Contribution of Lignan Analogues to Antioxidative Activity of Refined Unroasted Sesame Seed Oil

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A lignan compound, P3, having strong antioxidative activity was found to be formed in high concentration during the industrial bleaching process of unroasted sesame seed oil. P3 (named sesaminol) was identical to a minor constituent previously isolated from acetone extract of sesame seed. It was shown that sesamolin in unprocessed sesame oil is the source of sesaminol, and the formation of sesaminol was confirmed by the model experiment with corn oil to which sesamolin had been added. Sesaminol was not so greatly removed by the deodorization process that follows bleaching as was sesamol, and it was shown to be at a concentration of ca. 100 mg/100g in commercial refined unroasted seed oil. The antioxidative activity of sesaminol was roughly equal to those of sesamol and γ-tocopherol by the thiocyanate method. Therefore, it seems that the antioxidative activity of refined unroasted seed oil is mainly attributed to sesaminol.

Two kinds of sesame oils, from unroasted seed and roasted seed, are widely used. Both of these oils have been known to be resistant to oxidation. The oxidation of unroasted seed oil, commonly used throughout the world since ancient times, has remained obscure, in spite of chemical investigation of the oil by Budowski et al. (1-4) and Mathur et al. (5). They reported that sesamol was a strong antioxidant and was produced from sesamolin during a bleaching process with acid clay, but was nearly completely removed from the oil by the subsequent deodorizing process. We reported previously (6) that sesame oils from both unroasted and roasted seeds included notable amounts of γ-tocopherol, but in spite of decreases in the amount of tocopherol during refining processes, the refined unroasted seed oil had stronger antioxidative activity than the crude oil. However, the reason could not be shown.

The present work deals with investigation of the antioxidative principles in refined unroasted seed oil.

MATERIALS AND METHODS

Materials. Sesame oil at various stages of processing (crude oil, alkali-treated, washed, bleached and deodorized) and acid clay were donated by Takemoto Oil Co. Ltd., Gamagori, Japan. Corn oil was a gift from Oji Corn Starch Co., Tokyo, Japan, and γ-tocopherol was given by Eisai Co., Tokyo, Japan. Sesamol (reagent grade) was purchased from Sigma, St. Louis, Missouri. Sesamolin and sesamin were purified as reported previously (6) and their purity was confirmed by mass spectrometry and proton nuclear magnetic resonance (^{1}H-NMR). Linoleic acid was purified by distillation. Silica gel (BW 820-H from Fuji-Davison) was used for column chromatography.

High performance liquid chromatography (HPLC). The Millipore-filtered oil sample was injected onto a Develosil 10-ODS column, for analyses of γ-tocopherol, sesamin, sesamolin and sesamol. The eluting solvent was methanol for γ-tocopherol and 70% methanol for others. The mixture of sesamin and episesamin and that of P3 and its epimers were separated using Develosil SI 60-5, with 8:2 and 7:3 (v/v) *n*-hexane/ethyl acetate, respectivly, as solvents.

Thin layer chromatography (TLC). TLC was performed on a Merck 60F_{254} Silica plate.

Antioxidative activity. The thiocyanate method was employed to measure the degree of oxidation of linoleic acid in the samples as previously described (7).

Instruments. Those used were a Hitachi 200-10 Spectrometer for UV, JEOL JNM-FX-200 for NMR, JEOL JNM-D-100 for mass spectra, JMS-OISG for high resolution mass spectra and JASCO DIP-4 for specific rotation.

RESULTS

Isolation of antioxidants and some lignans from refined unroasted seed oil. Comparison of HPLC patterns at 290 nm between crude and refined unroasted seed oil was made to investigate chemical changes in sesamin, sesamolin, sesamol, etc. As shown in Figure 1, large

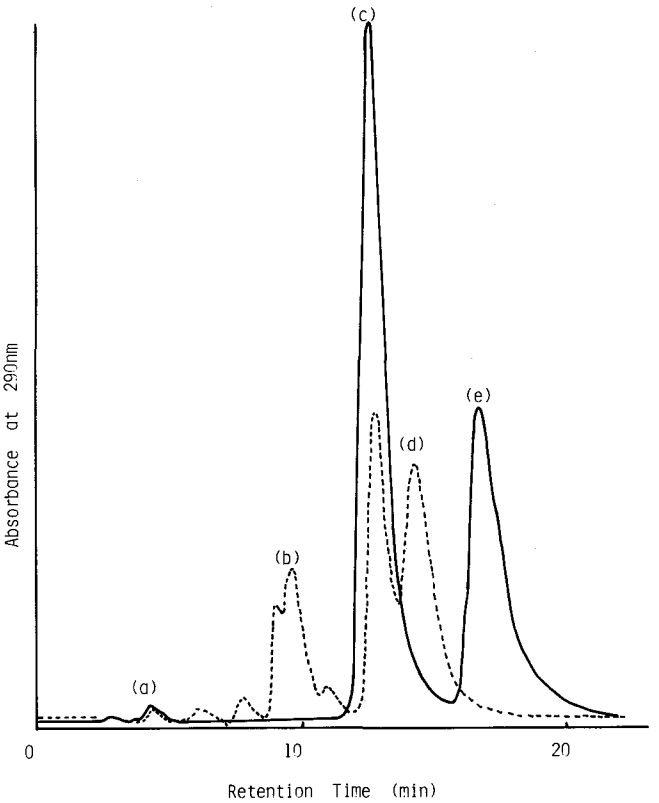

FIG 1. Comparison of HPLC patterns in crude and refined oils from unroasted sesame. Column, Develosil 10-ODS; eluent, MeOH-H_2O (7:3); flow rate, 3 ml/min; detector, UV 290 nm. ———, Crude oil; - - - -, refined oil. (a), Sesamol; (b), unknown; (c), (+)sesamin; (d), unknown; (e), sesamolin.

differences were observed. From comparison of retention times with authentic sesamin, sesamolin and sesamol, it was concluded that peak (a) was sesamol, peak (c) was sesamin and peak (e) was sesamolin. It was also shown that some refining process eliminated peak (e) and gave rise to several new peaks, including (b) and (d). Peak (b) gave a reddish-purple color with ferric chloride on the TLC plate and was considered to be a phenolic compound.

The procedure adopted for isolation and purification of the antioxidants from refined oil is outlined in Figure 2. The fraction of 9-1 (*n*-hexane-EtOAc) silica gel chromatography was oily eluents including a small amount of γ-tocopherol. The amount of soluble components in the more polar fractions beyond 7-3 (*n*-hexane-EtOAc) silica gel chromatography was very small; hence, in this report the isolation of those fractions was not carried out. F1 was crystallized from methanol and identified as (+)episesamin from the data of mass fragment ions, ^{1}H-NMR, optical rotation and mp in comparison with the literature (8,9). The retention time of F1 in HPLC agreed with that of peak (d) in Figure 1. F2 was identified as (+)sesamin by comparing with authentic (+)sesamin and agreed with that of peak (c) in Figure 1. F3 showed identical molecular formula and ^{1}H-NMR chemical shifts of the sesamin analogue P3 ($C_{20}H_{18}O_7$) isolated from sesame seeds (F3 was found to be identical to P3). F4 and F5 showed mol wt and fragmentation peaks identical with those of P3, but showed differences in H-2 and H-6 signals in the NMR spectra and in TLC Rf values ($CHCl_3$-Et_2O, 9:1). Hence F4 and F5 are assumed to be epimerica isomers of P3. The fact that episesamin was found in refined unroasted seed oil also supports P3 epimerization. F6 was identified as sesamol dimer upon comparison with the mol wt and ^{1}H-NMR spectrum reported in the literature (10). The confirmative studies of P3 and its epimer are under way.

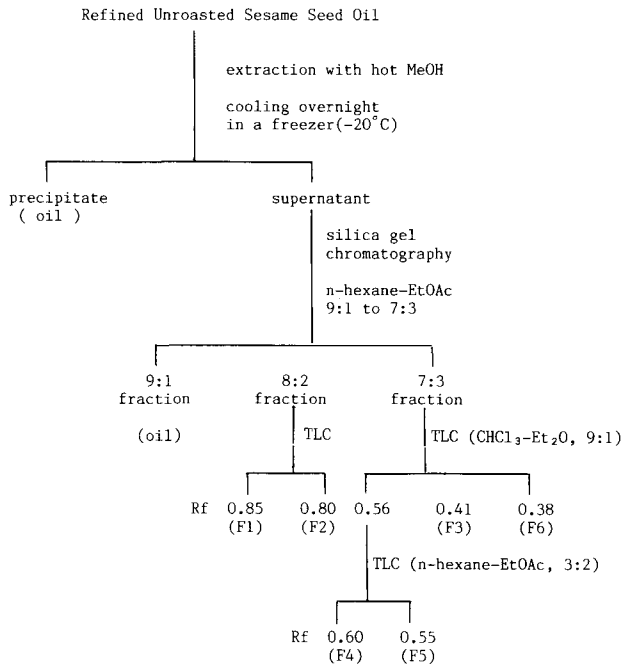

FIG. 2. Scheme for isolation of antioxidants from refined unroasted sesame seed oil.

Identification of P3. P3, presently identified as "Sesaminol," has the composition $C_{20}H_{18}O_7$ (cald. 370.3840, C 64.86%, H 4.90%, found 370.1057, C 64.90%, H 4.94%) and showed mp 130-131 C, $[\alpha]_D$ + 27.8 C (c = 1.0, $CHCl_3$), absorption maxa at 238 and 295 nm ($\log_\varepsilon$ = 3.99 and 4.17) in the UV spectrum and fragment ions, 370 (M$^+$100%), 353 (22.6), 203 (43), 186 (34.3), 149 (41.4), 135 (54.3). Though sesaminol seems to be a sesamolin-type lignan from the molecular formula, the ^{1}H-NMR spectrum of sesaminol was very similar to that of (+)sesamin. The ^{1}H-NMR spectrum of sesaminol and (+)sesamin is listed in Table 1 (11). The structure of (+)sesamin is apparently symmetrical in the identity of the ^{1}H-NMR signals for H-1/H-5, H-2/H-6 and the methylenedioxy signals (5.92, s), but in the ^{1}H-NMR spectrum of sesaminol, the signals for H-1/H-5 and H-2/H-6 were almost identical with those of (+)sesamin, but each of the methylenedioxy protons appeared as singlets at 5.90 (2H) and 5.97 (2H). The aromatic region of sesaminol showed, in addition to the signals seen in the (+)sesamin spectrum (6.83, m, 6H) due to the 1,3,4-trisubstituted ring, two singlets at 6.43 and 6.53, supporting the fine coupling due to para-relationships. This 2′,5′-placement, two singlets of methylenedioxy protons, production of monoacetyl ether of sesaminol (M$^+$412) on acetylation with acetic anhydride in pyrizine, and positive reaction with $FeCl_3$ reagent confirmed the structure of sesaminol as II in Figure 3. The position of the aromatic-OH was also confirmed by X-ray analysis of benzoate of sesaminol epimer. The stereochemistry of sesaminol and its epimers soon will be published elsewhere.

Analysis of lignans and tocopherol in unroasted seed oil during processing. Refining processes of the unroasted seed oil are as follows: alkali treatment, washing, bleaching with acid clay and deodorization. The amounts of the compounds (sesamin, episesamin,

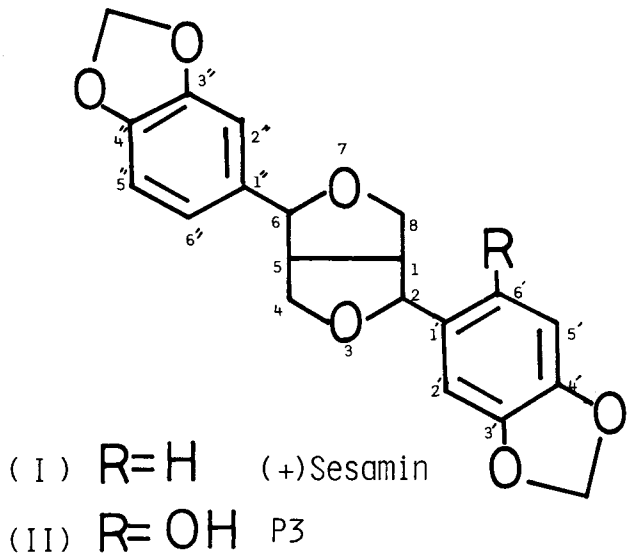

FIG. 3. Chemical structure of P3 and (+)sesamin.

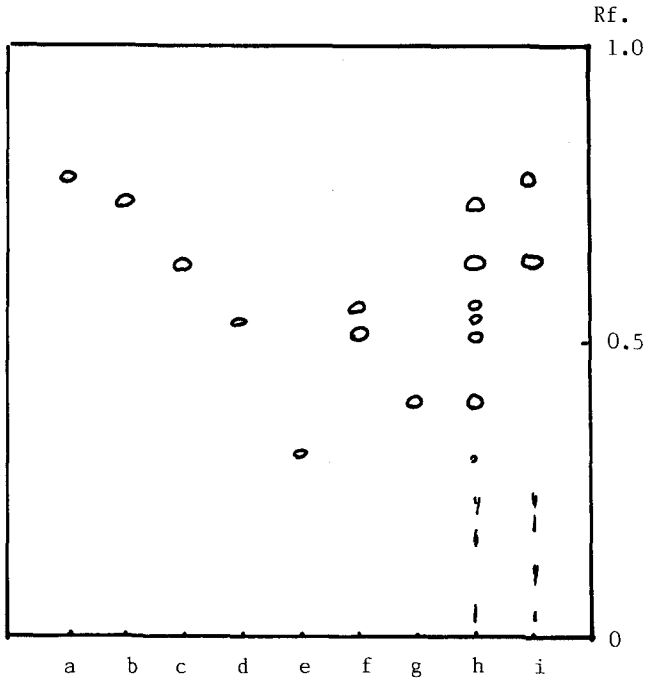

FIG. 4. TLC pattern of methanol extract of oil (oil no. 3 in Table 2) with addition of acid clay. Plate, Merck Silica plate 60F_{254}; solvent, $CHCl_3$-Et_2O (9:1); detector, UV lamp. a, Sesamolin; b, (+)episesamin; c, (+)sesamin; d, sesamol; e, sesamol dimer; f, episesaminol; g, sesaminol; h, MeOH extract from oil no. 3 add with acid clay, 1-hr heating, in vacuo in 90 C water bath; i, MeOH extract from oil no. 3, 1-hr heating, in vacuo in 90 C water bath.

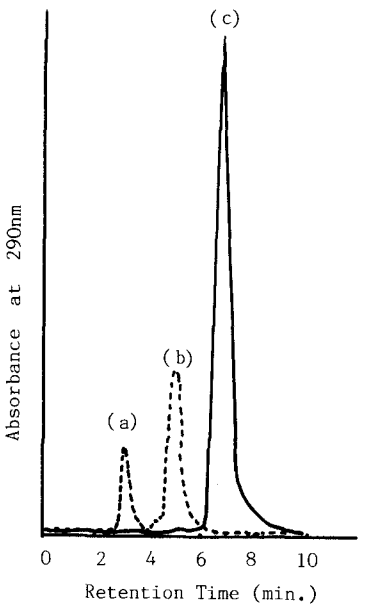

FIG. 5. HPLC pattern of corn oil added with sesamolin and acid clay. Column, Develosil 10-ODS; eluent, MeOH-H_2O (8:2); flow rate, 3.0 ml/min. ———; 2 ml corn oil + 5 mg sesamolin; heating 1 hr, in vacuo in 90 C water bath; - - - -; 2 ml corn oil + 5 mg sesamolin 0.5 g acid clay; heating 1 hr, in vacuo in 90 C water bath. (a), Sesamol; (b), sesaminol; (c), sesamolin.

TABLE 1

^{1}H-N,MR Data of Sesaminol (P3) and (+)Sesamin

Proton no.	Sesaminol (P3)	(+)Sesamin (11)
H-1/5	3.14 (2H,m)	2.88 (2H,m)
H-2/6	4.76 (2H,d, J=3.8)	4.75 (2H,d, J=4.0)
H-4a/8a	3.86 (2H,m)	3.74 (2H,d,d, J=4.0 and8.5)
H-4e/8e	4.36, 4.14 (2H,m)	4.10 (2H,d,d, J=6.0 and 8.5)
$-OCH_2O-$	5.90, 5.97 (4H,s)	5.92 (4H,s)
H-2′/5′	6.46, 6.53 (2H,s)	(Ar-H)
H-2″/5″/6″	6.80, 6.86 (3H,m)	6.83 (6H,m)
Ar-OH	7.6 (1H,s)	

δ Values, internal standard TMS, solutions in $CDCl_3$, 200 MHz spectra, J in Hz.

sesamolin, sesamol, sesamol dimer, sesaminol, its epimers and γ- tocopherol) in the oils at each step were determined by HPLC analysis. The results shown in Table 2 revealed that significant chemical changes took place mainly at the bleaching stage using acid clay. These were epimerization of sesamin (41%); disappearance of sesamolin; and production of sesamol, sesaminol, its epimers and a minor amount of sesamol dimer. That sesamol was produced from sesamolin during the bleaching step and was removed in the next deodorizing step has been reported by Budowski et al. (1-4), but the production of sesaminol after the bleaching step has not been reported before. Noteworthy is the fact that the significant amounts of sesaminol and its epimers produced were not as greatly decreased by the deodorization step as was sesamol.

The sesaminol and γ-tocopherol content in refined oil from three different sources is shown in Table 3. Sesaminol content varies from one product to another, probably because of the difference in bleaching procedures. Of six samples, two from the same source contained no detectable amount of tocopherol.

Epimerization of sesamin and formation of sesaminol and its epimers. To investigate whether the chemical changes, namely, epimerization of (+)sesamin and production of sesamol and sesaminol, in the bleaching stage as shown in Table 2 depend upon acid clay or not, 2 ml of the oil before bleaching (oil no. 3 in Table 2) was warmed in vacuo at 90 C with addition of 0.5 g acid clay for one hr. Formation of (+)episesamin, sesaminol and sesamol was confirmed by HPLC and TLC analyses as shown in Figure 4. If sesamolin were completely decomposed to sesamol, the amount of sesamol would be ca. 154 mg/100 g oil. But actually sesamol was 46.3 mg/100 g oil; hence, it was assumed that a part of sesamolin was converted to sesaminol or its epimers. To confirm this point, 2 ml of corn oil to which 5 mg of sesamolin was added was warmed in vacuo at 90 C with 0.5 g acid clay for one hr, and the formation of sesaminol and sesamol were confirmed by HPLC analysis, as shown in Figure 5. These results confirmed the production of sesaminol from sesamolin by acid catalysis. More detailed examination of chemical aspects is in progress.

Comparison of antioxidative activities of antioxidants present in refined oil from unroasted seed. The results of comparative study of sesaminol, episesaminol, sesamol,

TABLE 2

Amount of Lignans and Tocopherol in Unroasted Sesame Seed Oil During Refining Process (mg/100 g Oil)

Oil no.[a]	(+)Sesamin	(+)Episesamin	Sesamolin	Sesamol (its dimer)	Sesaminol (P3)	Episesaminol	γ-Tocopherol
1	813.3	0	510.0	4.3 (0)	0	0	33.5
2	730.6	0	458.0	2.5 (0)	0	0	23.4
3	677.8	0	424.8	0.7 (0)	0	0	22.6
4	375.5	277.6	0	46.3 (trace)	33.9	48.0	21.8
5	258.3	192.6	0	1.7 (trace)	28.4	34.3	18.4

[a]1, crude; 2, alkali-refined; 3, washed with warm water; 4, bleached; 5, deodorized. Amounts of antioxidants, sesamin and episesamin were analyzed by HPLC as described in the text.

TABLE 3

Amount of Sesaminol and Tocopherol in Different Commercial Sesame Oils (mg/100 g Oil)

Commercial sesame oil	Sesaminol	Episesaminol	Total sesaminol	γ-Tocopherol
A	61.2	81.6	142.8	25.5
B	58.2	76.0	134.2	29.3
C	52.2	69.6	121.8	25.2
D	17.9	23.9	41.8	23.5
E	52.2	69.6	121.8	n.d.
F	6.6	8.8	15.8	n.d.

Amount of antioxidants was analyzed by HPLC as described in the text.

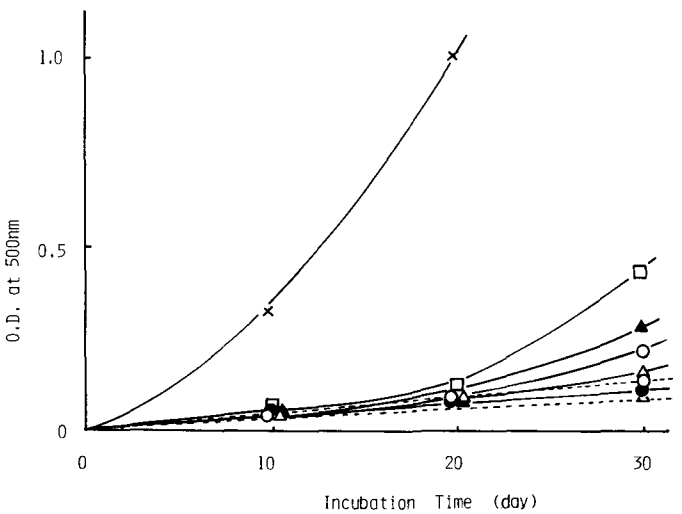

FIG. 6. Antioxidative assay of lignans in refined unroasted seed oil by the thiocyanate method. Amount of samples in the incubation mixtures (10 ml of 0.13 ml linoleic acid in 99% EtOH, 10 ml of 0.1 M phosphate buffer, pH 7.0, volume adjusted to 25 ml by H_2O). X——X, Control; □——□, sesamol dimer (0.2 mg); ▲——▲, sesaminol (0.2 mg); ●——●, γ-tocopherol (0.2 mg); O——O, sesaminol (0.2 mg); O- - -O, sesaminol (0.5 mg); △——△, episesaminol (0.2 mg); △- - -△, episesaminol (0.5 mg).

sesamol dimer and γ-tocopherol, using linoleic acid as substrate by the thiocyanate method (7), are shown in Figure 6. The antioxidative activities of sesaminol and episesaminols were roughly equal to sesamol and γ-tocopherol. It is clear than sesaminol and episesaminols are the dominant antioxidant in refined oil due to their potency and higher concentration than γ-tocopherol. Sesaminol was also highly heat-resistant because a relatively large amount of residual sesaminol remained after heating of the refined oil at 180 C for 10 hr.

DISCUSSION

The increase in sesamol content during the refining process of the oil from unroasted sesame oil has been noted earlier by Honig (12), Budowski (13) and Fujimura (14). A more quantitative and detailed picture of the antioxidant distribution was obtained in the present work by the use of HPLC. The results newly revealed show epimerization of (+)sesamin and catalytic conversion of sesamolin to sesaminol (a product of transformation) and its epimers.

The recognition of sesaminol (and its epimer) as the dominant antioxidant in refined oil appears to have considerable significance. Sesaminol, found in minor quantities in sesame seed and as its glycoside (7), is free of any taste, odor or color, has higher heat stability and might be considered one of the most important natural antioxidants for food. The antioxidative activity of sesaminol in biological systems is also an interesting topic related to the aging process, and is currently under study.

The conversion of sesamolin to sesaminol in oil systems seems to be an acid-catalyzed reaction, involving scission and transformation of a C-C bond, and is also of interest as a chemical reaction, the details of which will be reported elsewhere.

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♣Comparison of Acidic and Basic Volatile Compounds of Cocoa Butters from Roasted and Unroasted Cocoa Beans

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A total of nine acidic and 83 basic compounds was identified in the roasted and unroasted cocoa butter samples. Forty seven of the compounds identified are being reported for the first time in cocoa. The higher concentration of short chain fatty acids in the unroasted cocoa butter is responsible for its acidic aroma characteristics. The roasted cocoa butter generally contained greater numbers and higher concentrations of compounds whose formations would be favored by thermal processing. These compounds included pyrazines, thiazoles, oxazoles and pyridines. The aromas of many of these compounds are characteristic of the aroma differences between the two cocoa butters and contribute to the cocoa aroma of roasted cocoa butter.

The price and popularity of cocoa have made it one of the most studied natural flavors. More than 400 volatile flavor compounds have been identified as constituents of cocoa. The majority of research on cocoa has dealt with cocoa beans, cocoa powder, cocoa liquor and chocolate as sources of flavor. Only three publications exist which investigate cocoa butter as a source of flavor.

Van Elzakker and van Zutphen (1) identified 23 volatile flavor compounds in the neutral and basic fractions of a vacuum steam distillate of cocoa butter. Rizzi (2) identified nine alkylpyrazines in the basic fraction of a vacuum steam distillate of cocoa butter. Rostagno et al. (3) identified 30 volatile flavor compounds in a steam distillate of cocoa butter and developed an analytical method to evaluate the aroma intensity of different varieties of cocoa butter.

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The flavor of cocoa butter depends on the processing conditions to which the cocoa beans are subjected. Cocoa butter obtained from roasted cocoa beans has a strong flavor reminiscent of cocoa. Cocoa butter obtained from unroasted cocoa beans which have been given a steam treatment has a considerably milder, yet distinctive, flavor.

The objective of this study was to isolate, identify and semiquantitate the volatile flavor compounds of cocoa butters obtained from roasted and unroasted cocoa beans. This paper reports the isolation methodology employed and the acidic and basic compounds identified. The neutral compounds identified will be reported in a subsequent paper.

EXPERIMENTAL SECTION

Starting materials. The cocoa butter samples were supplied by Cadbury-Schweppes, Ltd. They were prepared from Ghanaian/Nigerian Grade 1 cocoa beans. The roasted cocoa butter was obtained from cocoa beans which were roasted at 145 C for 8 min in a continuous roaster. Cocoa liquor obtained from the roasted beans was hydraulically pressed at 100 C and 7,000 psi to obtain the roasted cocoa butter. The unroasted cocoa butter was obtained from the expeller pressing of whole cocoa beans. The beans were heated with steam to 85 C and held for 20 min before being pressed.

The aromas of the cocoa butter samples were evaluated by two trained flavorists. The roasted sample had a strong, well-rounded cocoa aroma with some burnt characteristics. The unroasted sample had a milder aroma with acidic, fruity, floral and fatty-waxy characteristics. It did not possess the total cocoa aroma.

Flavor isolation. The volatile flavor compounds of both cocoa butter samples were isolated using a semicontinuous, countercurrent, vacuum steam distillation apparatus (4). The volatile flavor compounds were isolated from a total of 80 lb of the roasted cocoa butter and 70 lb of the unroasted cocoa butter.