Minor Components Responsible for Flavor Reversion of Soybean Oil

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Edible refined, bleached and deodorized (RBD) soybean oil was fractionated by silicic acid column chromatography to identify minor components responsible for flavor reversion. Minor components from oil eluted with diethyl ether/n-hexane (1:1) were compared with those from corn and canola oils. All vegetable oils contain free fatty acids, diglycerides and sterols as major ingredients in this fraction. However, unusual triglycerides consisting of 10-oxo-8-octadecenoic acid and 10- and 9-hydroxy octadecanoic acids were detected in RBD and crude soybean oils.

KEY WORDS: Canola oil, column chromatography, corn oil, flavor reversion, hydroxy fatty acid, mass spectrometry, oxo fatty acid, photooxidation, soybean oil, thin-layer chromatography.

Flavor reversion of soybean oil is considered an obstacle for application of soybean oil to many lipid foods. Many undesirable volatile compounds have been observed in reverted soybean oil (1-6). In particular, 3-*cis*-hexenal (2) and 2-pentyl furan (4) reportedly contribute to reversion flavor. Several other hypotheses have been postulated for precursors and mechanisms responsible for flavor reversion. Fatty acid hydroperoxides and their decomposition products, unsaponifiables, phospholipids and polymers have been implicated as flavor reversion precursors (7,8). It is universally accepted that reversion is an oxidative phenomenon of polymers and hydroperoxides. Warner and Frankel (9) and Selke and Frankel (10) suggested that specific factors, such as photosensitizer (singlet oxygen) and metal, might contribute to flavor instability of soybean oil. Our previous data also have suggested that unknown minor components contained in soybean oil must play an important role on flavor reversion (11-13).

The purpose of this report is to isolate and identify minor components responsible for flavor reversion of soybean oil.

MATERIALS AND METHODS

The edible refined, bleached and deodorized (RBD) and crude soybean, corn and rapeseed (canola) oils were obtained from Ajinomoto Co., Tokyo, Japan. To separate minor components in soybean oil, the RBD soybean oil (PV<0.1) was subjected to silicic acid (deactivated with 5% water, w/w) column chromatography (Wakogel C-100, Wako Pure Chem. Ind., Osaka, Japan). The column was eluted stepwise with a diethyl ether/n-hexane mixture system: i) diethyl ether/n-hexane (2:98), ii) (5:95), iii) (1:9), iv) (1:4) v) (1:1), and vi) methanol, according to the method described previously (12,13). Corn and canola oils were also fractionated in a similar manner by silicic acid column chromatography. To determine the minor components responsible for flavor reversion, the soybean oil triglyceride supplemented with each fraction obtained on the column was exposed to a fluorescent lamp (100 lux) at 10°C. Sensory evaluations were made daily by staff members trained in our laboratory. Evaluators judged whether the sample oils exhibited odors and flavors described in the following scale: ++, strong; +, moderate; ±, faint; and −, bland. The occurrence of flavor reversion was expressed as the ratio of evaluators who judged a given sample oil as ++ or + for beany or grassy odor (11).

Minor components in the diethyl ether/n-hexane (1:1) fraction were qualitatively identified by thin-layer chromatography (TLC). TLC was carried out with Kieselgel 60 adsorbent and diethyl ether/n-hexane (3:2) developer. Minor components on a plate were visualized by spraying with 50% H_2SO_4, 1% N,N-dimethyl-p-phenylenediamine dihydrochloride (DPPD) reagent, Shiff's reagent or 0.4% dinitrophenyl hydrazine (DNPH) in 2N HCl solution (14).

Unusual triglycerides in soybean oil eluted with diethyl ether/n-hexane (1:1) from the silicic acid column were then isolated by silica dry gel column chromatography on Silica Woelm TSC (Woelm Pharma, Eschwege, Germany) with diethyl ether/n-hexane (2:3) as the eluting solvent (15), and they were repurified by TLC with the same developer as above. Unusual triglycerides were characterized by ultraviolet (UV) with hexane as the solvent and by infrared (IR) with CCl_4 as the solvent.

Oxo and hydroxy fatty acids isolated from unusual triglycerides were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) as methyl esters. GC and GC-MS separation were carried out on 10% Dexsil 300GC on Chromosorb W (0.3 × 50 cm). Injector/detector and column oven temperatures were programmed at 220 and 140-200°C (2°C/min), respectively. Mass spectral analyses were carried out at 70eV with JEOL JMS-HX110 (Japan Electron and Optics Laboratory Co., Ltd., Tokyo, Japan) at Tohoku University.

RESULTS AND DISCUSSION

To identify the precursors of flavor reversion of soybean oil, RBD soybean oil was fractionated by silicic acid column chromatography with diethyl ether/n-hexane mixtures as the eluting solvents (12). Table 1 shows sensory evaluations of photooxidized soybean oil triglycerides supplemented with the fractions obtained by column chromatography. As shown in Table 1, significant flavor reversion was observed when the soybean oil was supplemented with the diethyl ether/n-hexane (1:1) fraction, while other sample oils were not reverted during photooxidation. This result shows that minor components responsible for flavor reversion of soybean oil could be eluted with diethyl ether/n-hexane (1:1). Therefore, these fractions obtained from RBD and crude soybean oils were compared by TLC with those from rapeseed (canola) and corn oils, which are more resistant to flavor reversion than soybean oil under light irradiation (Fig. 1). Although all vegetable oils contained free fatty acids, diglycerides and sterols,

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TABLE 1

Sensory Evaluation[a] of Soybean Oil Triglycerides Supplemented with Fractionated Minor Components in RBD Soybean Oil During Photooxidation at 10°C

Day	Control[b]	Fraction eluted with: Diethyl ether/ n-hexane (2:98)	Fraction eluted with: Diethyl ether/ n-hexane (1:9)
1	2/8	3/8	3/8
3	2/5	3/5	2/5
5	2/7	4/7	2/7
8	2/8	4/8	2/8

Day	Control[b]	Fraction eluted with: Diethyl ether/ n-hexane (1:4)	Diethyl ether/ n-hexane (1:1)	Methanol
1	0/9	1/9	0/9	1/9
3	0/5	0/5	1/5	0/5
5	0/10	3/10	8/10[c]	0/10
7	3/9	6/9	9/9[c]	0/9

[a]The ratio of evaluators who judged the odor of a given oil to be beany or grassy.
[b]Control (soybean oil triglyceride) was eluted with diethyl ether/ n-hexane (5:95).
[c]Significant ($p<0.05$).

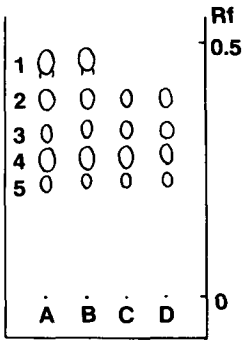

FIG. 1. Thin-layer chromatogram of the diethyl ether/n-hexane (1:1) fraction obtained from vegetable oils (A, soybean (RBD); B, soybean (crude); C, canola and D, corn oils) on silicic acid column. Minor compounds: 1) unknown compounds (unusual triglycerides); 2) free fatty acids; 3) 1,3-diglycerides; 4) sterols and 5) 1,2-diglycerides.

an unknown spot (Rf 0.48) was detected from the soybean oil fraction. Therefore, spot tests were employed for unknown compounds to characterize the unknown materials. The DPPD reagent, reactive with hydroperoxides, produced no coloration, while the DNPH reagent exhibited yellow coloration, which suggested that the unknown spot contained a carbonyl group. However, spot tests detected no α-keto and aldehyde groups.

These unknown compounds were also characterized by UV analysis after purification by dry column chromatography or silicic acid TLC. UV showed that the unknown compounds contained a carbonyl group, based on an absorption maximum at 268 nm, which is characteristic of conjugated carbonyl groups. The infrared (IR) spectrum showed the presence of a carbonyl group at 1650 cm^{-1} and ester-carbonyl at 1750 cm^{-1}. Moreover, the IR spectrum showed the characteristic broad absorption at 3400 cm^{-1} from associated hydroxyl groups. After saponification, TLC showed two spots in addition to glycerol corresponding to free fatty acids (Rf 0.38) and their high-polarity analogues (Rf 0.15). These results suggested that the unknown compounds were unusual triglycerides containing at least one mole of fatty acids with additional functional groups, such as carbonyl and hydroxyl groups.

To identify fatty acids with functional groups, they were analyzed by GC-MS after conversion to methyl esters. As shown in Figure 2, GC showed two peaks. Although the MS of the former (**I**) showed a fragmentation pattern similar to that of methyl 10-hydroxy-8-octadecenoate (16), they provided the molecular ion peak at *m/z* 310 $[M]^+$ and intensive ion peaks at 198 $[CH_3O_2C(CH_2)_6CH=CHC=O+H]^+$, 166 $[198-CH_3OH]^+$ and 113 $[CH_3(CH_2)_7]^+$ (Fig. 3). Based on these data, **I** was tentatively identified as methyl 10-oxo-8-octadecenoate as follows:

$$\text{I. } CH_3(CH_2)_7-\underset{\underset{O}{\|}}{C}-CH=CH-(CH_2)_6CO_2CH_3$$

The MS of the latter (**II**) showed the same fragmentation pattern as that of photosensitized oxidation products of methyl oleate after hydrogenation (17). **II** provided a significant mass ion peak at *m/z* 296 $[M-H_2O]^+$ and a series of intensive peaks at 201 $[CH_3O_2C(CH_2)_8COH]^+$, 172 $[201-COH]^+$, 169 $[201-CH_3OH]^+$, and 187 $[CH_3O_2C(CH_2)_7COH]^+$, 158 $[187-COH]^+$, 155 $[187-CH_3OH]^+$, based on a hydroxyl group at C-9 and C-10, respectively (Fig. 3). Thus, **II** was tentatively identified as a mixture of methyl 9- and 10-hydroxyoctadecanoate.

$$\text{II. } CH_3(CH_2)_8-\underset{\underset{OH}{|}}{C}-(CH_2)_7CO_2CH_3$$

$$\text{or } CH_3(CH_2)_7-\underset{\underset{OH}{|}}{C}-(CH_2)_8CO_2CH_3$$

From these results, the unusual triglycerides existing in soybean oil were thought to contain 10-oxo-8-octadecenoic, or 9- or 10-hydroxy octadecanoic acids.

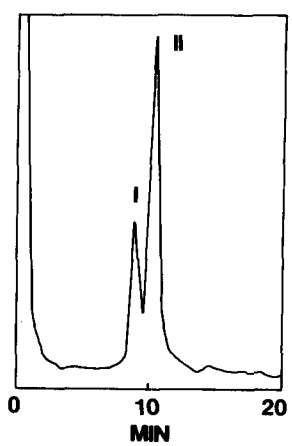

FIG. 2. Gas liquid chromatogram of methyl esters of hydroxy and oxo fatty acids prepared from unusual triglycerides.

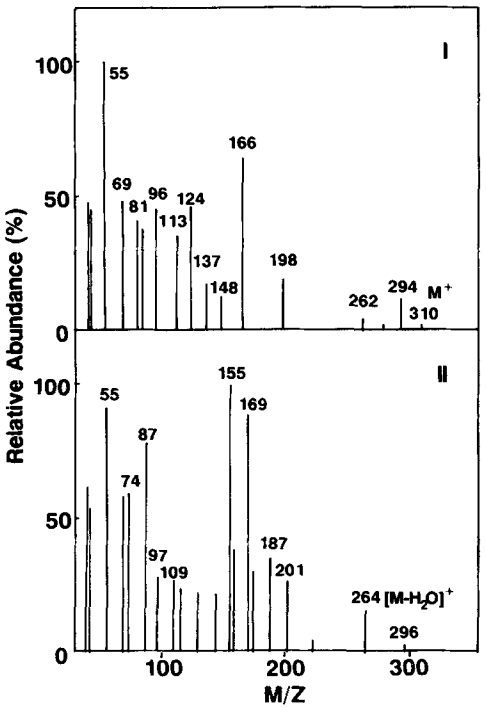

FIG. 3. Mass spectra of peaks I and II shown in Figure 2.

These unusual triglycerides are also interesting from a physiological point of view, because hydroxy and epoxy fatty acids are reported to have antimicrobial activity (20). Unusual triglycerides may be some kind of phytoalexins. Thus, researching for unusual triglycerides could broaden our knowledge of food chemistry and biology.

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