

THE CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF SESAME OIL

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I. INTRODUCTION

Sesame ranks ninth among the thirteen vegetable oil crops which account for approximately 90 per cent of the world's production of vegetable oils (98a). It is one of the two (the other is the coconut) oldest oilseeds known to man. Despite the significance of sesame to the agriculture of many areas of the world and also its economic and industrial importance, neither the seed nor its derived oil and meal have received the attention of the research scientist that its apparent importance would seem to warrant. This is true especially of the oil, which contains more unusual minor components and exhibits more unusual chemical and physiological properties than any other common edible oil. Such work as has been reported on sesame oil is scattered in many journals, the majority of which are in other languages than English. For example, less than half the titles included in Swingle's (215a) library list of two hundred and sixteen references covering the literature on genetics, cultural practices, history of production, marketing statistics, chemistry, nutrition, and utilization of sesame and its derived products are in English; and of the more than two hundred and fifty articles referred to by the present authors³ only about a third are in English. It is hoped that the following review of the present status of sesame research and the discussion of the unusual constituents of this oil will stimulate others to undertake work in this field, particularly with respect to the minor components

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of the oil, their unusual behavior during processing, and their physiological activity.

Sesame oil is obtained from the seed of several species of *Sesamum*, a member of the Pedaliaceae family. In addition to the cultivated *S. indicum*, seventeen wild species have been reported (254k) as occurring in Africa and two others in India that are not found in Africa. Food Inspection Decision No. 169 of the U. S. Department of Agriculture (228) refers to *S. indicum* De Candolle, *S. radiatum* Schum and Thorn, and *S. orientale* L. The nomenclature and the botanical classification of the different species and subspecies of sesame seem to be somewhat confused (144, 149, 200). The names of Linnaeus, Loew, or De Candolle are attached to *S. indicum*. According to *Standardized Plant Names* of the American Joint Committee on Horticultural Nomenclature, "*S. indicum* is the correct name under International Rules, whereas *S. orientale* (having line priority) was accepted under American Rules."

S. indicum is an herbaceous plant growing several feet high with a vegetative cycle of only 3 to 4 months. The botanical characters and cultivation of the sesame plant and the utilization of the seed have been described by several authors (32, 81, 100, 181) and especially by Langham and Rodriguez (144). A characteristic property of the sesame plant is the uneven ripening and dehiscence of its seed pods, which have heretofore made mechanical harvesting a difficult problem; consequently the cultivation of sesame has in the past been restricted to regions where labor was cheap and abundant.

The original home of sesame is unknown. Lewkowitsch (149) states that, according to De Candolle, sesame seed was brought from the Sunda Islands to India several thousand years ago and has migrated thence through the Euphrates to Egypt. The oil was used by the women of Babylonia as a cosmetic as well as a food. According to a reference given by Hedebrand (100), sesame is mentioned in old Hebrew and Egyptian scripts, such as the *Ebers Papyrus*, and different varieties and the manifold uses of sesame oil have also been described in ancient Sanskrit literature. The magic words "Open Sesame!" occurring in one of the tales of the *Arabian Nights* were probably inspired by the characteristic dehiscence of the sesame capsules. Marco Polo states in his travels through Persia that, "There is no oil of olives, but they express it from certain nuts, and from the grain called sesame, which resembles the seed of flax, excepting that it is light-coloured; and the oil this yields is better, and has more flavour than any other." Later in his travels he refers to the production of sesame oil in Abyssinia, Ceylon, and the Malabar Coast.

Sesame was introduced into the southern United States during the 17th century by Negro slaves who brought the seed with them from Africa. Some of the old Negro families still grow sesame for the confectionery markets in Charleston, South Carolina (254n).

Sesame is one of the highest-yielding, nonperennial oil plants. Langham and Rodriguez (144) have published data on the yields of sesame for the different countries in which it is cultivated. The grand average of all these figures is approxi-

mately 800 kg. per hectare, which is equivalent to about 400 kg. of oil per hectare (357 lb. per acre), considerably higher than the corresponding figures for peanuts, soybeans, and cottonseed (154a).

The seed of *S. indicum*, which is variously known as till or gingelly (India), simsim or benne (Africa), and ajonjoli (Latin America), is rich in oil (ca. 50 per cent) and protein (20–25 per cent). Other species of the genus *Sesamum* have been reported to contain as little as 31 per cent oil (13, 149, 200), and in some cases the derived oils do not give the Villavecchia test characteristic of the oil from the seed of *S. indicum*.

Sesame seed is mainly processed for oil, but it is also used extensively in confectionery and bakery products, in sesame butter, and for the preparation of milk-like drinks. The residual press cake is extensively used as a protein supplement for feeding livestock. The nutritive value of the seed and press cake has been investigated by several workers (2a, 57, 82, 83, 103, 120, 251, 254f, 254g).

The oil is processed for use as salad or cooking oil, shortening, margarine, and in the manufacture of soap. It is also used as a fixative in the perfume industry, and in the pharmaceutical industry it is used as a carrier for fat-soluble substances (9). At the turn of the century, sesame oil became an important article of commerce in Europe, when the admixture of a small amount of sesame oil to margarine was required by law. This requirement was based on the fact that sesame oil can be readily detected by a characteristic color reaction and the margarine therefore distinguished from butter. An important new use was found for sesame oil when its synergism with certain insecticides was discovered (63).

The world acreage devoted to the cultivation of sesame in the years immediately preceding World War II was approximately 11.5 million, of which about 5 million were in India, 3.5 million in China, and 1.5 million in Burma (240). The annual world production of sesame seed before World War II ranged from about 1.5 to 1.75 million tons. Asiatic countries accounted for from 90 to 95 per cent of this total (China about 55 per cent and India, with a larger acreage, 30 per cent).

International trade in sesame seed and oil is relatively small, as most of the seed produced is consumed domestically. The 1934–38 oil equivalent of sesame entering international trade was 68,800 tons (98a).

Varying and relatively small quantities of sesame seed, oil, and meal have been imported into the United States (71, 229, 230). Imports of sesame seed between 1923 and 1940 varied from 2.8 (1926) to 146.3 (1935) million pounds. Imports of sesame oil during the same period varied from 58,000 pounds (1933) to 39 million pounds (1937). Imports of sesame seed for 1947, 1948, and 1949 were 9.4, 22.6, and 10.8 million pounds, respectively, compared with the 5-yr. average (1937–41) of 10.6 million pounds. Imports of oil for the same years were 0.2, 1.4, and 0.25 million pounds, respectively, compared with the 5-yr. average (1937–41) of 9.9 million pounds. Prewar imports were mainly of Oriental (China and India) origin, whereas in recent years imports have originated principally in Latin America. For example, in 1948, 60 per cent of the imported sesame seed

came from Nicaragua and 15 per cent from El Salvador (230). The increasing amounts of seed imported into the United States in recent years from Central America reflect the rapid development of this crop in the latter area.

Edible sesame oil is dutiable at a rate of 2 cents per pound; inedible sesame oil (rendered unfit for use as food) is admitted free of duty but is subject to a 4.5 cents per pound import-excite tax. Imported sesame seed is also duty-free but is subject to an import-excite tax of 0.59 cent per pound, which is equivalent to a duty of 1.25 cents per pound on the oil content. Sesame meal is included in the 1930 Tariff, Paragraph No. 730, under the heading "All other vegetable oil cake and oil cake meals, not specially provided for," and is currently dutiable at a rate of 0.3 cent per pound. There are no data available on the proportion of imported sesame seed which is crushed and that which is consumed directly in culinary uses. The Tariff Commission has estimated that, of imports received in the 1930's, less than half was crushed, with the major portion being used in confectionery and in bakery products (152a).

Intensive breeding programs initiated recently in several countries, especially in the United States and Venezuela, have resulted in the development of improved varieties and strains, some of which have indehiscent capsules and are therefore better adapted for mechanical harvesting (144). Recent work on sesame, ranging from the genetics of the plant to the chemistry of the seed, was reported at the First International Sesame Conference, held at Clemson, South Carolina (254). The work reported at this conference reflected the increasing interest in this oilseed crop throughout the Western Hemisphere. In the United States the interest derives mainly from a desire to develop an oilseed crop especially adapted to the Cotton Belt as a replacement for declining cotton production. The successful introduction of sesame would provide not only an economic farm crop, but a source of raw material for the oil mills of the area, a high-quality edible oil, and an excellent source of protein feed.

II. EXTRACTION AND PROCESSING

In Europe, sesame oil has been traditionally obtained from the seed by successive expressions (11, 94, 122, 149) in hydraulic presses. The first expression, carried out in the cold, yields a high-grade oil, light in color and agreeable in taste and odor. Because of the high quality of this oil, it has been used much like olive oil without further processing other than clarification. The second expression, generally hot, yields a more highly colored oil, which is refined and used for edible purposes. The third expression, also hot, gives an oil of inferior quality, which is generally used for soap. Expellers of both high- and low-pressure types are likewise used for processing sesame. The former are now largely used in the United States and in Central and South America. Expeller-pressed oil is alkali refined, bleached, and deodorized. More recent practice in Europe is to use a combination of forepressing and solvent extraction for maximum recovery of oil.

An equation correlating the yield of oil expressed with the time and amount

of applied pressure, and the viscosity of the oil at the press temperature, has been proposed by Koo (135).

Direct solvent extraction of sesame does not seem to have been carried out commercially, probably because of the high oil content of the seed, but as long ago as 1903 and 1905, considerable amounts of press cake from damaged seed were extracted with carbon disulfide in the port of Marseille. The extracted oil was used for the manufacture of soap and the meal was used as fertilizer (149). Pilot-plant data on the extraction of sesame oil with commercial hexane were recently reported by Andraos *et al.* (6) and Menezes *et al.* (155).

A process involving forepressing, followed by extraction of the cake with hexane, has been patented by Schmidt and Webber (194). An extraction process based on the freezing of the moistened seed to rupture the cells has been patented by Musher (159).

The literature contains but few references (114, 171, 175, 189, 197, 224) to processing crude sesame oil. An investigation on alkali refining, bleaching, hydrogenation, and deodorization of different solvent-extracted sesame oils was reported by Andraos *et al.* (6) and Menezes *et al.* (155). All of the oils examined refined with very low losses and extremely light bleach colors. Investigations of the bleaching of sesame oil with different bleaching agents have been reported by Wagner (245), Honig (107), and Cohen (53).

The removal of free fatty acids from sesame oil with alcohol was investigated by Krebs (137), who reported that the oil could be practically completely freed from fatty acids by this means. Data on the steam consumption and alcohol losses were reported.

Paquot and Nagand (167) investigated the liquid-liquid extraction of sesame oil by solvents. Separation of the oil into fractions of different degrees of saturation was very inefficient. Furthermore, when the solvent consisted of a mixture containing ethanol, the extraction was "abnormal," i.e., the extracted fraction was more saturated than the insoluble fraction. When the solvent mixture did not contain ethanol, the extraction was "normal."

Hydrogenation of sesame oil has been investigated by several workers (6, 19, 23, 105, 124, 155) and was found to follow much the same course as cottonseed oil. The properties of sesame oil hydrogenated to shortening consistency have been reported by Andraos *et al.* (6) and by Menezes *et al.* (155).

III. COMPOSITION

Sesame seed and oil have been examined by numerous investigators (6, 10, 12, 27, 70, 100, 105, 118, 123, 130, 147, 155, 176, 186, 210, 212, 218, 219, 227). The data reproduced in table 1, taken from the work by Andraos, Swift, and Dollear (6), were obtained with a solvent-extracted sesame oil. Similar data were obtained by Menezes *et al.* (155) with the oils extracted from different varieties of sesame seed.

Hilditch and Riley (105) reported the presence of a small amount (0.5 per cent) of hexadecenoic acid in the unsaturated acid fraction of sesame oil. The

saturated acids are composed mainly of palmitic acid (about 8 per cent), stearic acid (4 per cent), and a small amount (0.4–1 per cent) of arachidic acid. Other acids present in the saturated acid fraction include 0.1 per cent myristic acid, reported by Hilditch and Riley (105), and a trace of lignoceric acid, reported by Jamieson and Baughman (123). Tsujimoto (226) found 1.2 per cent of highly unsaturated acids by a special lithium salt-acetone method. Hoover (108) reported 14.2 per cent of α -linoleic acid, using a bromination method. This value would be roughly equivalent to 28–30 per cent of total linoleic acid, a value which is abnormally low for sesame oil.

TABLE I
*Characteristics of crude sesame oil**

CHARACTERISTIC	VALUE
Free fatty acids (as oleic), per cent	1.0
Color, 5.25-in. cell, Lovibond yellow/red	35/4.3
Unsaponifiable matter, per cent	2.3
Smoke point, °F.	330
Flash point, °F.	607
Specific gravity, 25°/25°C.	0.918
Refractive index, n_D^{20}	1.4629
Optical activity, $[\alpha]_D^{20}$, 1-dm. cell†	1.44
Hydroxyl number	5.3
Reichert-Meissl value	0.51
Polenske value	0.47
Titer, °C.	22.0
Saponification value	185.8
Iodine value	112.0
Thiocyanogen value	76.3
Linolein, per cent‡	44.0
Olein, per cent‡	41.7
Saturated acid glycerides, per cent‡	14.3

* Andraos *et al.* (6).

† Value obtained by Menezes *et al.* (155) with oil of the same seed.

‡ Iodine-thiocyanogen method; saturated glycerides by difference.

The dextrorotation of sesame oil is unusual for an oil devoid of optically active fatty acid glycerides. The unsaponifiable fraction of sesame oil, however, contains minor constituents which are optically active and are responsible for the observed optical rotation of the oil (1, 25, 46a, 154).

The rapid determination of oil in sesame seed and meal has been the object of a number of investigations (20, 119).

IV. COLOR REACTIONS

A. Baudouin test

As early as 1850, Camoin observed that a red color was formed when sesame oil was shaken with concentrated hydrochloric acid to which a little cane sugar

had been added. This reaction was further investigated by several others, among them Baudouin, whose name has remained associated with this test. The literature prior to 1900 with respect to this and other color tests for sesame oil has been thoroughly reviewed by Utz (231).

B. Villavecchia test

In 1893, Villavecchia and Fabris (242, 243) modified the Baudouin test by replacing the cane sugar with furfural. These authors believed that in the presence of concentrated hydrochloric acid, the sugar condensed to yield furfural, which was supposed to be the reagent responsible for the test given by sesame oil. It has since been shown (157) that ω -oxymethylfurfural and not furfural is formed by the reaction between hydrochloric acid and sucrose. However, the test devised by Villavecchia and Fabris gained increasing acceptance; because it is a modification of the "Baudouin" test the latter name has also been retained, and the test is often referred to indiscriminately as the Baudouin or the Villavecchia test.

In 1897, the Villavecchia test acquired a legal status in Germany and other European countries when the addition of a small amount of sesame oil to margarine became obligatory to facilitate distinguishing the latter fat from butter. Similar laws are still in force in several European countries (90, 107, 190, 253) and in India. These laws generally specify that a margarine shall give a Villavecchia test of prescribed color intensity under specified conditions.

In the United States, a slightly modified Villavecchia test has been adopted as "official" by the American Oil Chemists' Society for the detection of sesame oil in mixtures with other oils (4).

Criticisms of the Villavecchia or Baudouin test have not been lacking. The question of whether or not furfural alone would produce red or purple colors with hydrochloric acid has been debated (203, 231). Actually, no color is formed with pure furfural in low concentrations in alcohol (133, 177).

The specificity of the Villavecchia test has been questioned, because olive oils from Algiers, Tunisia, Portugal, and elsewhere have been reported to respond positively to this test (62, 116, 173, 187, 225, 239). The following suggestions have been made to improve the specificity of this test: dilution of the colored acid layer with water [Carlifanti, referred to by Utz (231); Montefredine (158); Trevithick (225)]; addition of sodium hydrosulfite [Gassend, referred to by Utz (231)]; use of the fatty acids instead of the oil [Milliau, referred to by Utz (231)]; use of Domergue, referred to by Utz (231); Imbert and Durand (116)]; preliminary washing of the oil with sodium bicarbonate (116); preliminary treatment of the oil with alcoholic ammonia (173); dilution of the oil with cottonseed oil (109).

A number of workers have reported positive Villavecchia reactions for milk and butter fat from cows and goats fed with sesame press cake (7, 231, 241). Other workers (248) obtained negative reactions. The specificity of the test is important because of the legal aspect involved in its application. A related problem which has received no attention is the effect, if any, on the stability of

milk and butter fat obtained from animals fed on sesame press cake, because the compound responsible for the color test is also known to be an antioxidant for fats.

In cases where peanut oils were reported to give a positive Villavecchia test, it was shown that they were probably contaminated with sesame oil during processing (72, 195, 205). Pure peanut oil, extracted in the laboratory, does not give the test (205).

Curcuma and other coloring agents which may be present in butter or margarine interfere with the Villavecchia test by producing reddish colorations with hydrochloric acid (73, 235). This difficulty can be overcome, at least partially, by treatment of the fat with active carbon (248), by preliminary extraction of the fat with a more dilute hydrochloric acid (73, 161), or by adding a small amount of stannous chloride to the acid (16, 237). Casein has been reported to give a violet color under the conditions of the Villavecchia test; consequently, butter fat should be filtered to remove this substance before making the test (5, 41).

The influence of rancidity on the Villavecchia test has been discussed frequently. It is generally stated that rancidity appreciably reduces the intensity of the color formed or prevents its formation entirely (110, 211, 235, 237, 250). This, however, is not always true. Gravenhorst (90), in confirmation of Sprinkmeyer (211), states that the Villavecchia test given by a fresh sesame oil was partly inhibited by mixture with rancid oils, but that the original sesame oil still gave an equally intense test after it had become rancid. Lauffs and Huisman (146), as well as Utz (237), claim that interference in the color development due to rancidity can be largely overcome by the addition of fresh cottonseed oil. Alkaline washing of rancid oils has been claimed to remove substances which interfere with the Villavecchia test (90, 107). Hoyt (110) also recommended the use of a larger amount of furfural, preliminary extraction of the oil with hydrochloric acid, or heating to 200°C. for 30 min. in an inert atmosphere.

Such drastic treatments, however, also affect the intensity of the Villavecchia test. For example, prolonged heating of the oil is known to reduce the color intensity (35, 102, 208), and treatment of the oil with decolorizing agents has the same effect (35, 90, 102). Gravenhorst (90) reported that this reduction in the intensity of the color development may not become apparent until after deodorization, an observation which has been confirmed and explained by others (48, 107).

Hydrogenated sesame oil, according to Lewkowitsch (148), behaves capriciously with respect to the Villavecchia test. Prescher (174) observed that this test, as well as the Soltsien test, is still positive after sesame oils have been hardened by hydrogenation. Paal and Roth (166), as well as Jamieson (122), state that hydrogenation reduces the intensity of the color development, but that after storage the hydrogenated fat will give a more intense test. Other authors (17, 36, 112, 162) reported an increase in the intensity of the color development after hydrogenation. According to the most recent work (48), hydro-

generated sesame oils still give strong Villavecchia tests, but deodorization of the hardened oils produces marked decrease or complete absence of color development.

Besides furfural, a number of aromatic aldehydes have been shown to produce color reactions more or less similar to those given by furfural (40, 48, 65, 78, 81, 238). The absorption spectra of the corresponding colors have been investigated (48). Glucose (217, 232), fructose (66, 67, 249), and other sugars (66, 67) have also been used in place of furfural. Furfural, however, has remained the preferred reagent. Sulfuric acid has sometimes been used in place of hydrochloric acid (48, 133). Alcoholic hydrochloric acid has been claimed to produce stronger color development than aqueous acid (80). Some authors recommend the extraction of the oil with acetic acid (212) or alcohol (78, 80, 152) and carrying out the test on the extract. Wauters (247), as well as Hoyt (110), preferred to apply the Villavecchia test in the form of a "ring test" by slowly adding the oil to the furfural-hydrochloric acid mixture. Slight warming has been claimed to increase the intensity of the color obtained (59).

The influence of the concentration of the hydrochloric acid has been investigated by several workers (59, 90, 133, 180).

Some authors have attempted to adapt the Villavecchia test for the determination of the approximate content of sesame oil in mixed fats such as margarine (50, 136). It has been shown that sesame oils of different origins give different color intensities (48, 237) and that processing conditions markedly affect the Villavecchia test (47, 48, 252); therefore, the intensity of the color developed cannot be expected to bear a definite relationship to the amount of sesame oil present in a mixture of oils.

Many of the discussions reported in the literature concerning the value of the Villavecchia test have been obscured by the fact that the authors did not consider the nature of the compound responsible for the color reactions. This compound, sesamol, not only occurs in varying amounts in different sesame oils (48), but the amount present in a given oil has been shown to be markedly affected by different processing conditions (47, 48, 107). Furthermore, it may exist in either a free or a bound form, each of which has different properties (47, 48). It is not surprising, therefore, that margarines containing the prescribed amounts of added sesame oil may give Villavecchia tests of varying intensities (14). Because of these facts, difficulty may be experienced where a given intensity of the Villavecchia test is desired or set as a legal standard rather than a given percentage of sesame oil.

Probably some of these difficulties have led to attempts to replace the "sesame oil indicator" in margarine by specific chemical compounds. Phenolphthalein, starch, dimethylaminoazobenzene, and curcuma have been proposed (231) as such indicators. In this connection, it may be mentioned that oxyhydroquinone and its triacetic ester have been claimed to give a color test with furfural and hydrochloric acid which is very similar to that obtained with sesamol (115).

Instead of sesame oil, sesamol itself or a concentrate of sesamol produced by

distilling sesame oil under high vacuum could be used. Use of a concentrate fraction will give a more intense Villavecchia test, and can be employed with advantage for identifying margarine (117).

C. Soltsien test

In 1893 Soltsien (201) suggested the use of Bettendorf's reagent (solution of stannous chloride in concentrated hydrochloric acid) for the detection of sesame oil in olive oil. A rose to violet color was obtained if sesame oil was present. Subsequently, Soltsien (202, 207) elaborated details of the test, which various workers claimed to be more sensitive than the Baudouin or Villavecchia test, and claimed that it could be used where the latter test gave dubious results (37, 206, 232, 233, 235). Furthermore, according to some authors (235), the stannous chloride reaction is less subject to interference by the pigments present in butter or margarine. The intensity of Soltsien's test has also been claimed to be less affected by conditions of processing the oil (252), because the developed color is produced by the presence in the oil (209) of a different substance than that responsible for the Villavecchia test. It soon became apparent, however, that the stannous chloride reaction had serious drawbacks (42, 86, 149, 205, 212). Spices which are often present in chocolate and baked goods were found to interfere seriously with the reaction and the method soon lost prestige, while the Villavecchia test continued to be applied.

D. Behrens test

According to Tocher (222), Behrens' test for sesame oil was proposed in 1852. It consists in shaking an oil with a mixture of equal parts of sulfuric and nitric acids, whereupon a green color is obtained if sesame oil is present.

E. Tocher test

Tocher's test consists in shaking an oil with a solution of pyrogallol in concentrated hydrochloric acid, whereupon a purple color will form if sesame oil is present.

F. Bishop test

According to the test proposed by Bishop (30), if an oil is exposed to sunlight for a few days and then treated with concentrated hydrochloric acid a green color is observed if sesame oil is present. Kreis (138, 139, 141) suggested that this test could be used for the detection of rancid vegetable oils by observing whether a green color is formed upon addition of *fresh* sesame oil and hydrochloric acid to the oil suspected of being rancid. This test is known as the Bishop-Kreis test. The Baudouin and Bishop tests have been attributed to the presence in sesame oil of the same compound (146).

G. Cavalli test

A red color is observed when a fat containing sesame oil is treated with a mixture of hydrochloric and nitric acids (233).

H. Bellier tests

Three color tests for sesame oil were proposed in 1899 by Bellier (24). They were based on the reaction of (a) ammonium vanadate and sulfuric acid to give a green color turning to greenish black, (b) formaldehyde and sulfuric acid to give a bluish black color, and (c) resorcinol and nitric acid to give a bluish green color (55, 214).

I. Kreis "sesazo reaction"

Certain sesame oils give a red color when shaken with an aqueous solution of diazonaphthionic acid, followed by addition of alkali. This test is positive with all sesame oils, provided they have received a preliminary treatment with dilute sulfuric acid. This reaction was used by Kreis (140) to demonstrate the presence in sesame oil of a phenol (sesamol). A similar reaction was obtained by Malagnini and Armani (153), using diazo-*p*-nitroaniline.

J. Ambühl test

According to Ambühl (3), when rancid sesame oil or a rancid fat containing sesame oil is subjected to the Villavecchia test, an indigo-blue color is obtained instead of the crimson-red color given by fresh sesame oils. According to Kreis (138), the indigo-blue color is actually the result of blending the green color occurring in the Bishop test with the red color produced by the Villavecchia test.

K. Kreis test

If an oil is treated with a mixture of hydrogen peroxide and sulfuric acid, an olive-green color is observed in the presence of more than 5 per cent of sesame oil (38, 39, 140, 142).

L. Guarnieri test

When 1 ml. of an oil is treated with 2-3 drops of a solution of hydrogen peroxide in diethyl ether, followed by the addition of twice the volume of nitric acid (sp. gr. 1.40), a blue or green color is observed in the presence of sesame oil (93, 236).

M. Pavolini tests

T. Pavolini (170) found that treatment of an unknown oil with a mixture of sulfuric acid, diethyl ether, ethanol, and water would give a green color with a green fluorescence, if sesame oil was present. L. Pavolini (169) found that a green color was obtained when a fat containing sesame oil was shaken with a mixture of sulfuric acid and acetic anhydride. The same reaction had been used previously by Bömer for the detection of sesamin in mixtures of sesamin and phytosterol. If furfural was included in the mixture, a cherry-red color turning to blue-green was obtained.

N. Jacobson test

Jacobson *et al.* (121) obtained a greenish yellow color on treating sesame oil with a mixture of perchloric acid and hydrogen peroxide. They showed that the

same color was obtained with pure sesamin and used this reaction for the colorimetric determination of this substance.

V. MINOR CONSTITUENTS

In view of the practical significance of the color reactions of sesame oil, the problem of isolating and characterizing the compounds which are responsible for these reactions has attracted the attention of a number of workers. As the result of their investigations it soon appeared that sesame oil contained, in addition to phytosterol, two compounds which were not found in any other vegetable oil: namely, sesamin and sesamol. The latter compound upon acid hydrolysis yields sesamol, the methylene ether of oxyhydroquinone.

A. Sesamin

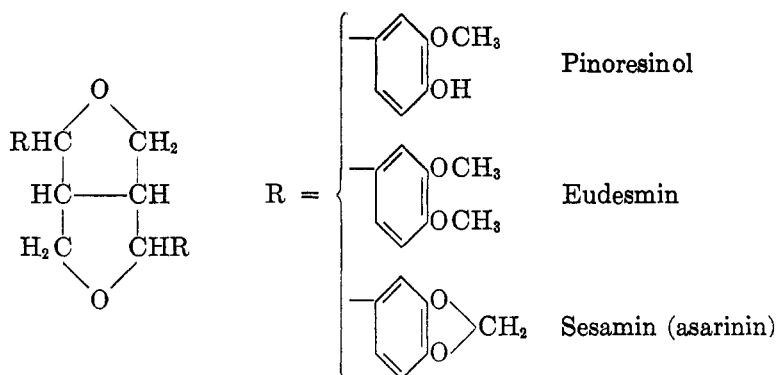
In 1890 Tocher (222, 223) isolated from the acetic acid extract of sesame oil a crystalline substance, melting at 117–118°C., which he called sesamin. The same compound, apparently in a purer state (m.p. 122.5°C.), was obtained several years later by Villavecchia and Fabris (242, 243) from the unsaponifiable fraction of sesame oil. The latter authors reported that sesamin was optically active, $[\alpha]_D^{22} = +68^{\circ}32'$. Although sesamin appeared to be rather inert chemically, Villavecchia and Fabris described the formation of two nitro derivatives. Heiduschka (101) found sesamin to have a molecular weight of 337–352 and proposed for it the formula $C_{20}H_{18}O_6$.

The correct molecular formula was established in 1928 independently by Adriani (1) and by Böeseken and Cohen (33) as $C_{20}H_{18}O_6$. The structure and chemical properties of sesamin were extensively investigated by Bertram, van der Steur, and Waterman (26), Böeseken and Cohen (33), and Cohen (54). Bertram *et al.* (26) found, and Böeseken and Cohen (33) confirmed the fact, that sesamin was isomerized under the influence of alcoholic hydrogen chloride to yield a compound, "isosesamin," which had a higher optical rotation than sesamin.

Kaku, Kutani, and Takahashi (126) showed that isosesamin was the optical antipode of *l*-asarinin, a compound which these authors had isolated from several species of *Asarum* (wild ginger). Following the publication of this work, investigations on the structure and chemical properties of sesamin (more correctly called *d*-sesamin) became an integral part of the work on *l*-asarinin. Erdtman (69) pointed out that sesamin is a member of the important group of "resinols," which may be considered as derivatives of 1,4-diphenylbutane or, by cyclization, of phenyl-naphthalene. This group of compounds includes conidendrin, matairesinol, hinokinin, cubebin, olivil, lariciresinol, guaiaretic acid, pinoresinol, and eudesmin.

Further work on sesamin, especially its relation to asarinin, was reported by Huang-Minlon (111). Kaku and Ri (127) isolated *l*-sesamin (isoasarinin) from *Asarum sieboldi* Miguel, var. *Seoulensis* Nakai. The same authors were able to convert *d*-sesamin into pinoresinol dimethyl ether and *l*-asarinin into eudesmin (128). As the result of the work of these and previous workers, the structure of

sesamin, and simultaneously those of asarinin, pinoresinol, and eudesmin, were established in 1939 by von Bruchhausen and Gerhard (45) as shown below:



According to the last-mentioned authors, the existence of the isomeric compounds, *d*-sesamin, isosesamin or *d*-asarinin, isoasarinin or *l*-sesamin, and *l*-asarinin, is explained by the presence of asymmetric carbon atoms in the sesamin molecule. However, Rolla and Marinangeli (183) recently denied the possibility of a centrosymmetrical formula for these compounds on the basis of the high value of the electric moment which they found for sesamin.

Sesamin does not give a positive Villavecchia test, but according to Kreis (143) it does give color reactions with the various reagents shown in table 2. As mentioned previously, Jacobson *et al.* (121) obtained a greenish yellow color when an oil containing sesamin was shaken with a mixture of perchloric acid and hydrogen peroxide, and they used the development of this color as a basis for the determination of sesamin in sesame oil. It has been shown, however, that the value of the test is vitiated by the fact that sesamol gives the same color as sesamin with this reagent (49).

The absorption spectra of *l*- and *d*-sesamin have been investigated (49, 68, 84, 113) and it has been found that ultraviolet absorption measurements of sesame oil can be used for the quantitative determination of sesamin (49). Application of this method to four sesame oils of different origins gave values ranging from 0.5 to 1.0 per cent sesamin.

B. Sesamol and sesamol

Villavecchia and Fabris (242, 243), Bömer (35), and Heiduschka (101) believed that the component in sesame oil responsible for the Villavecchia or Baudouin test was a yellow noncrystallizable oily substance. As early as 1903, Kreis (140) obtained evidence that this color reaction was due to a phenolic compound. Although he was unable to isolate this unknown substance, he proposed the name *sesamol* for it. The same year, Canzoneri and Perciabosco (51) succeeded in isolating from sesame oil a crystalline substance ("substance X") which melted at 92°C. and gave a strong Villavecchia test. This compound, which twenty-five years later was given the name *sesamol* (I) and the formula

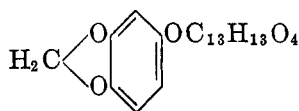
$C_{20}H_{18}O_7$ by Adriani (1), was readily hydrolyzed by mineral acids to yield two substances, one of which was a brown oil responding strongly to the Villavecchia test.

TABLE 2
Color reactions of sesamin*

REAGENT	COLOR	AUTHOR OF TEST
Nitric acid (with or without sulfuric acid)	Yellow	Behrens
Acetic anhydride + sulfuric acid	Red; then green	Bömer
Pyrogallol + hydrochloric acid	Violet	Tocher
Ammonium vanadate + sulfuric acid	Dark green	Bellier
Formaldehyde + sulfuric acid	Violet	Bellier
Stannous chloride	Red	Soltsien
Hydrogen peroxide + sulfuric acid	Green	Kreis

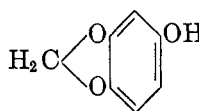
* According to Kreis (143).

In 1907 Malagnini and Armani (153) isolated from sesame seed the same "substance X" (m.p. 94°C.). From the products of hydrolysis of this compound they isolated a phenol melting at 57°C., which they proved to be the methylene ether of oxyhydroquinone. This was the phenolic compound which Kreis had called sesamol (II). These observations were confirmed by Adriani (1).



I

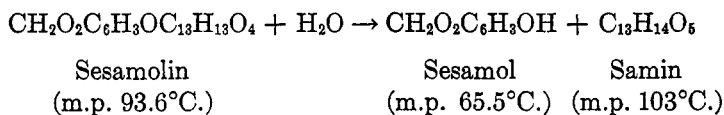
Sesamolin



II

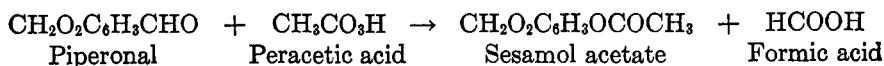
Sesamol

The equation for the hydrolysis reaction as established by Adriani (1) is as follows:



The second compound which Adriani (1) obtained by hydrolysis of sesamolin he named samin, m.p. 103°C. Both sesamolin and samin were optically active, the specific rotations being 218° and 103°, respectively.

In 1936 Böeseken, Cohen, and Kip (34) synthesized sesamol by the reaction of piperonal with peracetic acid. Sesamol was obtained in the form of the acetate, together with formic acid. The acetate was saponified with alkali, the alkaline solution acidified, and the sesamol recovered by vacuum distillation. The equation for the synthesis of sesamol acetate is as follows:



These authors (34) also synthesized the β -glucoside of sesamol and found that it gave a slow and gradual Villavecchia test instead of an almost instantaneous color development, as occurs with free sesamol. They concluded, therefore, that sesamolin was a glucoside, because sesame oil behaved very much like the synthetic glucoside with respect to the Villavecchia test. Although the authors referred to Adriani's work, they adopted an erroneous formula for samin. Furthermore, the formula for sesamolin, as deduced by Böeseken *et al.* (34), implied a structure for this compound for which there was no substantiating evidence but inferred instead that such a structure had been established by Adriani (1). Since no further work on the structure of sesamol or sesamolin has appeared in the literature, it seems advisable to adopt formula I for sesamolin, since it does not require making unwarranted assumptions.

The absorption spectra of sesamol and sesamolin have been reported recently (49), and it has been shown that both sesamolin and sesamin, and sometimes sesamol, contribute appreciably to the characteristic absorption peaks exhibited by sesame oil in the wave length region of 287 $m\mu$.

Sesamolin and sesamol can be determined colorimetrically by a modification of the Villavecchia test (48). Although both compounds respond positively to the test, the two can be separated readily by taking advantage of the solubility of sesamol in alkali.

Four crude oils obtained from different varieties of sesame seed were reported to contain 0.3–0.5 per cent sesamolin, with only traces of free sesamol (48). Bleached and hydrogenated sesame oils, however, may contain over 0.1 per cent free sesamol, because of the splitting of sesamolin during bleaching and hydrogenation (47).

Sesamol has been shown to possess pronounced antioxidant activity in lard and vegetable oils (46). Phosphoric and citric acids markedly enhance the antioxidant effect. Neither sesamolin nor sesamin was found to have any appreciable antioxidant activity when tested by the active oxygen method at 97.7°C. (46).

C. Phytosterol

The presence of a sterol in sesame oil has long been recognized, but it has never been investigated in detail. A number of authors reported melting points for this sterol(s) ranging from 130° to 139°C. (188, 198, 242, 243). Villavecchia and Fabris (242, 243) reported the sterol(s) to have an optical activity of $[\alpha]_D^{20} = -34^{\circ}23'$, while Marcusson and Meyerheim (154) found it to be $[\alpha]_D^{20} = -23^{\circ}$. The acetyl derivative has been reported to melt between 125° and 131°C. (164, 198, 243). According to Klamroth, as quoted by Lewkowitsch (149), the sterol is sitosterol.

Crude sesame oils from four different varieties of seed were found to contain 0.20–0.24 per cent free, 0.15–0.30 per cent bound, and 0.35–0.54 per cent total sterols (46a). Similar values were reported earlier by Klostermann and Opitz (134) for commercial sesame oil.

D. Other minor constituents

Besides sesamin, sesamolin, sesamol, and phytosterol, no other minor constituents have been isolated or characterized. Of interest is the curious observa-

tion made by Zimmermann (252), who obtained, from the oil of a "partially fermented" Chinese variety of sesame seed, a crystalline aldehydic compound which on heating to 200–220°C. yielded naphthalene.

Bailey (18) reported 0.050 per cent tocopherols in refined sesame oil, as determined by a modified Emmerie-Engel method. Karrer and Keller (129) found 0.005 per cent combined α - and β -tocopherols by potentiometric titration of the unsaponifiable matter with gold chloride. Furter and Meyer (85) could not detect any tocopherols in sesame oil by their colorimetric method involving nitric acid. In fact, they used sesame oil as a substrate for dissolving tocopherols used for the standard calibration of their method. It has been pointed out (47) that free sesamol responds to the Emmerie-Engel test, two parts by weight of sesamol being equivalent to approximately five parts of α -tocopherol. Consequently, when tocopherols are determined in sesame oil or mixtures containing sesame oil, care must be taken to eliminate any free sesamol which may be present. Beaver and Kaunitz (22) observed that sesame oil interfered with the polarographic determination of α -tocopherol, but no explanation is given for this phenomenon.

Sesame oil contains a small amount of phosphatides. Four crude hexane-extracted sesame oils were reported to contain 0.034–0.132 per cent phosphatides ($P \times 24.0$) (47). The composition of the phosphatides of sesame seed has been investigated by Rewald (179).

VI. STABILITY

Among the so-called yellow or soft oils, sesame oil is perhaps the most resistant to oxidative rancidity and especially so after hydrogenation. Johnston and Frey (125) and also Bhattacharyya (28) reported that the rate of absorption of oxygen by sesame oil was lower than for other oils. Kedvessy (131) found that sesame oil was more stable under prolonged storage than a number of other oils used in therapeutics, with respect to both rancidity and increase in free fatty acids. Fiero (74, 75) arrived at a similar conclusion in the case of hydrogenated sesame oil, which he found to be superior to lard and hydrogenated fats as an ointment base. Fiero also observed that the unsaponifiable fraction from hydrogenated sesame oil was devoid of any appreciable antioxidant activity, although the hydrogenated oil itself did inhibit rancidity in lard (76). More recently, sesame oils hydrogenated to shortening consistency have been reported (6, 47, 155) to vary in stability from 560 to 840 hr. by the active oxygen method.

Several patents have been issued which cover the addition of hydrogenated sesame oil to lard and vegetable shortenings for purposes of stabilization (2, 43, 91). Steam deodorization of the hydrogenated sesame oil destroys its antioxidant activity (91). The steam distillate from the deodorization, on the other hand, possesses antioxidant properties (92).

Basu and Ray (21) reported that the addition of unhydrogenated sesame oil to vegetable ghee fortified with vitamin A decreased the stability of this vitamin. No other reference to a similar effect is found in the literature, but the related work on β -carotene (provitamin A) would seem to contradict the work of the

Indian investigators. Baumann and Steenbock (21a) investigated the stabilizing action of various oils on β -carotene and found that after storage for 5 months in the dark at 4°C., the amount of carotene which disappeared was as follows: refined cottonseed oil, 8 per cent; sesame oil, 15 per cent; coconut oil, 25 per cent; olive oil, 38 per cent; corn oil, 45 per cent; crude wheat germ oil, 62 per cent. It would appear, therefore, that sesame oil is less destructive to β -carotene than are most vegetable oils. In view of the further fact that it has been a legal requirement of various European countries for more than fifty years to incorporate sesame oil in margarines and that for almost half as long they have been fortified with vitamin A, it would seem unlikely that this oil contributes appreciably to the destruction of vitamin A.

Kehren (132) found that sesame press cake was a poor antioxidant for vitamin A. Musher (160), on the other hand, patented a process of stabilizing fats and oils against rancidity by adding to them small amounts of an infusion of sesame seed.

Olcott and Mattill (163) suggested that sesamol might be responsible for the stability of sesame oil and for its antioxidant properties, but advanced no experimental evidence for the statement. Recently, the role of sesamol in the stability of sesame oil was investigated (47) and it was found that certain processing treatments, such as bleaching and hydrogenation, resulted in the formation of free sesamol from its bound form (sesamolin), a change which was accompanied by an increase in the stability of the oil. In samples, hydrogenated to shortening consistency, practically all the sesamol was found to exist in a free form. Deodorization, which removes the free sesamol, decreased the stability of the oils. In the case of the hydrogenated samples, however, the deodorized oils still retained unusually good stability (380-540 A.O.M. hours), although their content of free sesamol was reduced to very low values. Hydrogenation apparently produces some other product or introduces some factor in addition to the liberation of sesamol which contributes to the marked stability of the hardened oil.

It was shown that the antioxidant effectiveness of hydrogenated sesame oil in lard was roughly proportional to the addition of an equivalent amount of synthetic sesamol, indicating that free sesamol was mainly responsible for the inhibitory action of the added hydrogenated oil.

VII. SYNERGISTIC ACTIVITY WITH PYRETHRUM INSECTICIDES

In 1940 a patent was granted to Eagleson (63) describing the synergistic effect produced by the addition of sesame oil to insecticides such as rotenone, pyrethrins, and the like. The presence of sesame oil was said to reduce the concentration of the insect toxin required to produce 100 per cent mortality of the insects, by increasing the initial torpor and prolonging the duration of the torpor. This synergistic action was discovered in the course of screening forty-two animal and vegetable oils as constituents of livestock and household sprays (64). No oil, other than sesame, exhibited any effect upon the insects tested (houseflies).

Excellent insecticidal results were observed with an aerosol spray consisting

of pyrethrum and sesame oil in dichlorodifluoromethane (87-89). Such aerosols have been found to be toxic to adult *Culex*, *Anopheles*, and *Aedes* mosquitoes, to several species of flies (*Haematobia*, *Stromoxys*, and *Musca*), as well as to roaches and bed bugs (215). Good results have also been obtained against the adult cheese skipper, *Piophilina casei* L., the larvae of which often infect stored meat and cheese (29). The advantages of the pyrethrum-sesame oil aerosol have been pointed out by Sullivan *et al.* (215), Goodhue (87), and Haller (95). It is nontoxic to warm-blooded animals, noninflammable, easy to apply, and non-staining. During World War II, an aerosol consisting of 4 per cent pyrethrum extract (20 per cent pyrethrins) and 6 per cent sesame oil, dissolved in dichlorodifluoromethane (Freon 12), was extensively used, and approximately 35 million 1-lb. aerosol "bombs" were manufactured for the armed forces (95, 182).

In 1942 Haller (96, 98) and coworkers reported the results of an investigation which had for its objective the isolation and characterization of the principle(s) responsible for the synergistic activity of sesame oil. To this end the oil was fractionated by molecular distillation, and from the active fractions a crystalline solid was obtained which was shown to be sesamin. When tested as a synergist, sesamin was found to be highly active. This observation was also confirmed by other workers (60).

However, the data presented by Haller *et al.* (96, 98) showed that considerable synergistic activity was retained by the noncrystalline fraction after removal of most of the sesamin, indicating that sesamin may not be the only active principle present. This view was supported by Parkin and Green (168), who found that the effect produced by sesame oils of different origins is highly variable. One sample actually reduced the toxicity of pyrethrins to houseflies. The latter authors concluded that "there is no reason to doubt that the constituent of sesame oil chiefly responsible for activation is sesamin; but it seems that there may also be present a complex of secondary factors which, according to its composition, may increase or decrease the effect of the pyrethrins and/or sesamin in a fly spray."

Of interest in this connection is the observation made by Simanton (199), who reported the isolation from sesame oil of an unidentified crystalline solid which melted at 92°C. and had a marked synergistic activity. This unknown compound, for which no other properties are given by the author, may be sesamol (m.p. 94°C.). Simanton further reported the presence in sesame oil of another unidentified, noncrystalline, synergistically active compound (199).

Haller *et al.* (97) investigated several compounds closely related to sesamin, including asarinin and isosesarin. These optical isomers of sesamin were found to be as active as sesamin, when tested as synergists for pyrethrins, but when the methylenedioxy group in the sesamin molecule was altered, as in pinoselin and its dimethyl ether and diacetyl derivative, the activity was lost.

Following the establishment of the importance of the methylenedioxyphenyl group with respect to insecticidal activity, a large number of natural and synthetic compounds containing this group were examined, and a number of them have been found to be active, either alone or as synergists (31, 216, 244). Sesamol apparently has never been tested for this purpose.

A histological investigation of the effects of fly sprays and activators on the nerve and muscle tissues of adult houseflies was reported by Hartzell (99), who brought forth evidence that activation (by sesame oil or other activators) is a result of the destruction of at least two tissue components, the poison having an affinity for one component, and the activator for another component, and both at a lower concentration than either one alone.

VIII. NUTRITIONAL VALUE AND PHYSIOLOGICAL PROPERTIES

Sesame seed and oil have been important foods in Asiatic countries for thousands of years. Sesame seed or flour is very nutritious (57, 103, 254f, 254g) and has been recommended as a supplement for wheat and corn flours (251, 254f). Sesame meal and cake have been shown, particularly by Almquist (2a) and co-workers, to be especially valuable in supplementing soybean meal in chick diets. Soybean meal protein is deficient in methionine (1.8–2.0 per cent), whereas sesame meal protein contains 3.4 per cent of this essential amino acid. It has been found that soybean and sesame meals exert a marked mutual supplementary effect, as measured by gain or efficiency of food utilization in the chick.

Recently, a number of investigators have studied the nutritional properties of sesame oil, both hydrogenated and unhydrogenated. In all cases sesame oil has been reported to be satisfactory as a food product and nutritionally equivalent to other edible fats (145, 151, 185). As in the case of many other vegetable fats, sesame oil lacks vitamin A (184) and its content of vitamin E is low. However, the marked stability of this oil, especially after hydrogenation, makes it particularly valuable as an edible fat, in view of the fact that rancid fats, besides being unpalatable, tend to destroy certain vitamins existing in other ingested foods.

In therapeutics sesame oil is a preferred vehicle for fat-soluble substances, and in biological experiments it is often used as a standard or control fat. In view of the synergistic activity of sesame oil in pyrethrum insecticides, it may be presumed that the oil possesses some physiological activity, and in fact Bruce and Tobin (44) have pointed out that there are indications in the medical literature that sesame oil may not be physiologically inactive. These authors found that sesame oil contained alcohol-soluble substances, one of which was sesamin, that were toxic to adrenalectomized rats. Intraperitoneal injections in normal male rats of 0.25–1.0 ml. of sesame oil daily for several weeks were reported to produce toxic effects. In a subsequent communication Tobin (220) reported that daily intraperitoneal injections of sesame oil into adrenalectomized female rats increased the number of successful pregnancies, prolonged the survival time of those animals that had successful gestation, and increased their ability to rear young.

Spurr and Kochakian (213) reported that twice-daily injections of 0.1 ml. of sesame oil decreased the survival time of 1-month-old male adrenalectomized rats from 8.7 to 7.1 days. Pollia (172) reported that distilled sesame oil in some cases inhibited tumor growth in sarcoma-bearing rats, while no inhibition occurred with household brands of sesame oil. On the other hand, Steiner *et al.* (214a) observed that sarcomas were induced in three out of nine mice injected

with sesame oil that had been heated to 350°C. Armstrong *et al.* (14) found that injections of sesame oil had deleterious effects on the skeleton of mature rats and concluded that the oil was not metabolically inert.

Cruz *et al.* (58) reported purpuric lesions in the skin of dogs to which estradiol benzoate dissolved in sesame oil was given. These lesions did not occur when sesame oil alone was given, or when other vegetable oils were used as a vehicle, except, slightly, with soybean oil. According to Crafts (56), subcutaneous injections of sesame oil into immature male mice produced a reduction in weight of the prostate and seminal vesicles, and this effect occurred even when small amounts of androsterone were dissolved in this oil. Li and Baldwin (150) observed testicular tumors in three of forty adult male teleost (*Xiphophorus helleri*) that had received retroperitoneal injections of crude sesame oil. No neoplastic changes occurred in untreated adult male fishes. Sudden death of mice injected intraorbitally with minute amounts of sesame oil was reported by Figge (77). The oculocardiac reflex was believed to be responsible for the sudden and spectacular death.

Ratschow and coworkers (178) claimed that daily administration of 1.5 ml. of sesame oil to male and female rats for 53 days produced atrophy of the testes, shrinking of the ovaries with atresia of the follicles, and marked deposition of iron in the adrenal cortex, lymph follicles of the spleen, Kupffer's stellate cells of the liver and hypophysis. Ratschow also claimed that the necrosis which caused the American Council on Pharmacy to reject stilbene compounds was due to the sesame oil used as a solvent.

A curious physiological property of sesame oil has been reported by Schiff and Hirschberger (190-193). Following the oral administration of sesame oil to adults and children, as well as to rats, an increase in the number of blood platelets (thrombocytes) was observed. The activity of sesame oil was attributed to the presence in this oil of a fat-soluble "T-factor." The authors suggested that oxyhydroquinone, or a related compound, might be a constituent of this T-factor (191). This discovery, if confirmed, would be of importance with respect to the treatment of certain diseases accompanied by thrombocytopenia, such as morbus maculosus werlhofi. Several workers attempted to duplicate Schiff and Hirschberger's results, but mostly without success (165, 246). One investigator, however, obtained a positive result (52). Schiff and Hirschberger themselves had difficulty in duplicating their earlier results when they used a sample of sesame oil from a different source (193).

These observations on the physiological effects produced by sesame oil are especially interesting in connection with the fact that this oil contains several minor constituents (sesamin, sesamol, sesamol) which do not occur in other oils and which vary widely, not only for sesame oils of different sources, but also for any given series of oils having received different processing treatments, thereby offering a possible explanation for apparently contradictory observations.

IX. CONCLUSIONS

In the past, sesame oil has been investigated principally with respect to its color tests because of the practical significance of these reactions. Comparatively

little work has been reported in the literature on the effects of processing sesame oil and on other problems, such as its stability characteristics and the chemical and physiological properties of some of its minor constituents. These problems are, however, now beginning to receive attention and such work as has been done recently has been repaid by such discoveries as the synergistic activity of sesamin with pyrethrum insecticides and the antioxidant properties of sesamol. There is, however, need for more work on the chemistry and physiology of the minor constituents of sesame oil.

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