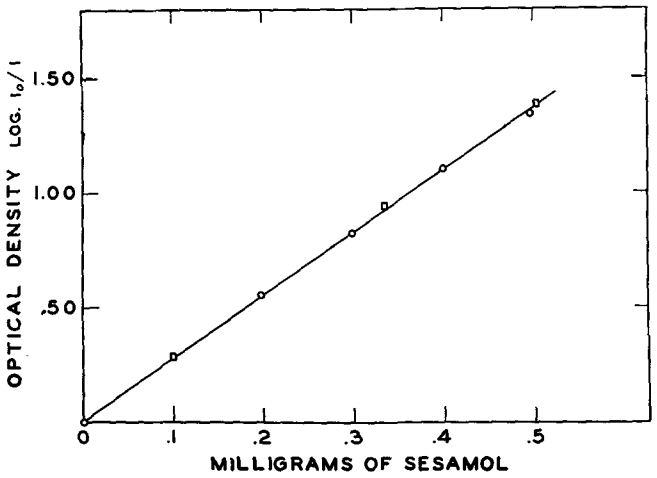

FIG. 3. Calibration curve for the spectrophotometer determination of sesamol in soybean oil ○, and in ethanol □.

The separatory funnel is shaken vigorously for three minutes and then allowed to stand for 30 minutes to permit the solution to separate into two layers. The alkaline layer is decanted through a small filter paper and used for the determination of free sesamol. The bound sesamol is determined in the residual oil solution.

A 50-ml. portion of the sulfuric acid solution is pipetted into a glass-stoppered flask and 1 ml. of furfural solution is added, followed by 0.6 ml. of the alkaline extract. Only a small amount of alkaline solution is used so that the color intensity will not be affected by the slight change in the strength of the sulfuric acid.

The flask is stoppered and inverted several times to mix the contents. A 1-cm. absorption cell is filled with a portion of the colored solution and the optical density read during the interval of 50 to 75 minutes after mixing the reagents, against a blank obtained under similar conditions with ethanol instead of furfural solution.

The content of free sesamol in the sample is calculated as follows:

$$\% \text{ free sesamol} = \text{optical density} \times V \times 0.0122$$

where V is the volume (ml.) of potassium hydroxide solution used for the extraction of the free sesamol. Here too a calibration curve will be necessary if an instrument other than the Beckman spectrophotometer is used.

TABLE II

Recovery of Sesamol Added to Two Refined and Bleached Sesame Oils

Sesamol added mg./100 g.	Sesamol found, mg./100 g.			Sesamol recovered mg./100 g.		Recovery, %	
	(free)	(bound)	(total)	(free)	(total)	(free)	(total)
0	20.3	71.6	91.5	.....	.....	.....	.....
39.5	62.2	72.1	131.8	41.9	40.3	106	102
80.6	104.9	72.1	172.1	84.6	80.6	105	100
0	13.6	116.6	128.1	.....	.....	.....	.....
19.0	31.8	116.8	146.2	18.2	18.1	96	95
40.9	56.7	116.8	170.6	43.1	42.5	105	104

C. Determination of Bound Sesamol. The alkali-treated oil solution from B, which contains only bound sesamol, is filtered if it is not clear, and the bound sesamol determined exactly as described for the total sesamol. The same blank may be used for the total and the bound sesamol.

The bound sesamol may be expressed as sesamolin using the relation

$$\text{Sesamolin} = \text{bound sesamol} \times 2.68$$

where the numerical factor represents the ratio of the molecular weights of sesamolin and sesamol, respectively.

Application of Method

The above-described method was applied to two refined and bleached sesame oils to which different amounts of synthetic sesamol had been added. The results which are shown in Table II indicate that the recovery varies from 95-106%. The method was also applied to determine the percentage of free, bound, and total sesamol in four different sesame oils whose characteristics have been described elsewhere (2). In Table III the value obtained for the total sesamol is compared with the sum, "free plus bound sesamol." The maximum difference between the two values for the total content of sesamol thus obtained is 0.004%, for the samples tested. It is believed that the above-described method yields results which are accurate to about 0.005%. The slight increase in the values for the total and bound sesamol which is noted in two of the samples during refining is within this range and is therefore not significant. The sesamol content of a sample of sesamolin pre-

TABLE III

Percentage of Free, Bound, and Total Sesamol in Crude and Processed Sesame Oils

Sesame oil	Free (A)	Bound (B)	Total (C)	Free+Bound (A+B)
SO-1				
Crude	0.001	0.144	0.144	0.145
Refined	0.000	0.143	0.142	0.143
Bleached[a]	0.014	0.117	0.132	0.131
Deodorized	0.001	0.119	0.119	0.120
SO-2				
Crude	0.003	0.133	0.135	0.136
Refined	0.001	0.139	0.137	0.140
Bleached[a]	0.011	0.117	0.125	0.128
Deodorized	0.001	0.099	0.096	0.100
SO-3				
Crude	0.001	0.135	0.135	0.136
Refined	0.001	0.134	0.133	0.135
Bleached[a]	0.006	0.124	0.130	0.130
Deodorized	0.001	0.001	0.002	0.002
SO-4				
Crude	0.001	0.161	0.162	0.162
Refined	0.001	0.164	0.165	0.165
Bleached[a]	0.011	0.151	0.161	0.162
Deodorized	0.001	0.007	0.008	0.008

[a] The refined oils were bleached with 2% of B.-C. clay.

pared from sesame oil was found to be 35.1%, which agrees reasonably well with the calculated value of 37.3%.

The method has been applied to the determination of free and bound sesamol in a number of samples of crude, refined, bleached, hydrogenated, and deodorized sesame oils as part of an investigation of the role played by sesamol in the stability of these oils.

Summary

The Villavecchia test was adapted for the quantitative determination of sesamol in sesame oil. Of the several aromatic aldehydes tested, furfural, in the presence of aqueous sulfuric acid (sp. gr. 1.37 at 15°C.), proved to be the most suitable for the quantitative measurement of the color produced with sesamol.

The oil, dissolved in iso-octane (10 g. per 100 ml.), is shaken with dilute aqueous-alcoholic potassium hydroxide to remove the free, but not the bound sesamol. The modified Villavecchia test is applied to a) the original iso-octane solution of the oil, b) the iso-octane solution after extraction with alkali, and/or c) the aqueous-alcoholic extract. The optical densities, read at a wavelength of 518 mμ, correspond to the total bound, and free sesamol, respectively.

The method was applied to the determination of free, bound, and total sesamol in a number of crude, refined, bleached, and deodorized sesame oils. Agreement between the values for total and free plus bound sesamol was close. Four crude sesame oils of different origins had total contents of sesamol ranging from 0.13 to 0.17%, of which nearly all was in the bound form. The yields of added sesamol varied from 95 to 106%.

REFERENCES

1. Andraos, V., Swift, C. E., and Dollear, F. G., J. Am. Oil Chem. Soc. *27*, 31-34 (1950).
2. Menezes, F. G. T., Budowski, P., and Dollear, F. G., J. Am. Oil Chem. Soc. *27*, 184-186 (1950).
3. Budowski, P., J. Am. Oil Chem. Soc., *27*, 264-267 (1950).
4. Honig, P., Chem. Weekblad, *22*, 509-512 (1925).
5. American Oil Chemists' Society, Official and Tentative Methods, 2nd ed., Chicago, 1946.

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Molecularly Distilled Monoglycerides[1] I. Preparation and Properties[2]

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THE widespread use of monoglycerides has developed in the last decade although methods for preparing them have been known since the mid-19th century. In the presence of an alkaline catalyst, either direct esterification of fatty acids or interesterification of fats provides a convenient method for preparing monoglycerides. The reaction product is a mixture of monoesters, diesters, and triesters as well as free glycerol, free fatty acid, and the catalyst in the form of metallic soaps. The term monoglyceride has been loosely applied to this total reaction mixture in which the glyceryl monoester is frequently a minor constituent.

Up to the present there has been little or no concentration of monoester from such a mixture on a commercial basis. The wide range in molecular weights of the components of the mixture suggests a distillation process, but ordinary distillation is difficult because of the low vapor pressure and thermal instability of the monoester. Molecular distillation however is readily effective for separating the monoglyceride from the reaction mixture.

Molecular distillation is well-known and should not need any discussion in this paper. Essentially pure monoglycerides were obtained by molecular distillation of the reaction products of glycerol and a partially hydrogenated vegetable oil. A sample of one of the well-known products of the commercial monoglyceride mixture type was distilled on a 5-inch centrifugal molecular still (1).

Table I shows the temperature and pressure during the distillation of the monoglyceride mixture. Two small 5% cuts were taken to remove the low molecular weight components. Three 10% fractions, assaying over 90% monoester, were obtained from the original 41% monoglyceride mixture. The monoester was determined by the periodic acid oxidation method of Pohle, Mehlenbacher, and Cook (2) as modified by Handschumaker and Linteris (3).

The graph in Figure 1 shows the concentration of the various components. The bulk of the glycerol and fatty acids shown by the hatches is removed in the forepart of the distillation. The free fatty acids were determined by the A.O.C.S. procedure (4) and the glycerol by the method of Bradford, Pohle, Gunther, and Mehlenbacher (5). About 50% of the volatile glycerol escapes condensation on the condenser surface and is collected in the dry ice trap which is ahead of the pumping system. The major portion of the monoester is obtained in a substantially pure form and only small amounts of monoester are found in the di- and triglyceride fractions.

The above example is typical of the method used to prepare purified monoglyceride concentrates from a variety of oils and fatty acids. Data are presented

TABLE I

Operation Data for the Separation of Monoesters by Molecular Distillation of a Partially Hydrogenated Vegetable Oil Monoglyceride Mixture

Fraction	Temperature °C.	Pressure, mm. Hg.	% Cut	% Monoester
Charge	.....	.....	(100)	41
1	80-140	0.018	5.2	67
2	140-142	0.016	4.8	84
3	142-148	0.011	9.2	93
4	148-153	0.04	10.0	97
5	153-168	0.04	9.9	94
6	168-200	0.04	10.0	23
7	200-206	0.04	9.9	12
Residue	.....	.....	39.6	1

[1] Presented at the 23rd Fall Meeting, American Oil Chemists' Society, Chicago, Ill., Oct. 31, Nov. 1 and 2, 1949.

[2] Communication No. 165 from the Laboratories of Distillation Products Industries.