reacts rapidly with them to liberate free fatty acids, without neutralization of the fatty acids liberated, or the addition of buffers, or any other substance although the rate of fat splitting is enhanced if about 2% of water is added. The rate of splitting of cottonseed oil at 10° and 20° by this lipase cream indicates that the temperature coefficient of this enzyme is about half of the usual temperature coefficient. The lipase is inhibited by a soluble synthetic cephalin, egg phosphatide, and salmon egg phosphatide, but not by soybean phosphatide.

Acknowledgments

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Sesame Oil. VI. Determination of Sesamin

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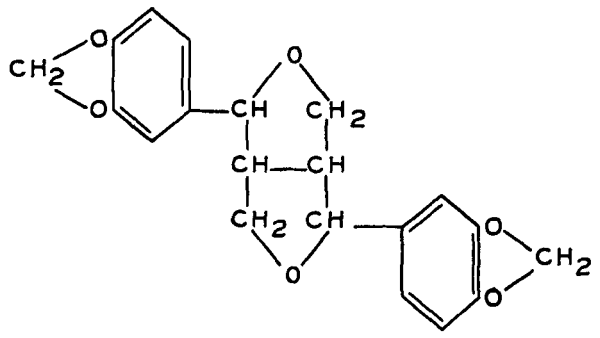
SESAMIN, one of the minor constituents of sesame oil, was first isolated from the acetic acid extract of sesame oil by Tocher (20) in 1890. Villavecchia and Fabris (21) in 1892 obtained sesamin from the unsaponifiable matter of sesame oil. These authors described some of its properties, but no further significant work on sesamin was reported until 1928 when Adriani (1), and Boeseken and Cohen (3), independently, indicated that the correct empirical formula for sesamin was $C_{20}H_{18}O_6$. The structure and properties of sesamin were extensively investigated by Bertram *et al.* (2), Boeseken and Cohen (3), and Cohen (8). The relationship between sesamin and other naturally occurring compounds, especially asarinin, was investigated and discussed by Erdtman (10), Kaku *et al.* (17), Huang-Minlon (14), and Bruchhausen and Gerhard (4). The last mentioned authors proposed a complete structure for sesamin as shown in the accompanying formula.



In 1937 Eagleson (9) found that the insecticidal activity of pyrethrum insecticides was markedly enhanced by the addition of sesame oil. The oil alone was inactive as an insecticide, and of 42 animal and vegetable oils tested only sesame oil acted as a synergist for the pyrethrins. Haller and co-workers (13) fractionated sesame oil by molecular distillation in an effort to isolate the active principle. From the active fractions a crystalline compound was obtained, which was shown to be sesamin. Insecticidal tests with pure sesamin showed that this compound possessed marked synergistic activity with pyrethrins.

Although other unidentified minor constituents of sesame oil have been reported to exhibit synergistic effects (19), sesamin is the only component which has been shown definitely to act as a synergist with pyrethrins; therefore its quantitative determination in this oil is of practical importance.

A colorimetric method for the estimation of sesamin was described in 1944 by Jacobson, Acree, and Haller (16). Since this method has certain disadvantages which will be discussed below, an improved method appears to be desirable. Such a method has been developed and is described in the present report. It is based on the measurement of the ultraviolet absorption of sesame oil (11, 15, 18) following the removal of sesamol by treatment with alkali (6) and correction for the absorption resulting from the presence of sesamolin.

Experimental

Sesame oil contains, besides sesamin, other minor constituents, the absorption characteristics of which are not known. Therefore, the following compounds were prepared: sesamin and sesamolin, by extraction from sesame oil as described elsewhere (5); and sesamol, by synthesis (5). The absorption spectra of these compounds in the ultraviolet region are shown in Figures 1 and 2 together with the absorption spectrum of a crude sesame oil.

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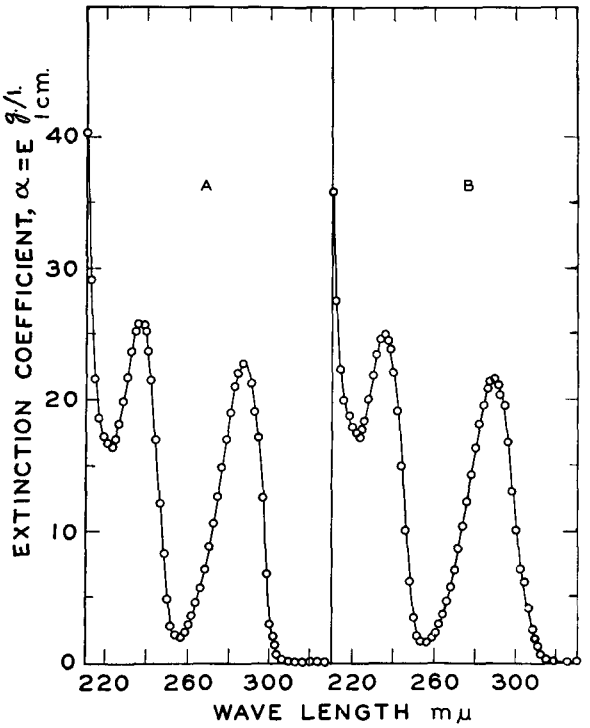

FIG. 1. Ultraviolet absorption spectrum of (A) pure sesamin, (B) pure sesamolin in isooctane.

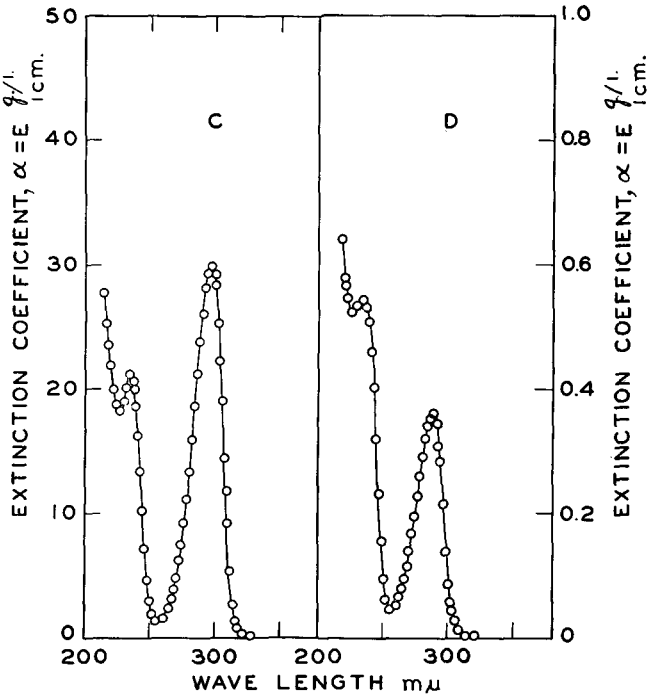

FIG. 2. Ultraviolet absorption spectrum of (C) pure sesamol, (D) crude sesame oil in isooctane.

The extinction coefficients and wave lengths of the maximum and minimum absorption of sesamin, sesamolin, and sesamol in isooctane solution are given in Table I. The extinction coefficient observed for sesamin at 287 mμ is identical with that reported by Fuchs (12).

TABLE I

Spectral Absorption Characteristics of Sesamin, Sesamolin, and Sesamol in Isooctane Solution

Compound	Characteristic absorption, mμ	Extinction coefficient
Sesamin	Maximum 287	23.03
	Minimum 255	2.02
	Maximum 236	26.01
	Minimum 221	16.46
Sesamolin	Maximum 288.5	21.79
	Minimum 255	1.54
	Maximum 235	24.85
	Minimum 223	17.25
Sesamol	Maximum 296	29.74
	Minimum 256-257	1.35
	Maximum 233	21.18
	Minimum 225-226	18.32

The data presented in Figures 1 and 2 and in Table I show that sesamin and sesamolin possess nearly identical absorption characteristics while sesamol presents a somewhat different absorption pattern; however the absorption bands produced by sesamol are situated in the same wave length regions as those obtained from sesamin and sesamolin.

Sesamol and sesamolin may be expected to contribute to an appreciable extent to the characteristic absorption peak of sesame oil at 287-288 mμ. It may be observed from the absorption curve of sesame oil (Figure 2D) that the band at 287-288 mμ is superimposed upon a general "background" absorption, which also had to be taken into account if the sesamin content of sesame oil is to be calculated from the maximum absorption value of the oil in the region of 287-288 mμ. The interference resulting from the presence in the oil of sesamol, sesamolin, and of oil "background" absorption was overcome as follows:

Interference of Sesamol. Although crude sesame oils contain only very small amounts (less than 0.005%) of free sesamol, it has been shown that bleached or hydrogenated oils may contain more than 0.1% sesamol (6, 7).

The removal of free sesamol from a solution of sesame oil in isooctane is readily accomplished and constitutes an important step in the determination of free and bound sesamol (6). The determination of sesamin can be carried out on an aliquot of the same solution which is used for the determination of sesamolin (bound sesamol) because this solution has been treated to remove free sesamol.

Interference of Sesamolin. Sesamolin is not readily removed from the oil. Strong mineral acids will hydrolize this compound, but when the oil or its solution is shaken with such acids, they also react with any sesamin present in the oil. The interference resulting from the presence of sesamolin is best eliminated by applying a correction for the absorption of this compound. This is easily accomplished if the concentration of sesamolin in the oil is known. For this reason it is also advantageous to determine sesamin in conjunction with the estimation of sesamolin, *i.e.* of bound sesamol multiplied by 2.68.

Interference of Oil "Background" Absorption. Since the "background" absorption of the oil cannot be measured directly at the wave length of maximum absorption, the assumption is made that the variation of extinction coefficient (or optical density) resulting from oil "background" is roughly linear throughout the absorption band. Two wave lengths are then selected, situated on both sides of the absorption band, and at equal distance from the wave length of maximum absorption, and the unknown

"background" absorption at 287-288 mμ is calculated as the arithmetic means of the background absorptions at the two selected wave lengths. The wave length corresponding to the minimum absorption (about 255 mμ) is always selected for the first measurement. The corresponding absorption does not however represent the pure "background," but includes slight contributions from sesamin and sesamolin, the absorption of which is not negligible at this wave length. Corrections must therefore be applied. The second wave length is then selected at about 320 mμ. In this region the absorption of sesamin and sesamolin is so small that no correction for these compounds is necessary. The arithmetic means of the corrected extinction coefficients at the two selected wave lengths is taken as the extinction coefficient of the oil "background" at the absorption maximum.

Procedure. If the determination of sesamin is carried out in conjunction with the determination of free and bound sesamol, as described in a previous article (6), no additional apparatus, glassware, or reagents are required.

The solution of the unknown oil in isooctane (10 g./100 ml.) is freed from sesamol, as described for the determination of bound sesamol, and an aliquot further diluted with optically pure isooctane to produce a solution containing 2-4 g. of oil per liter.

The extinction coefficients of this solution are then determined with the aid of a Beckman spectrophotometer, using 1 cm. cells, at about 287-288 mμ, 255 mμ, and 320 mμ. In practice, the extinction coefficients are recorded for the actual maximum and minimum (at about 287-288 and 255 mμ, respectively), and the third wave length, at about 320 mμ, is then selected accordingly.

Calculation. The following equation is used:

$$C = \frac{100\left[K_{288} - \frac{21.75 \times C_1}{100} - \frac{1}{2}\left(K_{255} - \frac{2.02 \times C}{100} - \frac{1.54 \times C_1}{100} + K_{320}\right)\right]}{23.03}$$

C and C_1 represent the percentage concentrations of sesamin and sesamolin, respectively, contained in the oil. The K's are the observed extinction coefficients of the oil at the maximum (about 288 mμ), minimum (about 255 mμ) and 320 mμ absorption regions. The figures 21.75 and 1.54 are the values of the extinction coefficients of pure sesamolin at 288 and 255 mμ, respectively, while 23.03 and 2.02 are the corresponding values for sesamin.

The second term in the brackets is included to correct for the absorption at 288 mμ contributed by sesamolin. The third term is included to correct for the "background" absorption of the oil. The latter term also includes corrections for the absorption of sesamin and sesamolin at 255 mμ.

The equation may be rearranged to the following more convenient form:

$$C = 4.541\ K_{288} - 0.953\ C_1 - 2.271\ (K_{255} + K_{320})$$

Results Obtained. The above-described method was tested by determining the sesamin content of a) cottonseed oil to which known amounts of sesamin and sesamolin had been added, and b) sesame oil to which known amounts of sesamin had been added, with the results shown in Table II.

The cottonseed oil used in these tests was purified by passage through a column of activated alumina to reduce its general absorption and make it comparable in background absorption to that of sesame oil.

TABLE II

Determination of Sesamin in the Presence of Sesamolin

Type of oil	Sesamin added, %	Sesamin found, %	Sesamolin added, %	Sesamolin found, %
Cottonseed	0	0.017	0	.....
	0.498	0.516	0.399	.....
	1.001	1.008	0.402	.....
Sesame (SO-1)	0	0.496	0	0.386
	0.400	0.886	0	0.386
	0.800	1.301	0	0.386

The results shown in Table II indicate that the recovery of added sesamin in the presence of sesamolin is satisfactory in the case of both cottonseed and sesame oil.

This method was also applied for the determination of sesamin in a series of crude and processed sesame oils with the results shown in Table III.

TABLE III

Percentage of Sesamin in Sesame Oils at Various Stages of Processing

Sesame oil	Crude	Refined and bleached	Refined, bleached, and hydrogenated
SO-1	0.496	0.477	0.098
SO-2	0.709	0.698	0.389
SO-3	0.690	0.624	0.650
SO-4	0.963	0.975	0.978

It is seen from the data in this table that the sesamin content of the crude oils ranged from 0.5-1%. Alkali refining and bleaching did not significantly affect the sesamin content of the oils. Hydrogenation to shortening consistency however, produced a considerable decrease in sesamin content in two of the samples.

Discussion

Jacobson *et al.* (16) have described a method for the determination of sesamin based on the measurement of the greenish-yellow color produced by sesamin in the presence of a mixture of hydrogen peroxide and perchloric acid. However some of the older literature indicates that sesamol may also produce green or yellow colorations with oxidizing agents. Samples of sesamol and sesamolin, dissolved in refined kerosene ("Deobase"), were tested as described by Jacobson *et al.* for the determination of sesamin. Both compounds were colored greenish-yellow in the presence of this reagent. The color produced by sesamol, although very intense at first, faded rapidly and disappeared after a few minutes while that obtained with sesamolin increased slowly. It was found that two parts of sesamolin were equivalent to one part of sesamin with respect to color formation when tested under the conditions described by Jacobson *et al.*

Since sesame oils are known to contain appreciable amounts of sesamolin (6), the method of Jacobson *et al.* would be expected to yield high results for sesamin. When the same four crude sesame oils (SO-1 to SO-4 of Table III) were tested by the method of Jacobson *et al.*, the respective values for sesamin were found to be 0.96, 1.03, 1.37, and 1.12% respectively. These values are much higher than those shown in Table III, which were obtained by the ultraviolet absorption method.

While it should be possible to apply a correction for sesamolin, the colorimetric method presents a number of other disadvantages. The most serious of these are a) the development of gas bubbles in the mixture at the time the color is measured, b) rapid fading of the color, and c) handling the hazardous reagent prepared with 30% hydrogen peroxide and 72% perchloric acid. The centrifugation of this mixture, which is part of the method, is a hazardous operation. For these and other reasons it is believed that the present method constitutes an improvement over the colorimetric method both with respect to simplicity, accuracy, and lack of hazards.

Summary

A new method for the determination of sesamin in sesame oils is described. It is based on the measurement of the ultraviolet absorption of sesame oil following the removal of sesamol by treatment with alkali and correction for the absorption resulting from the presence of sesamolin. The advantages of the new method over the previously described colorimetric method are discussed. The accuracy of the method is attested by a comparison of the determined values with those for known added amounts of sesamin in cottonseed and sesame oils. When applied to four crude oils, the content of sesamin was found to range from 0.50 to 0.96%.

Ultraviolet absorption spectra curves are reported for sesamin, sesamolin, sesamol, and sesame oil.

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Sesame Oil. VII. Optical Rotation and the Minor Components of Sesame Oil

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THE unsaponifiable matter of sesame oil is known to contain sesamin, sesamolin, and phytosterols, each of which is optically active and therefore assumed to be responsible for the observed optical rotation of this oil. It seemed of interest to compare the observed optical rotation of the four crude sesame oils described in the preceding publication (1) of this series with the optical rotation calculated from the known amounts of sesamin, sesamolin, and sterols in these oils. The sesamin content of these oils is given in Table I together with the sesamolin content of the same oils calculated from the values for bound sesamol given in a previous paper (2) of this series.

The content of free sterols for each oil was determined by the method of Klostermann and Opitz (3) as follows: A solution of the oil in a mixture of ethyl ether and petroleum naphtha was treated with an alcoholic solution of digitonin, and the precipitated sterol digitonides determined gravimetrically. Total sterols were similarly determined using the fatty acids obtained after saponification of the oil and acidification of the soaps. The bound sterols were calculated by difference.

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TABLE I

Minor Constituents and Optical Rotation of Crude Sesame Oils

Sesame oil	Sesamin, %	Sesamolin, %	Sterols			Optical Rotation[a]	
			Free, %	Bound, %	Total, %	Found[b]	Calculated
SO-1	0.496	0.386	0.201	0.154	0.355	0.93	0.97
SO-2	0.709	0.356	0.215	0.161	0.376	0.95	1.04
SO-3	0.690	0.362	0.199	0.192	0.391	1.02	1.04
SO-4	0.963	0.431	0.239	0.300	0.539	1.44	1.30

[a] Angle of rotation, α_D^{25} in a 1 dm. cell. [b] See Reference 6.

The specific rotations in chloroform solution of sesamin, sesamolin, and phytosterols of sesame oil have been reported by several authors. The following reported values which were used for the present calculation are: sesamin +68.6 (4), sesamolin +218 (4), and phytosterol −34.4 (5). These values may not be strictly comparable since they were obtained under conditions which may not have been identical, but they can be used to calculate the rotations of the crude sesame oils whose compositions with respect to these constituents have been determined. The following equation was used:

$$\alpha = \frac{\text{Specific gravity} \times (68.6\ C_1 + 218\ C_2 - 34.4\ C_3)}{100}$$