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Flavoglaucin, a Metabolite of *Eurotium chevalieri*, its Antioxidation and Synergism with Tocopherol

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ABSTRACT

Screening tests of fungal metabolites were performed for developing new types of antioxidants and synergists for tocopherol (Toc). Flavoglaucin has been found to be an excellent antioxidant and synergist. It is a phenolic compound isolated from mycelial mats of *Eurotium chevalieri*. Under autoxidation conditions, flavoglaucin remarkably synergized with Toc and stabilized many edible oils and fats. After the addition of flavoglaucin (0.05 %) the vegetable oils retained their original stabilities even after thermal treatment at 180 C for 25 hr. During the oxidation of lard containing Toc (0.04%) under the simulated deep-fat frying conditions, the addition of flavoglaucin didn't retard the oxidative decomposition of Toc. However, the stability of lard always was higher in the presence of flavoglaucin than in its absence. Flavoglaucin is not mutagenic to Salmonella typhimurium TA 100 and TA 98.

INTRODUCTION

Natural antioxidants and synergists are required to maintain the stability of edible oil and fat as safer food additives than synthetic antioxidants like butylated hydroxyanisole because carcinogenic activity of the latter has been in doubt. Screening tests of many substances of plant origin (1-9) have been carried out. Much attention has been paid to herbs and spices such as rosemary as possible sources of safe and potent antioxidants (10-13). Consequently, carnosol, rosmanol and their related compounds were isolated and determined as antioxidative substances (14-18).

Little work has been published on antioxidants of microbial origin, although microorganisms may offer great possibilities in the formation of potent antioxidants. Zaika and Smith reported that of microorganisms tested including fungi, yeasts and Streptomyces, one substance in *Aspergillus niger* mycelia had antioxidative activities and synergistic effects (19). Recently, Aoyama et al. isolated curvulic acid from the culture filtrate of *Penicillium* species (20). However, its antioxidative activities were not strong. Because tocopherol (Toc) is one of the most useful natural antioxidants, use of synerigism between Toc and its synergists is one way to inhibit the oxidation of edible oil and fat. Therefore, we have investigated synergism between Toc and the following substances: trimethylamine oxide (TMAO) (21-23), tri-n-octylamine (TOA) (24), phospholipids (25) and amino acids (26).

This paper deals with synergism between Toc and microbial metabolites in the inhibition of oxidation of edible oil and fat. Flavoglaucin, one of the metabolites of *Eurotium chevalieri*, was found to be a potent antioxidant and synergist for Toc.

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EXPERIMENTAL PROCEDURES

Materials

The fungal metabolites used in this experiment were isolated from *Aspergillus* and *Eurotium* species and identified by one of the authors. Flavoglaucin (I) and isodihydroauroglaucin (II) were isolated from mycelial mats of *E. chevalieri* (27), and dihydroflavoglaucin (III) was a hydrogenated product of I (28). L-Alanyl-2-(1,1-dimethyl-allyl)-L-tryptophyl (IV) (29) and L-alanyl-L-tryptophyl (V) (30) were isolated from culture filtrate of *E. chevalieri*. Parasiticolide (VI) (31), shamixanthone (VII) (32), and echinulin (VIII) (33) were isolated from mycelial mats of *Asp. parasiticus, Asp. nidulans* and *E. chevalieri*, respectively. Sydwic (IX) (34,35) and sydnic acids (X) (36) were isolated from culture filtrate of *Asp. sydowi*. The structures of all the compounds are shown in Figure 1. Methyl linoleate, commercially available (Tokyo Kasei Co.), was passed through a silica gel column equilibrated with n-hexane to remove peroxides. Natural Toc mixture (ca. 80% in purity) of a commercial product was supplied by Eisai Co. γ-Toc was prepared from Toc mixture as shown in the previous paper (37).

Weight Gain Method

Methyl linoleate (50 C), lard (60 C) and corn oil (50 C) were used as substrate, and the former two were added with γ-Toc (0.1%) and Toc mixture (0.04%, adjusted to the real concentration of Toc), respectively. Each oil (850 mg) was placed in a petri dish, id 45 mm, and autoxidized in the dark. An induction period was defined as the days required to increase the weight of the substrate by 0.5%.

Simulated AOM Test

Lard (7 g) containing Toc mixture (0.04%) was placed in a test tube, id 18 mm, and kept at 100 C. Then, air passed through silica gel was gently blown through the oil (Air flow was not regulated, but the results were reproducible). An induction period was expressed as the hr required for peroxide value to reach 100 meq/kg.

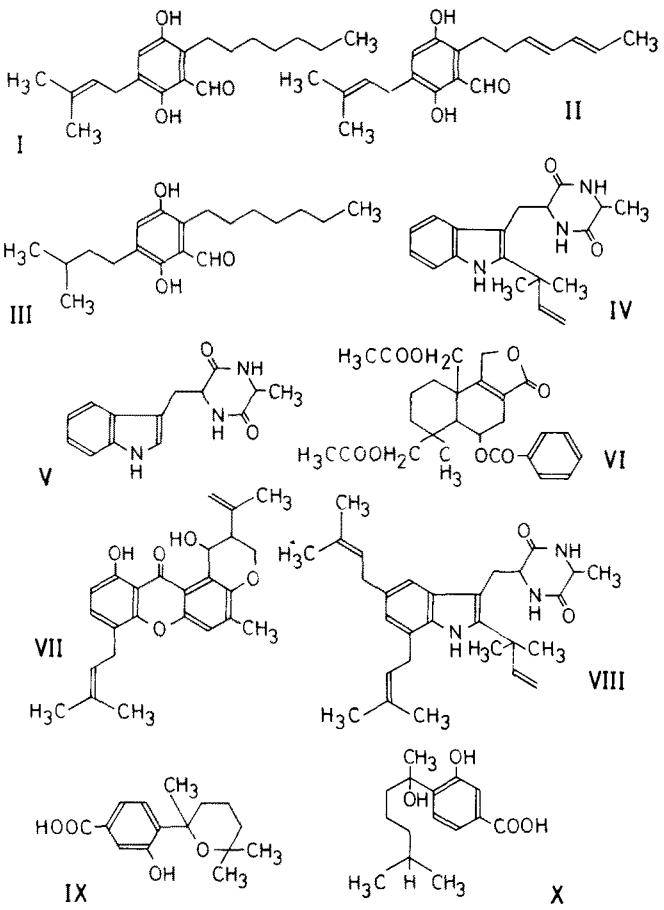

FIG. 1. Structures of Fungal Metabolites.

Thermal Stability Test

Each oil (11.1 g) with air-contacting area of 0.159 cm^2/g was thermally treated at 180 ± 1 C for 25 hr. The effect of flavoglaucin was estimated according to the total carbonyl and acid values and the weight gain method (60 C, 1 g of each oil was used). Even trace amounts of silicon oil (ca. 0.03 ppm) influence the results obtained under thermal oxidation conditions (38,39). In order to avoid the influence of silicon oil, lard and refined palm oil were added with 2.5 ppm of the silicon oil (Toshiba Silicon TSA-750, MW 10,000-20,000). This amount was almost the same as that in commercial vegetable oils. Lard and refined palm oil had no silicon oil. They were supplied by Kanegafuchi Chem. Co. and Fuji Oil Mills Ltd., respectively. Soybean, rapeseed and corn oils were silicon oil-added commercial products of Ajinomoto Co.

Simulated Deep-Fat Frying Test

Effects of flavoglaucin (0.05%) on the oxidation of lard containing Toc mixture (0.04%) and silicon oil (2.5 ppm) were tested under simulated deep-fat frying conditions (40). The oil (400 g) was placed in a stainless steel beaker, id 9 cm, and kept at 180 ± 2 C. 60 g of water/hr was sprayed continuously on the surface of the oil. These heating operations were performed for 5 hr/day. 10% of the treated oil in the beaker was removed for analysis and the same amount of new oil was added. These procedures were repeated three times. Analyses of Toc by HPLC were done on a silica gel (Merck LiChrosorb SI 60,5μm) column (4 × 250 mm) at 1 ml/min. The effluent was monitored with a Hitachi fluorescence spectrophotometer with Ex 300nm, Em 328 nm and a slit of 10 nm. Mobile phase was n-hexane/diisopropyl ether, 85:15. The amounts of γ- and δ-Toc were calculated from the height of each peak as compared to the height of a known amount of each standard.

RESULTS

Screening of Fungal Metabolites

The antioxidative activities of fungal metabolites and their synergism with Toc were investigated using methyl linoleate, lard and corn oil as substrate. As Table I shows, flavoglaucin was the most excellent antioxidant and synergist, followed by dihydroflavoglaucin in the inhibition of autoxidation of methyl linoleate and lard. They also retarded the autoxidation of corn oil. The antioxidative activity of isodihydro-

TABLE I

Screening of Fungal Metabolites for Synergists of Tocopherol

	Methyl linoleate		Lard		
Metabolite	-Toc	+Toc	-Toc	+Toc	Corn oil
Flavoglaucin	11	39	32	>242	82
Isodihydroauroglaucin	3	16	3	>242	49
Dihydroflavoglaucin	11	30	25	99	74
L-Alanyl-2-(1,1-dimethyl-allyl)-L-tryptophyl	1	11	2	34	39
L-Alanyl-L-tryptophyl	1	11	2	27	35
Parasiticolide	1	11	2	29	35
Shamixanthone	1	11	2	31	39
Echinulin	1	11	2	27	39
Sydwic acid	2	11	2	29	39
Sydnic acid	1	11	2	29	39
Control	1	11	2	27	39

Figures show induction periods (day) due to the weight gain method. Each oil and fat (850 mg) was added with each metabolite (0.5 mg). Methyl linoleate containing γ-Toc (0.1%) and corn oil were kept in the dark at 50 C and lard containing Toc mixture (0.04%) was at 60 C.

auroglaucin was weak, but it showed remarkable synergism with Toc in inhibiting the autoxidation of lard. The other metabolites didn't show any effect.

Autoxidation Condition

The antioxidative activity of flavoglaucin and its synergism with Toc were investigated according to the simulated AOM, using lard as substrate. As Figure 2 shows, the antioxidative activity of flavoglaucin was almost the same as that of Toc mixture and reached a maximum at ca. 0.1% of flavoglaucin. However, it was found to synergize remarkably with Toc and to act as a potent synergist.

Effects of TMAO and TOA on synergism between γ-Toc and flavoglaucin also were investigated according to the weight gain method, using methyl linoleate as substrate. As Table II shows, the antioxidative activity of flavoglaucin and its synergistic effect on γ-Toc increased with an increase of its concentrations. Ternary synergism including γ-Toc, TOA and flavoglaucin was observed, but the replacement of TOA with TMAO did not show any effect.

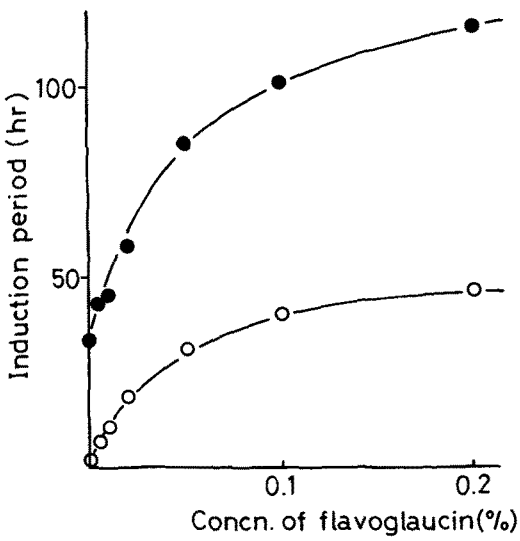

FIG. 2. Synergism between tocopherol and flavoglaucin during the autoxidation of lard. Lard containing M-Toc (0.04%) and each concentration of flavoglaucin was autoxidized at the simulated AOM conditions. •, with Toc; ○, without Toc.

Thermal Oxidation Condition

Effects of flavoglaucin on the thermal stabilities of various oils and fats were investigated by comparing the extent of oxidation before and after thermal treatment at 180 C for 25 hr. As Table III shows, palm oil was stabilized by the addition of 0.05% of flavoglaucin. The other oils and fats retained approximately their original stabilities in the presence of 0.05% of flavoglaucin, even after thermal treatment.

Deep-Fat Frying Condition

During the oxidation of lard containing Toc mixture (0.04%) under the simulated deep-fat frying conditions, the addition of flavoglaucin slightly increased carbonyl and acid values (Fig. 3) and decreased the residual amounts of Toc (Table IV).

The composition of original Toc mixture was γ-Toc (3.2%) and δ-Toc (96.8%). The amounts of Toc in Table IV show the total amounts of both, but γ-Toc disappeared after 5 hr treatment. However, the stabilities due to AOM and the weight gain method were always higher in the presence of flavoglaucin than in its absence (Table IV).

TABLE II

Effects of TMAO and TOA on Synergism between γ-Tocopherol and Flavoglaucin during the Autoxidation of Methyl Linoleate

Concn. of flavoglaucin	None	Toc	TMAO	TOA	Toc+TMAO	Toc+TOA
0%	1	8			7	13
0.05	13	62	11	13	64	>80
0.1	24	>80				

Figures show induction periods (day) due to the weight gain method at 50 C. Methyl linoleate was added with γ-Toc (0.1%) and TMAO or TOA (0.05%).

TABLE III

Effect of Flavoglaucin on the Thermal Stabilities of Various Oils and Fats

		Before heating			After heating		
Oil and Fat	Flavoglaucin (ppm)	IP[a] (day)	CoV[b]	AV[c]	IP[a] (day)	CoV[b]	AV[c]
Lard	–	3	11.5	0.03			
	500	65	13.0	0.03	12	37.8	0.27
(Toc 0.04%)	–	38	11.6	0.03	19	31.8	0.21
(Toc 0.04%)	500	130	13.1	0.03	73	25.4	0.17
Soybean	–	16	6.1	0.06	6	46.1	0.20
	50	17	6.2	0.06	8	45.1	0.20
	125	20	6.4	0.06	8	46.4	0.20
	250	21	6.9	0.06	11	44.0	0.20
	500	30	7.6	0.06	15	43.3	0.20
	1000	36	9.0	0.06	21	44.4	0.20
Rape seed	–	13	6.3	0.05	5	44.3	0.27
	500	26	7.5	0.05	15	36.8	0.24
Corn	–	24	5.4	0.10	11	28.8	0.28
	500	49	6.5	0.11	26	28.3	0.28
Palm	–	41	9.1	0.03	38	18.2	0.17
	500	147	10.0	0.03	128	17.5	0.17

Each oil (air-contacting area, 0.159 cm2/g) was thermally treated at 180 ± 1 C for 25 hr.
[a]Induction periods (IP) were measured due to the weight gain method at 60 C.
[b]CoV (meq/kg) and [c]AV are total carbonyl and acid values, respectively.

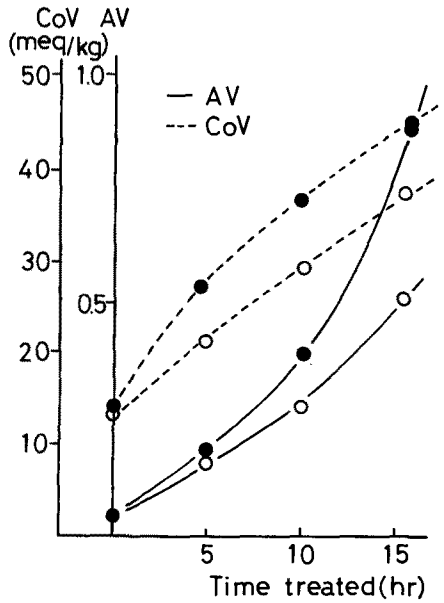

FIG. 3. Changes of total carbonyl and acid values during the oxidation of lard containing tocopherol and flavoglaucin under the simulated deep-fat frying conditions. •, with flavoglaucin; ○, without flavoglaucin.

TABLE IV

Effect of Flavoglaucin on the Stability of Lard Containing Tocopherol under the Simulated Deep-Fat Frying Conditions

		Heating time (hr)			
		0	5	10	15
+Flavoglaucin	AOM	59.8	44.3	34.5	25.2
	WGM[a]	130.0	63.5	47.6	33.4
	Toc[b]	39.2	24.3	16.0	11.2
- Flavoglaucin	AOM	28.1	27.4	22.0	15.7
	WGM[a]	28.0	25.3	19.3	13.0
	Toc[b]	39.2	25.4	18.1	13.1

[a]WGM (the weight gain method) and AOM show the induction periods of day and hr, respectively.

[b]Toc (mg/100 g of oil) are the total amounts of γ- and δ-Toc.

DISCUSSION

Flavoglaucin is one of the metabolites produced by *E. chevalieri* when grown in surface culture on a malt extract medium at 24 C (27). The proton magnetic resonance data and yellow color of flavoglaucin show that the hydroxyl and formyl groups in the ortho position form hydrogen bonds with each other, and that the antioxidative activities of flavoglaucin and its synergism with Toc are based on the other hydroxy group in the para position of the hydroxy group bonded with the formyl group (41). Flavoglaucin has been known as one of the common metabolites of the *Asp. glaucus* group (42), which has been used in the manufacture of Katsuobushi (dried bonito), a traditional marine product in Japan. It also can be isolated from *Penicillium charlesii* (41). Isodihydroauroglaucin is isolated together with flavoglaucin from *E. ruber* (28) and *E. chevalieri* (27).

Though few works have been done on the antioxidation and synergism of microbial metabolites, it has been known that oil and fat in foods fermented with microorganisms are very stable to oxidation. Tempeh is a soybean product fermented with *Rhizopus oligosporus* (43). The oil extracted from Tempeh was used to stabilize other edible oils (44,45). Ikehata et al. isolated isoflavon as antioxidant (46). Extracts of Miso, a commonly used food in Japan and a fermentation product of soybean by, mainly, *Asp. oryzae*, have antioxidative activities (47-49).

We reported that extracts from soybean fermented with *Actinomucor elegans*, used for Sufu production (43), synergized with Toc in inhibiting the autoxidation of lard and that they stabilized vegetable oils (50). Though marine products are very sensitive to oxidation (51), the oil in dried bonito was not oxidized so much (52). This fermentation product is tasty and is used for Japanese soups.

The utility of flavoglaucin as an excellent antioxidant and synergist is based on the fact that it is not mutagenic to *Salmonella typhimurium* TA 100 and TA 98 (27). Such a safe natural antioxidant might be used as an anticarcinogen and antimutagen against oxygen radicals and lipid peroxidation (53).

The fact that flavoglaucin synergized with Toc under autoxidation conditions indicates that it has a good chance of inhibiting the oxidation of edible oil and fat, including corn oil (Table I). TMAO has been found to be an effective constituent for promoting the shelf-life of cuttlefish fried with soybean oil (54,55). TMAO is widely present in fish. TMAO itself acts as a prooxidant and didn't always synergize with a lower concentration of γ-Toc during the autoxidation of methyl linoleate (56). However, the addition of phospholipids such as phosphatidylethanolamine and phosphatidylserine to this reaction system inhibited the oxidative deterioration of γ-Toc, and a remarkable synergism was observed (25). As Table II shows, flavoglaucin didn't synergize with γ-Toc and TMAO. These facts suggest that the simultaneous addition of flavoglaucin and TMAO could not be expected for higher stabilization of oil and fat, compared with the addition of either of them.

Flavoglaucin stabilized palm oil under thermal oxidation conditions (Table III). Though the reason for this unexpectedly favorable result is not yet understood, synergism of flavoglaucin with tocotrienols and unknown minor components in palm oil may be worth further consideration. As Table IV shows, the presence of flavoglaucin slightly decreased the residual amounts of Toc under the simulated deep-fat frying conditions. However, lard treated with flavoglaucin always was more stable than that without it in the stability tests under autoxidation conditions. These facts indicate that its loss at higher temperature is small, and the rest shows excellent synergism with Toc.

Consequently, it must be pointed out that screening of useful microorganisms make them very attractive as sources for natural and safe antioxidants and synergists.

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Effect of Selected Storage Conditions and Packaging Materials on Olive Oil Quality

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ABSTRACT

Transparent glass and polyethylene plastic bottles filled with virgin olive oil were stored in diffused room light and direct sunlight (ca 4 hrs a day in sunlight and the remainder of the day in diffused light). In each case one-half of the containers were covered completely with aluminum foil. The oxidation of all oil samples proceeded slowly in darkness, faster in diffused light and even faster in direct sunlight. Glass packaging materials gave better protection against oxidation than polyethylene plastic bottles. Significant destruction of the color of oil was observed under different light conditions.

INTRODUCTION

Light causes significant deterioration of olive oil quality in the presence of air (1-6). In the absence of air, however, direct sunlight causes a decrease in peroxide and Kreis values of the oil (7). Further, Cucurachi (1) noted that peroxide formation in olive oil stored in closed tins is generally insufficient to lead to development of the typical rancid odor because of the limited amount of oxygen in the headspace.

One study with olive oil stored in different types of containers revealed similar results in glass and PVC containers (8,9) each of which were better than other types of plastic containers (8).

Unal (5) reported that the peroxide value of olive oil stored in cans or glass bottles decreased during storage, whereas peroxide values of samples stored in PVC bottles increased. This effect was attributed to the O_2 permeability of PVC (5). In addition, an increase in the free fatty acids and decreases in the β-carotene and chlorophyll contents were observed. The destruction of these two pigments was greater in illuminated samples than in those stored in darkness (5).

The purpose of this work was to study the effect of some storage conditions involving light and of different packaging materials (glass and polyethylene plastic) on olive oil quality.

MATERIALS AND METHODS

Six samples of oil from olive fruits of the cultivar "Koro-