other end. The total effective length of the column should be at least 35 cm. so as to accommodate the entire sample.
2. Flasks—250 ml. capacity extraction type fitted with 24/40 standard taper ground glass joint (Soxhlet type) and 100 cc. Erlenmeyer or extraction type.
3. Beakers—150 ml. and 400 ml., 1 liter.
4. Funnels—Corning No. 6380—250 ml. capacity.
5. Ring stand with duplex burrette clamp supports.
6. Vacuum oven.
7. Greiner—Friederichs Distillation Apparatus—Scientific Glass J 1210.

REAGENTS:

1. U.S.P. ethyl ether.
2. Absorbent cotton—preferably defatted.
3. Aluminum oxide—activated alumina—Grade F-20—Mesh 80-200 (Aluminum Ore Company, East St. Louis, Illinois) was found suitable as received.

METHOD:

Weigh a 2 to 3 gr. (accuracy ± 0.1 mg.) *representative* sample of the oil to be tested into a 100 cc. flask and dissolve the oil in 25 ml. diethyl ether. A representative sample for this analysis should be withdrawn from the 1-gal. official sample only after it has been heated to steam bath temperature and agitated thoroughly and vigorously.

Prepare the adsorption column, by plugging the drawn-out end with a piece of cotton, then add to it through a funnel a slurry consisting of 20 gr. ± 1 gr. of alumina and 30 ml. ether, and wash the column enough with ether to form a compact bed of adsorbent. Use liberal amounts of ether to effect the transfer because the column packs best if the slurry is maintained in a fluid condition as it flows into and distributes itself in the column. It is usually helpful to have some ether present at the bottom of the column to receive the first alumina to arrive. Under these conditions, air pockets, which impair the efficiency of the column, are generally avoided. Maintain a level of ether not less than ½ cm. in depth above the top of the alumina until such a time that the column is of no further use. This precaution must be taken to avoid air pockets brought about by the evaporation of ether.

Transfer the oil-ether solution to the column and then use 25 ml. of ether in at least 4 portions to effect a quantitative transfer. It is important to wash the lip of the beaker carefully with ether because the oil tends to creep. Wash the column with an additional 100 ml. of ether added in 3 or 4 portions from a 250 cc. Erlenmeyer, collecting the extract in a *clean, dry, tared*, ground glass, jointed 250 cc. extraction flask. The clean, dry, tared extraction flask used to collect the extract should be heated in a 105° oven and cooled in a desiccator before its tare weight is taken.

When the wash-ether has passed through, rinse off the tip of the column with a little ether and evaporate the solvent on a steam or water bath or preferably distill off the ether in a suitable all-glass still of the type frequently used to recover inflammable solvents. A stream of inert gas can advantageously be used to speed up the evaporation of the last traces of solvent. When practically all of the ether has been eliminated, wash down the sides of the flask with a little ether to consolidate the neutral oil and evaporate the solvent as before. Remove the flask from the bath, wipe the outside of it with a clean absorbent towel and bring the sample to constant weight at 105°C. *in an atmosphere of nitrogen.* Cool in a desiccator and weigh quickly to the nearest 0.1 mg. About 1 hr. in the oven is generally sufficient.

CALCULATION:

$$\% \text{ Neutral Oil} = \frac{\text{Weight of Extract}}{\text{Weight of Sample}} \times 100$$

NOTES:

1. Up to three columns can be conveniently carried along at one time when an operator becomes familiar with the technique.
2. The alumina may be reused by burning off the organic matter for 5 hrs. at 550°C. in a muffle furnace. After repeated use the alumina has to be screened to remove fine particles. Material passing a 200-mesh screen should be discarded.
3. Questionable batches of unused alumina, which have been exposed to air for long periods of time, must be reactivated by heating in a muffle furnace at 550°C. for several hours.
4. When high room temperatures prevail, it will be convenient to surround the column with a cold water jacket.
5. Method specifications for crude oils having 5% or less of loss constituents:

Weight of Sample	Diameter of Column-ID	Height of Column	Weight of Alumina	Volume of Ethyl Ether		
				To Dissolve	To Transfer	To Elute
gm.	*mm.*	*cm.*	*gm.*	*ml.*	*ml.*	*ml.*
2- 3	18	30	20	25	25	100
15-16	26	30	50	50	50	300
20-21	26	30	60	50	50	300

6. Method specification for high loss crude oils, tank settlings, and lecithins.

Approx. Amount of Loss Constituents	Weight of Sample	Diameter of Column-ID	Height of Column	Weight of Alumina	Volume of Ethyl Ether		
					To Dissolve	To Transfer	To Elute
%	*gm.*	*mm.*	*cm.*	*gm.*	*ml.*	*ml.*	*ml.*
5-15	2-3	18	40	36	50	50	150
15-25	1-2	18	40	36	50	50	150
25-50	0.7 -1	18	40	36	50	50	150
50-100	0.45-0.55	18	40	36	50	50	150

Sesame Oil. III. Antioxidant Properties of Sesamol[1]

PIERRE BUDOWSKI,[2] Southern Regional Research Laboratory,[3]
New Orleans, Louisiana

THE unsaponifiable fraction of sesame oil contains certain compounds which are not found in other natural fats and which confer on this oil certain unusual properties. For example, it has been known for 40 years that the characteristic Villavecchia or Baudouin test is caused by sesamol, a component of sesamolin, one of the unsaponifiable substances present in the oil. Another characteristic property of sesame oil is its unusual resistance to oxidative rancidity, especially after hydrogenation to shortening consistency. The present report is concerned specifically with the antioxidant properties of synthetic sesamol.

Considerable importance has been attached to the color reactions of sesame oil, particularly in Europe where this oil has been used to adulterate olive oil and where the addition of a certain amount (5 to 10%) of sesame oil to margarine has been obligatory for more than 50 years in order to render possible a rapid distinction between butter and margarine.

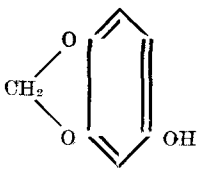
Older literature on the color tests given by sesame oil has been reviewed by Utz (1). Of the many different color tests proposed and discussed about 50

[1] Presented at the International Sesame Conference, Clemson Agricultural College, Clemson, South Carolina, August 15-16, 1949.
[2] Rockefeller Foundation Fellow from the Ministerio de Agricultura y Cria, Division de Quimica, El Valle, D. F., Venezuela.
[3] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

years ago, the Villavecchia test is the one which is widely used at the present time. The American Oil Chemists' Society (2) has adopted this test as official for the qualitative detection of sesame oil in mixtures of this and other oils.

The first crystalline compound to be isolated from sesame oil was sesamin (3-5), a compound which does not give the Villavecchia test, but which has been shown in recent years to produce a remarkable synergistic effect on the insecticidal action of the pyrethrins (6). Accompanying sesamin, a phytosterol was obtained which likewise was not responsible for the Villavecchia test.

In 1903 Kreis (7) obtained evidence of the presence in the oil of a phenolic compound which appeared to be responsible for the color test. His attempts to isolate this compound were unsuccessful. However he named the unknown substance "sesamol," pointing out that it might be present in the oil in a bound form, possibly as a glucoside. The same year Canzoneri and Perciabosco (8) succeeded in isolating from sesame seed a "substance X" melting at 92°C., which gave a strong Villavecchia test. This compound was resistant to the action of alkali but was easily hydrolyzed by mineral acids. One of the hydrolysis products, an oily substance, gave a very intense color test. In 1907 Malagnini and Armanni (9) isolated the same compound (m.p. 94°C.) from sesame oil; and from its hydrolysis products they were able to isolate a phenol which was proved by analysis and synthesis to be the methylene ether of hydroxyhydroquinone (I). It

I 

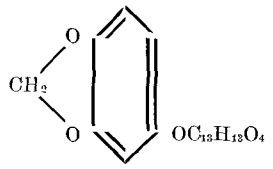
gave a strong color test and proved to be the phenol which Kreis had designated as "sesamol."

In 1926 Honig (10) reported that certain acid bleaching earths behaved like mineral acids toward "sesamin," splitting off sesamol, also that the steam distillate of sesame oils previously treated with such earths produced a strong Villavecchia test while the steam distillate from untreated oils gave no reaction with this test. Honig apparently confused Canzoneri's "substance X" with sesamin although these two substances were then known to be quite different. The same "substance X" was isolated and examined by Adriani (11) in 1928, who assigned to it the formula $C_{20}H_{18}O_7$ and gave it the presently accepted name, *sesamolin*. The melting point of sesamolin was reported to be 93.6°C. and the optical rotation in chloroform solution was [d] = 218.4° (4.0 g./100 ml.). By comparing the color intensities given by sesamol and sesamolin respectively, Adriani concluded that one mole of sesamolin yielded one mole of sesamol in the presence of concentrated hydrochloric acid, according to the reaction:

$$\underset{\text{Sesamolin}}{C_{20}H_{18}O_7} + H_2O \longrightarrow \underset{\text{Sesamol}}{C_7H_6O_3} + \underset{\text{Samin}}{C_{13}H_{14}O_5}$$

Adriani isolated the compound $C_{13}H_{14}O_5$ which he called "samin" (m.p. 103°C.), thus substantiating the hydrolytic reaction.

In 1936 Boeseken *et al* (12) described the synthesis of sesamol from piperonal, using peracetic acid, as an oxidizing agent. They also synthesized the β-glucoside of sesamol and showed that his compound gave the Villavecchia test with a slowly increasing intensity, very much like sesame oil, while sesamol produced the color rapidly. On the basis of this evidence it was presumed that sesamolin probably was a glucoside of sesamol. Unfortunately the authors while referring to Adriani's work, wrote the formula of samin with 14 carbon atoms instead of 13, leaving the equation for the hydrolysis reaction of sesamolin unbalanced; furthermore the structures which were assumed for sesamolin and samin lacked any justification whatsoever. As no further work on sesamolin or sesamol has appeared since then, a certain amount of confusion exists at present, concerning the structure of sesamolin. The following formula (II) ac-

II 

counts for the few known properties of sesamolin without making unjustified assumptions.

The Antioxidant Activity of Synthetic Sesamol

Although mention of the inhibitol effect of sesamol has been made by Olcott and Matill (13), Lundberg (14), and Ralston (15), no data appear to have been published on this subject. The antioxidant activity of synthetic sesamol was investigated with lard and refined peanut, cottonseed, and sesame oils as substrates. The inhibitory action of sesamin, sesamolin, and a phytosterol obtained from sesame oil was also determined.

Synthesis of Sesamol. Boeseken *et al.* (12) reported the synthesis of sesamol acetate by oxidation of piperonal with peracetic acid, a reaction somewhat similar to the "Dakin reaction" between hydrogen peroxide and aromatic aldehydes having a free phenolic group in the ortho or para position (16). Boeseken *et al.* (12) showed that the reaction proceeded according to the following equation:

$$CH_2O_2C_6H_3CHO + CH_3CO_3H = CH_2O_2C_6H_3OCOCH_3 + HCOOH$$

By saponification of the sesamol acetate they obtained a yield of 60% of sesamol.

The procedure followed in the present work was essentially similar to that described by Boeseken *et al.* except that an 11% peracetic acid solution was used instead of the less readily prepared 20% solution.

The 11% peracetic acid solution was prepared by adding dropwise a 30% hydrogen peroxide solution containing 0.3% of *p*-toluenesulfonic acid (as catalyst) to the exact amount of acetic anhydride required by the equations:

$$H_2O_2 + (CH_3CO)_2O \longrightarrow CH_3CO_3H + CH_3CO_2H$$
$$H_2O + (CH_3CO)_2O \longrightarrow 2CH_3CO_2H$$

The rate of addition was regulated in such a manner that the temperature of the reaction mixture did not rise above 35°C.

Piperonal was then allowed to react with a slight excess (10% of theory) of the peracetic acid solution, following the procedure indicated by Boeseken *et al.* (12).

The yield of sesamol obtained by this method was 22%, and the product had a light brown color. After two recrystallizations from a chloroform-petroleum naphtha mixture, colorless needles (m.p. 63°C.) were obtained in a final yield of 15%. In subsequent syntheses of sesamol, yields of more than 50% were obtained using an anhydrous 20% peracetic acid reagent prepared from 40% commercial peracetic acid by addition of acetic anhydride to remove water and hydrogen peroxide, sodium acetate to neutralize the free sulfuric acid, *p*-toluene sulfonic acid as catalyst, and glacial acetic acid to adjust the concentration.

Analysis. Calculated for $C_7H_6O_3$ C 60.87 Found C 60.48; 60.55
H 4.38 H 4.39; 4.40

As noted by Boeseken *et al.*, the pure product had a tendency to darken in contact with the air and the color will be retained on recrystallization, but sublimation under 1 mm. vacuum at 60°C. yields a colorless product.

Isolation of Sesamolin From Sesame Oil. The isolation of sesamolin has been reported by Canzoneri and Perciabosco (8), Malagnini and Armanni (9), and Adriani (11). Kaku *et al.* (17) also obtained sesamolin as a by-product in the preparation of sesamin from sesame oil. Aside from the time-consuming and tedious procedure described by Canzoneri and Perciabosco (8), who used sesame seed as a starting material, no experimental data on the isolation of sesamolin appear in the publications referred to above.

The following procedure was used to obtain sesamolin, together with sesamin, and a phytosterol as a by-product.

The oil was dissolved in acetone (1:8) and the mixture allowed to stand overnight at −50°C. The liquid fraction separated from the glyceride crystals was freed from solvent, mixed with five parts of iso-octane, and allowed to stand in the ice box (about 5°C.) for three days. Crystals separated, which after filtration and recrystallization from ethanol proved to be sesamin (m.p. 123°C.). However sesamin is more simply obtained by extraction of the oil with acetic acid, a procedure used by Tocher (4), Bertram *et al.* (18), and Jacobsen *et al.* (19).

Analysis. Calculated for $C_{20}H_{18}O_6$ C 67.80 Found C 67.62; 67.73
H 5.13 H 5.14; 5.21

The filtrate, after evaporation of the iso-octane, was saponified with alcoholic potassium hydroxide, the mixture diluted with water and extracted with ethyl ether. The ether was evaporated and the residue taken up in chloroform. Petroleum naphtha was added until turbidity appeared. After standing for several hours, sesamolin crystallized from the solution in colorless crystals which after recrystallization from a mixture of chloroform and petroleum naphtha melted at 93-94°C.

Analysis. Calculated for $C_{20}H_{18}O_7$ C 64.86 Found C 64.80; 64.80
H 4.90 H 4.98; 4.84

The mother-liquors contained a phytosterol which was obtained by removing the mixed solvent by evaporation and taking up the residue in hot, 95% ethanol. After cooling phytosterol crystals separated which after two recrystallizations from 95% ethanol melted at 137.5°C.

Stability Tests. Antioxidant activity was determined by the active oxygen method at a temperature of 97.7°C. The different compounds tested were dissolved directly in the lard or vegetable oil substrates. Phosphoric and citric acids, when used, were added in the form of alcoholic solutions containing 1.0 mg. of 85% phosphoric acid and 1.0 mg. crystallized citric acid per ml., respectively.

The time in hours required to reach a peroxide value of 20 and 100 milli-equivalents per kilogram of lard and vegetable oil, respectively, was taken as the "keeping time."

TABLE I

Antioxidant Activity of Sesamol in Prime Steam Lard

Antioxidant added		Stability, A.O.M., hours[a]	
Type	%	Lard A	Lard B
Sesamol	0.0	1.75	1.2
Sesamol	0.005	11	16
Sesamol	0.01	23	28
Sesamol	0.05	36	52
Sesamol	0.50	115	....
N.D.G.A.[b]	0.01	20	....

[a] Time required to attain a peroxide value of 20 m.e./kg.
[b] Nordihydroguaiaretic acid.

Results

The keeping times of two different samples of lard to which varying amounts of sesamol were added are given in Table I. For purposes of comparison, the protective action of nordihydroguaiaretic acid in a concentration of 0.01% is included. As can be seen from this table, the antioxidant activity of sesamol in lard at a concentration of 0.01% is comparable to that of nordihydroguaiaretic acid. Of particular interest is the fact that the antioxidant efficiency of sesamol continues to increase with increasing concentrations, even at comparatively high levels, which behavior contrasts with that of α-tocopherol which reaches a maximum protective efficiency at about 0.05% concentration (20).

It was observed that, during aeration at elevated temperature, a reddish brown color was formed, which increased in intensity up to the end of the induction period but disappeared again shortly after the onset of organoleptic rancidity. The same phenomenon occurred with the vegetable oils containing sesamol. It was also found that sesamol was destroyed during the aeration and disappeared entirely at the end of the induction period.

The stabilities of lard containing added sesamin, phytosterol, and sesamolin are given in Table II. A combination of sesamolin and phosphoric acid was also tested, as phosphoric acid might be expected to split off sesamol from sesamolin. However none of

TABLE II

Effect of Unsaponifiable Components of Sesame Oil on the Stability of Prime Steam Lard

Compound added		Stability A.O.M., hours[a]
Type	%	
None	.....	1.0
Sesamin	0.05	1.0
Phytosterol	0.05	1.0
None	.....	0.85
Sesamolin	0.02	0.86
Phosphoric acid (85%)	0.005	1.8
Phosphoric acid (85%) plus sesamolin	0.005 0.02	2.0

[a] Time required to attain peroxide value of 20 m.e./kg.

these compounds and combinations thereof proved to possess any significant activity. The sesamolin-phosphoric acid combination was only very slightly superior to phosphoric acid alone. It must be emphasized however that the active oxygen method is carried out under conditions very different from those prevailing in actual storage and that over long periods of time free sesamol might be liberated in sufficient amounts to affect significantly the stability of the substrate. While this question cannot be decided at present, the data presented here indicate that sesamolin is inactive as an antioxidant, even in the presence of small amounts of phosphoric acid.

The synergistic effect of phosphoric and citric acid on the antioxidant action of sesamol in lard is shown in Table III. A pronounced effect is noted for both acids.

TABLE III

Effect of Phosphoric and Citric Acids on the Antioxidant Activity of Sesamol in Prime Steam Lard

Compound added		Stability A.O.M., hours[a]
Type	%	
None	.....	1.0
Sesamol	0.01	21
Phosphoric acid (85%)	0.005	1.6
Phosphoric acid (85%) plus sesamol	0.005 0.01	32
Citric acid	0.005	1.9
Citric acid plus sesamol	0.005 0.01	32

[a] Time required to attain a peroxide value of 20 m.e./kg.

The antioxidant activity of sesamol in various vegetable oil substrates is shown in Table IV. It can be seen that sesamol is appreciably active in all of the oils in which it was tested. Of particular interest is the marked response of sesame oil to the addition of sesamol. The deodorized sample kept 40 hours upon the addition of 0.050% sesamol while the undeodorized sample kept 34 hours, with a similar free sesamol content. The explanation for this difference in behavior probably resides in the fact that the undeodorized sample had an initial peroxide value of 5.5 m.e./kg., while the deodorized oil had a value which was less than 1.

TABLE IV

Effect of Sesamol on the Stability of Peanut, Cottonseed, and Sesame Oils

Type of oil	Sesamol added, %	Stability A.O.M., hours[a]
Peanut, refined	none	6.0
Peanut, refined	0.05	19
Peanut, RBD[b]	none	10.5
Peanut, RBD[b]	0.05	27
Cottonseed, refined	none	6.3
Cottonseed, refined	0.05	10
Cottonseed, RBD[b]	none	7.5
Cottonseed, RBD[b]	0.05	11.5
Sesame, refined, bleached[c]	none	14.5
Sesame, refined, bleached[c]	0.045	34
Sesame, RBD	none	8.5
Sesame, RBD	0.05	40

[a] Time required to attain a peroxide value of 100 m.e./kg.
[b] Refined, bleached, and deodorized.
[c] Original sesamol content, 0.005%.

Summary and Conclusions

Sesamol possesses marked antioxidant activity in lard and also exhibits a pronounced protection for vegetable oils, especially for sesame oil.

The antioxidant activity of sesamol strengthens the assumption that free sesamol is responsible for the unusual stability of hydrogenated sesame oil, a subject which has been reported in another article in this series.

The use of sesamol as a commercial antioxidant would probably not be permitted until it has been shown to possess no undesirable physiological activity. However the presence of sesamol in concentrations up to 0.2% in sesame oil, one of the oldest edible oils known to man, would probably indicate that no serious adverse physiological problem exists in this respect. Although usually present in a bound form (sesamolin), this sesamol is known to be liberated by the action of mineral acids, even when quite dilute.

Sesamol is a very low molecular weight compound which is sufficiently volatile to be removed by deodorization, consequently it should be added to the fat after deodorization.

Acknowledgment

The author is greatly indebted to F. G. Dollear, W. G. Bickford, and C. E. Swift for advice and helpful suggestions; and to L. E. Brown, Analytical, Physical Chemical, and Physical Division, for the microanalytical data reported here.

REFERENCES

1. Utz, F., Apoth.-Zeit., *15*, 28-29, 38-39 (1900).
2. American Oil Chemists' Society, Official and Tentative Methods, 2nd ed., 1946.
3. Tocher, J. F., Pharm. J. and Trans., 1890/91, 638-640.
4. Tocher, J. F., Pharm. J. and Trans., 1892/93, 700-702.
5. Villavecchia, V., and Fabris, G., Zeitschr. angew. Chem., *17*, 505-506 (1893). See also *J. Soc. Chem. Ind.*, *1894*, 69.
6. Haller, H. L., McGovran, E. R., Goodhue, L. D., and Sullivan, W. N., J. Org. Chem., *7*, 183-184 (1942).
7. Kreis, H., Chemiker-Zeit., *27*, 1030-1031 (1903).
8. Canzoneri, F., and Perciabosco, F., Gaz. chim. ital., *33 II*, 253-260 (1903).
9. Malagnini, G., and Armanni, G., Rend. soc. chim. ital., *5*, 133-137 (1907). See also Chemiker-Zeit., *31*, 884 (1907).
10. Honig, P., Chem. Weekblad., *22*, 509-512 (1925).
11. Adriani, W., Z. Untersuch. Lebensm., *56*, 187-194 (1928).
12. Boeseken, J., Cohen, W. D., and Kip, C. J., Rec. trav. chim., *55*, 815-820 (1936).
13. Olcott, H. S., and Mattill, H. A., Chem. Revs., *29*, 257-268 (1941).
14. Lundberg. W. O., A Survey of Present Knowledge, Researches, and Practices in the United States Concerning the Stabilization of Fats, The Hormel Institute of the University of Minnesota, Publication No. 20, August, 1947.
15. Ralston, A. W., Fatty Acids and Their Derivatives, Wiley, 1948.
16. Dakin, H. D., Am. Chem. J., *42*, 477-499 (1909).
17. Kaku, T., Kutani, N., and Takahashi, J., Pharm. Soc. J. (Japan), *56*, 80-91 (1936).
18. Bertram, S. H., Van der Steur, J. P. K., and Waterman, H. I., Biochem. Z., *197*, 1 (1928).
19. Jacobson, M., Acree, F., Jr., and Haller, H. L., Ind. Eng. Chem., *16*, 166-167 (1944).
20. Oliver, G. D., Singleton, W. S., and Bailey, A. E., Oil & Soap, *21*, 188-193 (1944).

[Received January 5, 1950]