Many solvents were tested for extracting the unsaponifiables after saponification. Cyclohexane was selected because it is not toxic, does not form peroxides as does diethyl ether, and above all because it does not form stable emulsions if the procedure is followed exactly.

### Vegetable Oil Analysis

We analyzed 13 vegetable oils purchased in the Washington DC area for their tocopherol and sterol contents, to evaluate the applicability of the method and possible separation problems. With a few exceptions, duplicate analyses agreed well (Table III). The chromatograms of 9 of these oils appear in Figure 2. Not all peaks were completely separated but the major sterols and tocopherols were either completely separated or separated well enough for quantitative estimation. A peak that interfered with α-tocopherol appeared in some of the chromatograms, and was most prominent in sesame oil. A complete separation of the two peaks could be obtained by using temperature programming and lowering the carrier gas linear velocity.

### GLC Retention Data

Kovats retention indices and retention ratios relative to 5,7-dimethyltocol were determined or calculated for tocopherols, tocotrienols and the major sterols (Table IV). Separation would be possible for all compounds listed, except for α-tocotrienol and campesterol.

TABLE IV

**Retention Indices and Relative Retention Times of Tocopherols and Sterols**

| | Retention indices | Relative retention time |
|---|---|---|
| Internal standard | | |
| 5,7-Dimethyltocol | 3129.7 | 1.000 |
| Tocopherols | | |
| α | 3183.9 | 1.161 |
| β | 3022.9 | 0.746 |
| γ | 3034.7 | 0.771 |
| δ | 2935.6 | 0.587 |
| Tocotrienols | | |
| α | 3309.6 | 1.632 |
| β | 3147.4[a] | |
| γ | 3160.2 | 1.082 |
| δ | 3058.2 | 0.822 |
| Sterols | | |
| Cholesterol | 3209.7 | 1.245 |
| Campesterol | 3310.2 | 1.634 |
| Stigmasterol | 3333.0 | 1.738 |
| Sitosterol | 3389.3 | 2.024 |

[a]Calculated.

ACKNOWLEDGMENT

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REFERENCES

1. Sheppard, A.J., D.R. Newkirk, W.D. Hubbard and T. Osgood, JAOAC 60:1302 (1977).
2. Nelson, J.P., A.J. Milun and H.D. Fisher, JAOCS 47:259 (1970).
3. Govind Rao, M.K., and E.G. Perkins, J. Agric. Food Chem. 20: 240 (1972).
4. Hartman, K.T., JAOCS 54:421 (1977).
5. Mordret, F., A. Prevot, N. Le Barbanchon and C. Barbati, Rev. Fr. Corps Gras 24:467 (1977).
6. Karrer, P., and H. Fritzsche, Helv. Chim. Acta 21:1234 (1938).
7. Slover, H.T., and E. Lanza, JAOCS 56:933 (1979).

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# ♣Participation of Sesamol in Stability of Sesame Oil

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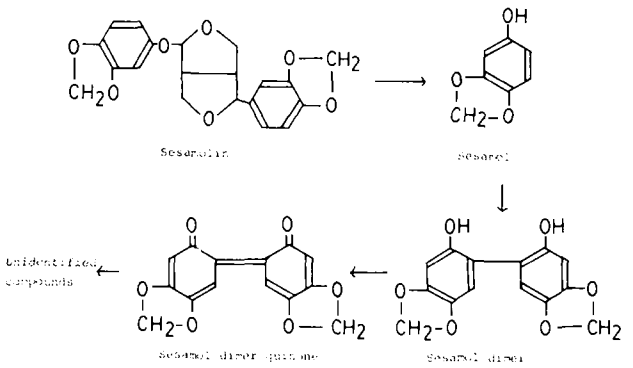
## ABSTRACT

Sesame oil is known to be the most resistant to oxidative rancidity. Constituents of sesame oil such as sesamolin, sesamol and sesamol dimer (a possible intermediate of oxidative degradation of sesamol) were determined by high performance liquid chromatography using a reverse-phase column. Sesamol was specifically determined in an alternative way by use of hydrogen peroxide/horseradish peroxidase. Sesamolin was relatively stable but sesamol and sesamol dimer were unstable when irradiated in benzene, and the final degradation products were identical. Whereas sesamolin was inactive, sesamol and sesamol dimer showed significant antioxidant activity in several kinds of fat and oils. Stability of Japan Pharmacopoeia sesame oil free from sesamol was relatively low; antioxidant activity of sesamol incorporated in the oil was unexpectedly low and was rapidly lost in the oil activated by oxygen. Edible sesame oil with intrinsic sesamol was highly stable. Activation of the edible oil gradually increased the sesamol content with concomitant decrease of sesamolin. High stability of edible sesame oil could not be ascribed to sesamol, but it could be explained by another powerful antioxidant(s) which might stabilize both the oil and unstable sesamol.

## INTRODUCTION

Among the several vegetable oils, sesame oil is known to be most resistant to oxidative rancidity (1,2). It was suggested that sesamol, one of the constituents of sesame oil, might be responsible for the stability of sesame oil, and the comparative antioxidant activity of sesamol and other antioxidants in certain fats and oils has been evaluated (3-7). It was found that certain processing treatments resulted in the formation of free sesamol from its bound form (sesamolin) (8,9). We have demonstrated that sesamol was readily oxidized into sesamol dimer and successively into its quinone by mild treatment with hydrogen peroxide/horseradish peroxidase (10). It is readily conceivable that sesamol is converted into its dimer during the progress of peroxidation of sesame oil (see Scheme 1), since butylated hydroxyanisole was oxidized into the dimers during the peroxidation of oils (11,12).

Selective determination of the constituents of sesame oil hitherto reported has been very troublesome (1,2,13-15). Due to the lack of an excellent method for determination of the constituents of sesame oil, it is difficult to monitor the changes of these constituents during the peroxidation of sesame oil. We attempted to separate sesamolin, sesamol and the possible oxidation product, sesamol dimer, in sesame oil by high performance liquid chromatography (HPLC), and to elucidate the relevance of these constituents



*SCHEME 1*

to stability of sesame oil.

This paper demonstrates (a) determination of the constituents by high performance liquid chromatography and by specific colorimetric determination; (b) stability of sesamolin, sesamol and sesamol dimer in benzene irradiated by ultraviolet light; (c) comparisons of antioxidant activity of sesamolin, sesamol and sesamol dimer in several fats and oils; and (d) changes of sesamolin and sesamol in sesame oil, and their participation in stability of sesame oil.

## EXPERIMENTAL

### Materials

Sesamol (Aldrich Chemical Company, Ltd.) was recrystallized from chloroform/petroleum ether for use (16). Sesamolin and sesamin were obtained from edible sesame oil according to the method of Haslam and Haworth (16). Sesamol dimer was prepared from sesamol according to the method described elsewhere (10). Horseradish peroxidase was a product of Toyobo Company, Ltd. (Grade III, 100 units/mg). Japan Pharmacopoeia sesame oil (JP sesame oil) and edible sesame oil (Takemoto Yushi Company, Ltd.), both exhibiting peroxide value (POV) of zero, were used. Methyl oleate with POV of 2.33 meq/kg was a product of Tokyo Kasei Kogyo Company, Ltd. Edible soybean oil with POV of 1.22 meq/kg was a product of Showa-Sangyo Company, Ltd. Fresh lard was prepared by warming and squeezing for use and stored in a freezer.

### Determination of Sesamolin, Sesamol and Sesamol Dimer by HPLC

Determination of sesamolin, sesamol and sesamol dimer by HPLC was carried out by use of a Shimadzu LC-2 liquid chromatograph equipped with a stainless steel column (4.6 mm id × 25 cm) of ZORBAX ODS and a Shimadzu spectrographic detector SPD-1. The chromatograph was operated with mobile phase (75% methanol) at a flow rate of 0.8 mL/min. A sample solution (5 μL) in benzene or *n*-hexane was injected. The amount of each compound was determined by the peak height observed at 300 nm. For determination of each of the compounds in sesame oil, 1.0 g of the oil was dissolved in *n*-hexane and the solution was made up to 5.0 mL.

### Determination of Sesamol in Sesame Oil by Hydrogen Peroxide/Horseradish Peroxidase

Sesamol in sesame oil was determined by the colorimetric method previously described (10), which is selective for free sesamol. Thus, 1.0 g of sesame oil was mixed with 3.5 mL of 0.1 M phosphate buffer (pH 7.0), 0.5 mL of 100 mM hydrogen peroxide and 0.5 mL of 10 μM horseradish peroxidase, and the mixture was vigorously shaken to stand for 10 min in the dark and it was then extracted with 5.0 mL of chloroform. The absorbance at 530 nm of the extract (A) was determined by a Shimadzu double-beam spectrophotometer, UV-200S. The same sesame oil was mixed with 0.3 mg/g of sesamol and similarly treated and extracted to measure absorbance (B). The absorbance of the extract of the mixture of 1 g of sesame oil, 3.5 mL of phosphate buffer, 0.5 ml of 100 mM hydrogen peroxide and 0.5 mL of water was also determined (C). The amount of sesamol in the sesame oil was calculated according to the equation: sesamol (%) = A-C/B-A × 0.3 × 1/10.

### Ultraviolet Irradiation of Sesamolin, Sesamol and Sesamol Dimer

A 25 mL solution of 1.0 mM sesamolin, sesamol or sesamol dimer in benzene was irradiated at room temperature and at a distance of 40 cm from two ultraviolet lamps (325 nm) (Toshiba Company, Ltd.). Decreased amount of the solvent was frequently supplied and the content of each compound in the solution was estimated by HPLC at 300 nm. After irradiation for 2 days, the mixtures revealed no peaks when detected at 300 nm, but they revealed many peaks at 230 nm. The mixtures were applied to thin layer chromatography by use of Wakogel B-5F (Wako Pure Chemical Industries, Ltd.) and a developing solvent system: *n*-hexane/ethyl acetate (2:1). Spots were detected by irradiation of ultraviolet lamps (254 and 365 nm) and by spraying BQC reagent, which was specific to phenolic compounds and prepared by dissolving 1% 2,6-dibromoquinone monochlorimide in ethanol.

### Stability of Fat and Oil

Each constituent of sesame oil was incorporated in lard, methyl oleate, soybean oil and JP sesame oil at a concentration of 0.01% with the aid of a small amount of ethanol. An equivalent amount of ethanol was added to the control sample. Portions were placed in tubes designed as described (11) and aerated with purified air at the rate of 2.3 mL/sec and at 98 C (active oxygen method) (11,17). At regular intervals, a 0.5-1.0 g sample was removed and POV was determined according to the method of Wheeler (18).

## RESULTS

### Determination of Sesamolin, Sesamol and Sesamol Dimer

Sesamolin, sesamol, sesamin and sesamol dimer were clearly separated by HPLC using a reverse-phase column. A representative profile of the mixture containing pure compounds is illustrated in Figure 1. When the concentrations of each compound in benzene were varied, the peak heights showed straight lines against the concentrations up to 1 mM.

Both JP sesame oil and edible sesame oil revealed many peaks in the chromatography (Fig. 2). JP sesame oil did not reveal sharp peaks corresponding to sesamol and sesamol dimer. The absence of sesamol dimer was confirmed by detection at the wavelength of 314 nm, the absorption maximum of the dimer (10). Edible sesame oil revealed the peaks corresponding to sesamolin, sesamin and sesamol, but no peak corresponding to sesamol dimer. Contents of sesamolin and sesamol in JP sesame oil and edible sesame oil were calculated by the standard calibration curves (Table I). Edible sesame oil revealed many small peaks around that of sesamol, which interferred with accurate determination of the content of sesamol. Thus, the content of sesamol was determined in an alternative way; specific colorimetric

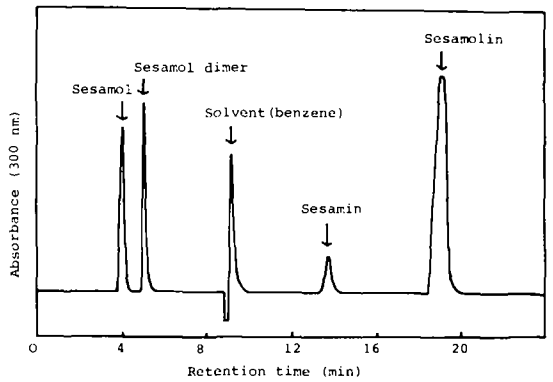



**FIG. 1. Separation of sesamolin, sesamol, sesamin and sesamol dimer by HPLC.**

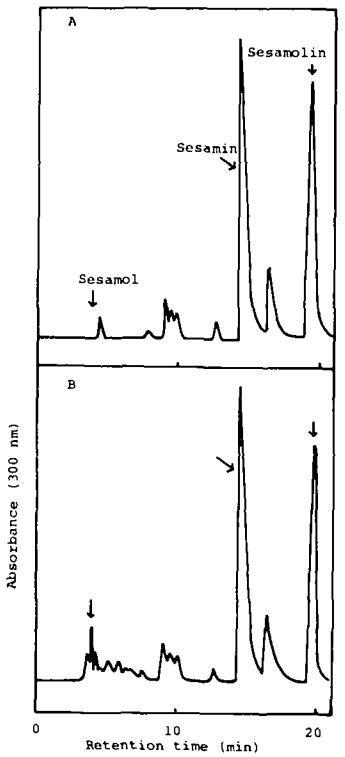



**FIG. 2. HPLC of JP sesame oil (A) and edible sesame oil (B).**

determination by use of hydrogen peroxide/horseradish peroxidase (Table I). JP sesame oil contained 0.21% sesamolin and 0% sesamol, and edible sesame oil contained 0.29% sesamolin and ca. 0.01% sesamol. The contents of sesamolin in both oils were near to those reported by other researchers (13-15, 19). The possible oxidation product, sesamol dimer, could not be detected in either JP or edible sesame oils.

### Stability of Sesamolin, Sesamol and Sesamol Dimer in Ultraviolet Irradiation

A solution of 1.0 mM sesamolin, sesamol or sesamol dimer in benzene was irradiated by ultraviolet light, and the residual amount of each compound in the solution was etimated by HPLC. Time courses of the decrease of each compound are shown in Figure 3. All the compounds decreased by irradiation and the decrease was faster in the order of sesamol dimer > sesamol > sesamolin. After 24-hr irradiation, sesamol and sesamol dimer were completely lost, but 30% of sesamolin still remained in the solution. When detected at 300 nm, each solution revealed no peaks other than those corresponding to the initial compounds during the irradiation for up to 24 hr.

The solution of sesamol dimer turned violet after 2-3 hr irradiation, showing absorption maximum at 530 nm, and then gradually turned brown, which indicated that sesamol dimer turned into unstable sesamol dimer quinone (10). The solutions of sesamolin and sesamol were not significantly colored violet throughout the irradiation but they gradually turned brown. After irradiation for 2 days, each solution was applied to HPLC and the peaks were detected at 230 nm (Fig. 4). Every irradiated solution revealed many

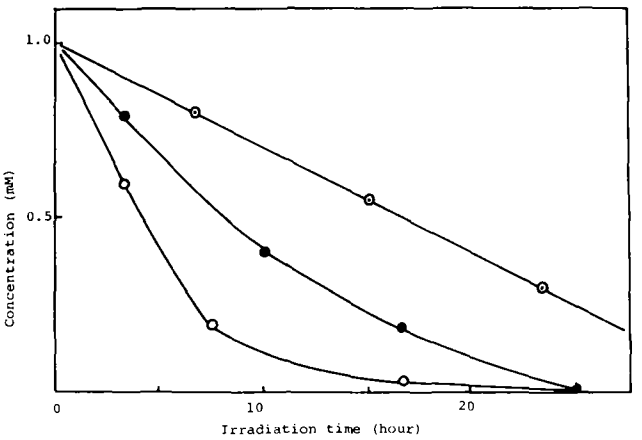



**FIG. 3. Decreases of sesamolin (⊙), sesamol (●) and sesamol dimer (○) by ultraviolet irradiation. The content of each compound was determined by HPLC.**

**TABLE I**

**Content of Sesamolin and Sesamol in Sesame Oil**

| | Method | Content (%) | |
|---|---|---|---|
| | | Sesamolin | Sesamol |
| JP sesame oil | HPLC[a] | 0.21 | 0 |
| | $H_2O_2$/HRPO[b] | – | 0 |
| Edible sesame oil | HPLC[a] | 0.29 | 0.01 |
| | $H_2O_2$/HRPO[b] | – | 0.0064 |

[a]High performance liquid chromatography.
[b]Horseradish peroxidase.

peaks and the chromatograms resembled each other. Each solution was applied to thin layer chromatography and the spots were detected by ultraviolet light (254 and 365 nm) and BQC reagent (Fig. 5).

Each solution revealed many unidentified spots, and the chromatograms of the solutions resembled each other. These results indicated that the final products of sesamolin, sesamol and sesamol dimer were identical, suggesting that these three compounds were degraded through the sequence illustrated in Scheme 1. Although the intermediate compounds in degradation could not be detected in HPLC, they might be degraded much faster than the parent compounds.

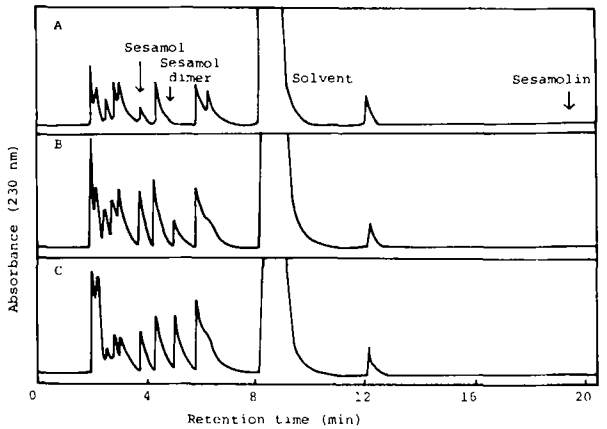



**FIG. 4. HPLC of sesamolin (A), sesamol (B) and sesamol dimer (C) irradiated for 2 days.**

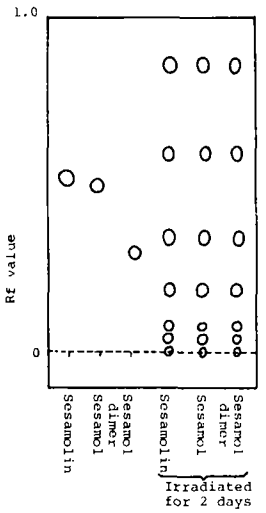



**FIG. 5. Thin layer chromatography of sesamolin, sesamol and sesamol dimer irradiated for 2 days. Solvent: *n*-hexane/ethyl acetate (2:1). Spots were detected by BQC reagent.**

## Comparison of Antioxidant Activity of Sesamolin, Sesamol and Sesamol Dimer in Lard, Methyl Oleate and Soybean Oil

Sesamolin, sesamol and sesamol dimer were incorporated in lard, methyl oleate and soybean oil at a concentration of 0.01%, and stability of the fat and oils was evaluated by active oxygen method. POV of these fat and oils was measured at the regular intervals, and the results are shown in Figure 6.

Sesamolin showed no significant antioxidant activity in any of the fats or oils. On the other hand, sesamol and sesamol dimer exhibited extensive antioxidant activity with clear demonstration of prolonged induction periods, especially in lard and methyl oleate. While the activity of sesamol dimer was higher than that of sesamol in lard, it was lower in methyl oleate. In soybean oil, sesamol exhibited low but significant activity, and sesamol dimer had a slightly higher activity. It may be noted that sesamol dimer, an oxidation product of sesamol, has an extensive antioxidant activity and the activity was much higher than that of sesamol in certain oils.

## Relevance of Sesamolin and Sesamol to Stability of Sesame Oil

Stability of sesame oils was evaluated by measuring POV at the regular intervals in active oxygen method (Fig. 7). Edible sesame oil containing both sesamolin and sesamol resisted peroxidation: POV did not increase and was less than 5 meq/kg even after 94-hr activation of the oil (Fig. 7A). On the other hand, JP sesame oil containing sesamolin and free from sesamol was relatively unstable; POV rapidly increased to ca. 100 meq/kg after 23-hr activation without exhibiting a clear induction period. When 0.01% sesamol was incorporated in JP sesame oil, the stability of the oil was slightly increased but was far from that of the edible oil. JP sesame oil mixed with 0.01% seasmol dimer was more stable but was not so stable as the edible oil (Fig. 7A). High stability of the edible oil could not be explained by the presence of ca. 0.01% sesamol.

When the JP oil was mixed with 5% of the edible oil (total sesamol content: 0.0005%), stability of the oil was as same as the JP oil with 0.01% seasmol. When the JP oil was mixed with 10% of the edible oil (total sesamol content: 0.001%), stability of the oil was higher (Fig. 7B). These results suggest that the edible oil contains autioxidative compound(s) other than sesamol, and the highly stable character of the edible oil can be explained by the presence of another strong antioxidative compound(s).

Changes in amounts of sesamolin, sesamol and sesamol dimer in JP sesame oil under the activated conditions were monitored by high performance liquid chromatography (Fig. 8). Sesamolin in JP oil mixed with sesamol decreased gradually, and sesamol decreased rapidly to reach 30% of the initial content during the period of 20 hr, while POV gradually increased to 50 meq/kg. Sesamol dimer mixed in the JP oil decreased more rapidly and was completely lost after 20 hr, while POV of the oil gradually increased to 30 meq/kg. The order of degradation speed of these compounds in the activated JP oil was sesamol dimer > sesamol > sesamolin; the tendency was similar to the case of ultraviolet irradiation (Fig. 3). No peaks other than those of the parent compounds were detectable in HPLC. It was found that the rate of decrease in the activated JP oil correlated

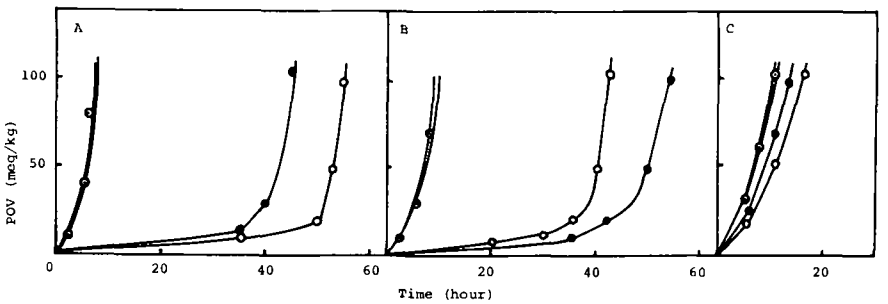



**FIG. 6. Increases in POV of fat and oils by active oxygen method. (A) Lard; (B) methyl oleate; and (C) soybean oil. ——, Control; —⊙—, +0.01% sesamolin; —●—, +0.01% sesamol; and —○—, +0.01% sesamol dimer.**

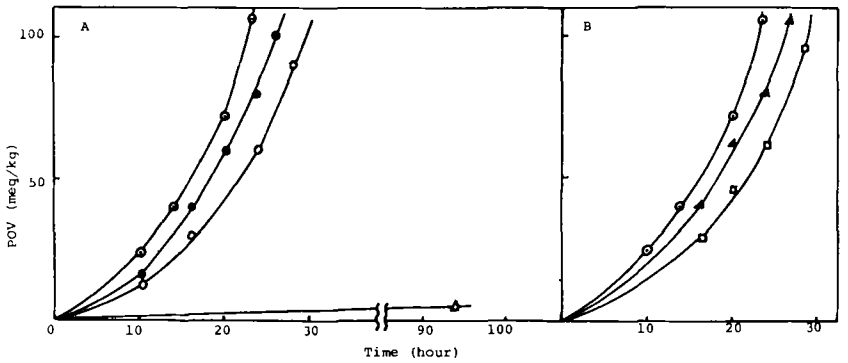



FIG. 7. Increases in POV of sesame oil by active oxygen method. —⊙—, JP sesame oil; —●—, JP sesame oil + 0.01% sesamol; —○—, JP sesame oil +0.01% sesamol dimer; —△—, edible sesame oil; —▲—, JP sesame oil +5% edible sesame oil; and —□—, JP sesame oil +10% edible sesame oil.

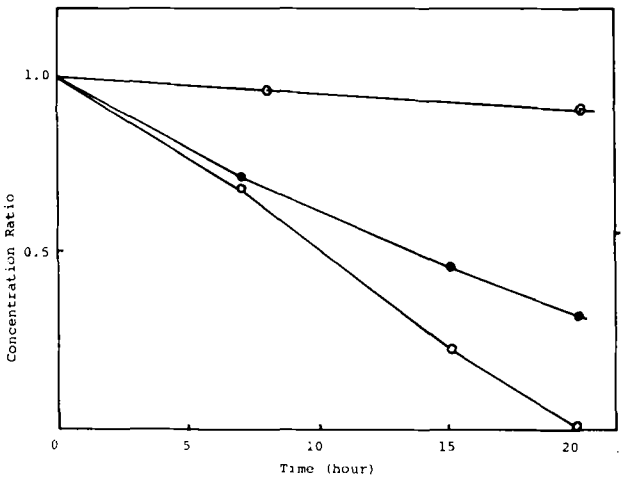



FIG. 8. Decreases of sesamolin, sesamol and sesamol dimer in JP sesame oil by active oxygen method. The amounts of sesamolin (⊙) and sesamol (●) in JP sesame oil containing 0.01% sesamol and the amount of sesamol dimer (○) of the oil containing 0.01% sesamol dimer were determined by HPLC.

with the development of antioxidant activity of these compounds.

In contrast to the case of JP oil, changes in the amounts sesamol by HPLC indicated the content of sesamol was increased to 0.036%, and that by hydrogen peroxide/horseradish peroxidase indicated it was increased to 0.028%. POV of the edible oil did not increase during the period of 94 hr. The results indicate that sesamolin was transformed into sesamol by the activation of the oil, and the unidentified powerful antioxidant(s) endogenously contained in the oil might prevent degradation of unstable sesamol and peroxidation of the oil.

## DISCUSSION

### Determination of the Constituents of Sesame Oil

Sesame oil contains several constituents, among which sesamol, bound sesamol (sesamolin) and sesamin were regarded as compounds with antioxidant activity and synergistic activity with pyrethrin insecticides (2). Colorimetric assay according to Baudouin or Villavecchia test has been adapted for the determination of sesamolin and sesamol (1,2,13,14). However, these classical methods were not selective and troublesome, and partial separation with organic solvent was needed prior to the colorimetric determination. Recent works by Yoshida and Kashimoto (19) demonstrated that HPLC by a silica gel column was useful for determination of sesamolin, sesamol and sesamin in sesame oil. In the present study, we successfully separated and determined these constituents by HPLC using a reverse-phase column.

In the previous paper (10), it was shown that sesamol was converted into violet-colored sesamol dimer quinone by mild treatment with hydrogen peroxide/horseradish peroxidase system. Sesamolin was not colored and sesamol was selectively colored and determined in this system. The method may provide a versatile tool for determination of free sesamol in sesame oil.

### Antioxidant Activity of Sesamolin, Sesamol and Sesamol Dimer

Bound sesamol (sesamolin) was inactive as an antioxidant in lard, methyl oleate and soybean oil (Fig. 6), and the results were consistent with those by Budowski (4). Since liberation of sesamol from sesamolin in activated oil was very gradual (Fig. 9), sesamolin could not serve as an antioxidant by itself.

Sesamol was very active in lard and methyl oleate but much less so in soybean oil and JP sesame oil (Figs. 6 and 7). Sesamol showed an unexpectedly low antioxidant activity in JP sesame oil. High activity in lard has been described previously (4,5). Sesamol decreased relatively rapidly demonstrating antioxidant activity in JP sesame oil (Figs. 7 and 8).

Sesamol dimer has been isolated in a crystalline form (10). This compound is a possible intermediate of the oxidative degradation of sesamol, since its structure was close to that of the dimeric compounds of butylated hydroxyanisole (11) which was produced in the activated oils containing butylated hydroxyanisole (12). Sesamol dimer showed a potent antioxidant activity comparable to that of the parent compound in lard, methyl oleate, soybean oil and JP sesame oil (Figs. 6 and 7). This strong antioxidant activity of sesamol dimer was not surprising since the dimers of butylated hydroxyanisole had the same activity as the parent compound (11). The compound decreased more rapidly in JP sesame oil demonstrating more powerful antioxidant activity than sesamol (Figs. 7 and 8).

Conversion of sesamolin into sesamol was demonstrated in edible sesame oil, while the transformation could not be observed in the experiments with activated JP sesame oil or irradiated benzene solution, which may be due to the high-

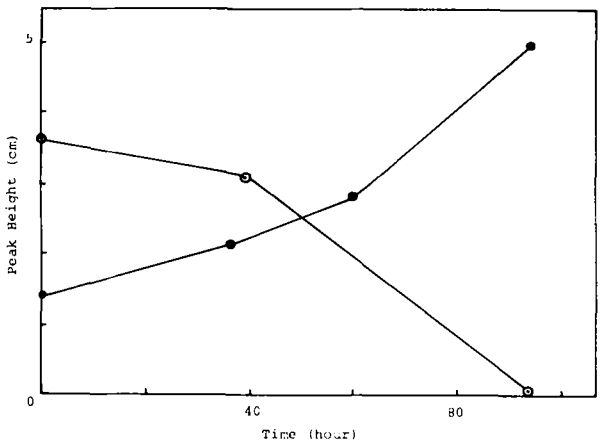



**FIG. 9. The changes of the amount of sesamolin and sesamol in edible sesame oil by active oxygen method. The contents of sesamolin (○) and sesamol (●) were determined by HPLC.**

ly unstable nature of sesamol. Detection of sesamol dimer also failed, probably due to much higher instability. The final products observed in each irradiated solution of sesamolin, sesamol and sesamol dimer were identical, suggesting that the degradation of these compounds proceeded as illustrated in Scheme 1.

### Stability of Sesame Oil

It has been long believed that high stability of sesame oil toward peroxidation was due to the presence of free sesamol, based on the observation that the oil contained sesamol with high antioxidant activity (1-5).

JP sesame oil was the purified sesame oil and freed from various constituents by certain processing treatments, and it contained no sesamol. Stability of JP sesame oil was relatively low. The antioxidant activity of sesamol incorporated in JP sesame oil was not so strong as has been described. In contrast, edible sesame oil contained many constituents as well as sesamol, and the oil had much higher stability than JP sesame oil mixed with sesamol. It is unlikely that sesamol is responsible for stability of sesame oil. High stability of the edible oil could only be explained by the presence of another powerful antioxidant(s). Sesamol incorporated in the JP oil rapidly disappeared and simultaneously POV of the oil increased, while sesamol content in the edible oil increased during the much longer period. Presence of sesamol in sesame oil seemed to be a mere index of whether the sesame oil is protected against peroxidation by another antioxidant(s). It may be concluded that both peroxidation of sesame oil and loss of sesamol were prevented by another powerful antioxidant(s).

REFERENCES

1. Budowski, P., and K.S. Markley, Chem. Rev. 48:125 (1951).
2. Budowski, P., JAOCS 41:280 (1964).
3. Olcott, H.S., and H.A. Mattill, Chem. Rev. 29:257 (1941).
4. Budowski, P., JACOS 27:264 (1950).
5. Moore, R.N., and W.G. Bickford, JAOCS 29:1 (1952).
6. Sahasradbudhe, M.R., J. Sci. Ind. Res. (India) 121B (1953), Chem. Abstr. 47:7238b (1953).
7. Fukuzumi, K., and N. Ikeda, JAOCS 46:64 (1969); JAOCS 47: 369 (1970).
8. Budowski, P., F.G.T. Menezes and F.G. Dollear, JAOCS 27: 377 (1950).
9. Beroza, M., JAOCS 32:348 (1955).
10. Kurechi, T., K. Kikugawa and S. Aoshima, Chem. Pharm. Bull. 29:2351 (1981).
11. Kurechi, T., Eisei Kagaku 15:301 (1969).
12. Maga, J.A., and W.C. Monte, Lebensm. Wiss. Technol. 10:102 (1977).
13. Budowski, P., R.T. O'Connor and E.T. Field, JAOCS 27:307 (1950).
14. Suarez, C.C., R.T. O'Connor, E.T. Field and W.G. Bickford, Anal. Chem. 24:668 (1952).
15. Budowski, P., JAOCS 28:51 (1951).
16. Haslam, E., and R.D. Haworth, J. Chem. Soc. 827 (1955).
17. King, A.E., H.L. Roschen and W.H. Irwin, Oil Soap 10:105 (1933).
18. Wheeler, D.E., Ibid. 9:89 (1932).
19. Yoshida, M., and T. Kashimoto, Shokuhin Eiseigaku Zasshi 23: 142 (1982).

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