were not variable depending on the difference of the culture conditions (203-254 mg/400 ml). A higher level of linoleic acid was extracted from ETL grown on cellulose (74.5-81.5%), compared with 22.5% linoleic acid in ETL using sugar cane bagasse medium under any cultivation conditions.

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⁂Sensory Characteristics and Oxidative Stability of Soybean Oil and Flour Extracted with Aqueous Isopropyl Alcohol

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ABSTRACT

Soybean flakes extracted with hexane or aqueous isopropyl alcohol (85%, 87.7% and 90.5% IPA by weight) were processed to toasted flours and the miscellas to refined soybean oils. These products were evaluated for sensory characterisitics and oxidative stability. Sensory analyses of initial oils and flours indicated good quality products. Initial flavor scores of IPA-extracted oils and flours were not significantly different from those of hexane-extracted oil and flour. Flour samples aged at 49 C for 1 mo and 37 C for 3 mo were rated slightly lower in flavor score than the initial flours. Flavor scores of oils decreased after aging but remained acceptable. Oils extracted with aqueous IPA concentrations of 85% and 90.5% received significantly lower scores than oils extracted with hexane or 87.7% IPA after 8 hr of fluorescent light exposure. Oxidative stability measured by the induction of weight increases of the oils during aging was similar. Residual solvent flavors were slightly detectable in unaged IPA flours and in those aged 3 mo at 37 C.

INTRODUCTION

In the extraction of oil from soybeans and other oilseeds, hexane is used almost exclusively as the solvent in this country. Hexane is an excellent solvent for such extractions. However, concerns about availability, flammability and toxicity have stimulated interest in alternative extraction solvents. Some of the solvent systems examined include alcohols (1), water (2), halogenated hydrocarbons (3) and supercritical carbon dioxide (4).

Extraction of soybeans with ethanol was evaluated at the Northern Regional Research Center in the mid-1940's. Low solubility of oil in 95% ethanol at ambient temperature required the extraction to be carried out under slight pressure to raise the temperature above 90 C, where the oil and ethanol are completely miscible. Isopropanol has better solvent properties than ethanol for the extraction of oilseeds. Recently, Shell Development Company (5) and NRRC scientists (6) have developed a pilot-plant process for extracting soybeans with isopropanol. The purpose of this study was to evaluate the sensory properties of the oil and meal products prepared by this process.

MATERIALS AND METHODS

Soybean flakes were extracted with hexane or aqueous isopropyl alcohol at concentrations of 85%, 87.7% and 90.5% IPA by weight, with a solvent-to-meal ratio of 2:1. The complete process of extraction, oil refining and meal desolventizing was presented in detail by Baker and Sullivan (6). Characteristics of the crude and refined oils such as free fatty acids and metal content were included in that paper, as was information on the desolventized meal such as residual alcohol, nitrogen solubility index and trypsin inhibitor content. The oils contain 0.01% citric acid added on the cooling side of deodorization.

Oils were aged for 8 days at 60 C or for 8 hr of fluorescent light exposure. For the storage tests, 8-oz narrow-mouth clear glass bottles were filled 2/3 with oil and loosely stoppered (air in the headspace) with cellophane-covered corks. The 60 C storage samples were aged in a forced draft oven in the dark for 8 days. For the fluorescent light exposure test, the bottles were placed on a revolving platform in the middle of a 17.5-in. diameter stainless steel drum, 17.5 in. high, which contained six 15-in., 14-watt daylight fluorescent bulbs mounted on the perimeter (7). The light intensity was 7,535 lux or 800 ft candles.

Soy flours were evaluated for flavor initially and after aging at 49 C for 1 mo or at 37 C for 3 mo. Flours were packaged in 4-oz wide-mouth clear glass bottles and sealed with screw-cap closures. Packaging was done with air in the headspace.

Sensory Evaluation

Oils were evaluated for flavor by a trained, experienced 15-member panel. Each tester was given 10 ml of oil maintained at 50 C in a 50-ml clear glass beaker covered with a watch glass. Overall flavor intensity of each oil was rated on a 1-10 scale, with 10 as bland and 1 as extreme intensity. Panelists also described the predominant flavors detected and rated the intensity of each description on a

scale of 0=none, 1=weak, 2=moderate and 3=strong. A weighted average known as a flavor intensity value (FIV) was calculated for each description by the following formula: FIV = No. weak responses + 2 × no. moderate responses + 3 × No. strong responses divided by the number of testers. The flour samples were evaluated for flavor as 2% dispersions in charcoal-filtered tap water and rated on a 1-10 intensity scale as previously described by Warner et al. (8).

Oxidative Stability

Oxidative stability of the oils was measured both by peroxide value (9) at time of sensory panel testing and by a modification of a gravimetric method to measure the induction period of oxidation (10). The induction method consisted of weighing 10 g oil into a 100-ml beaker and aging the oil at 60 C in a forced draft oven in the dark. The beaker with the oil was weighed periodically, after cooling to room temperature, on an analytical balance to measure the increase in weight caused by the oxidation of the oil. Induction time (in days) was defined as the time to reach a rapid acceleration in weight increase.

TABLE I

Flavor Scores[a] of Soybean Oil Extracted with Hexane and Aqueous Isopropyl Alcohol (IPA)

Solvent	Treatments		
	Initial[b]	8 Days, 60 C[c]	8 hr light[d]
Hexane	7.8f (0)[e]	6.8g (0.6)	6.9 (1.0) h
85% IPA	7.9f (0)	6.1g (1.3)	5.9 (3.0) i
87.7% IPA	7.5f (0)	6.2g (0.8)	6.4 (5.0) i h
90.5% IPA	7.5f (0)	6.1g (0.8)	6.2 (2.0) i

[a]Scores for each treatment type with letters (f, g, h or i) in common are not significantly different.
[b]LSD (Least Significant Difference) = 0.7.
[c]LSD = 0.8.
[d]LSD = 0.7.
[e]Peroxide value at time of tasting given in parentheses.

Statistical Design

Balanced incomplete block designs were used as testing patterns for the 4 flours and 4 oils (11). Each storage series was tested separately, with each tester evaluating two samples at each panel sitting. Each sample received a total of 15 scores that were used to calculate an overall mean score. Two-way analysis of variance and least significant difference (LSD) were used to determine statistical significance in overall flavor scores (12).

RESULTS AND DISCUSSION

The sensory tests were designed to determine the flavor characteristics of soybean oils and flours when 3 levels of IPA were substituted for the standard hexane extraction of soybean flakes. Scoring on a 1-10 scale (10 as bland and 1 as strong) as well as descriptive analysis were used to measure, characterize and quantify any flavor differences. The flavor quality and stability of the 4 oils initially and after storage are shown in Table I. All oils had initial flavor scores of 7.5 or higher and no detectable peroxides. These results indicate good initial quality. Most commercially available soybean oils are usually scored by our panel in the range of 6.5-7.0 initially. Although lower in score, the IPA-extracted oils were not significantly different from the hexane-extracted oil. After the oils were aged at 60 C for 8 days, the flavor scores decreased by about 1 unit for the hexane-extracted oil compared to 1.8 units for the 85% IPA-extracted oil; peroxide values ranged from 0.6-1.3. No significant differences were noted between overall flavor scores of hexane and IPA-extracted oils after this storage, although the scores for the IPA oils had a consistently lower score-trend. The 8-hr fluorescent light exposure test showed a significantly higher overall flavor score for the hexane-extracted oil than the scores of the 90.5% and 85% IPA-extracted samples. All IPA-extracted oils had higher peroxide values than the hexane-extracted sample. No significant differences were noted among the scores of the three IPA-extracted oils by the light exposure test.

The predominant flavors of the oils initially were buttery and nutty with a variety of "off" flavors, including heated oil and acrid (Table II). The buttery flavor characteristic increased slightly after 8 days aging at 60 C along with the appearance of rancid and painty flavors and "off"

TABLE II

Flavor Descriptions and Intensity Values[a] of Soybean Oil Extracted with Hexane and Aqueous Isopropyl Alcohol (IPA)

Solvent	Treatments		
	Initial	8 Days, 60 C	8 hr light
Hexane	0.5 Buttery 0.3 Nutty 0.3 Other "off" flavors	0.3 Buttery 0.5 Rancid 0.3 Painty 0.4 Other "off" flavors	0.6 Buttery 0.2 Rancid 0.6 Other "off" flavors
85% IPA	0.5 Buttery 0.5 Other "off" flavors	0.7 Buttery 0.9 Rancid 0.3 Painty 0.3 Other "off" flavors	0.8 Buttery 0.3 Rancid 0.7 Other "off" flavors
87.7% IPA	0.2 Buttery 1.0 Other "off" flavors	0.5 Buttery 0.5 Rancid 0.3 Painty 0.4 Other "off" flavors	0.7 Buttery 0.4 Rancid 1.1 Other "off" flavors
90.5% IPA	0.5 Buttery 0.4 Nutty 0.6 Other "off" flavors	0.6 Buttery 0.9 Rancid 0.4 Painty 0.3 Other "off" flavors	0.5 Buttery 0.5 Rancid 0.8 Other "off" flavors

[a]Based on 0-3 scale of 0=none, 3=strong intensity.

flavors such as: fishy, sour, bitter, metallic. After 8 hr light exposure, the predominant flavors were buttery and rancid and a variety of "off" flavors, typical of light-exposed oils, including: metallic, waxy and "light-struck." The number of "off" flavor descriptions was higher for the 87.7% IPA oil initially and after 8 hr fluorescent light exposure than for any of the other samples. The descriptions for the 87.7% IPA oil were the same as for the other oils, but were present in greater numbers. The overall flavor scores were not significantly affected by the higher intensity of "off" flavors in some of the oils. No descriptions indicative of residual solvent were noted for the oils.

The oxidative stability of the oils, measured by the induction of weight increase during aging, was similar for all oils (Fig. 1). The days of storage at 60 C to reach induction were: 16.0 days, hexane; 16.6 days, 90.5% IPA; 16.4 days, 87.7% IPA, and 16.4 days, 85% IPA. Until 17 days of storage the rate of weight increase for the oils was constant at an average of 2.7 mg/day (range of 2.6 mg for 90.5% IPA to 2.9 mg for 87.7% IPA). The final rates for the oils were: 28.8 mg/day, 85% IPA; 29.8 mg/day, 87.7% IPA; 25.7 mg/day, 90.5% IPA, and 11.1 mg/day, hexane. Even though the number of days to induction was similar for the 4 oils, the hexane sample did have somewhat better stability because of the slow rate of weight increase after induction began. The 85% IPA and 87.7% IPA oils had the greatest slopes after induction began, whereas the rate increase for the 90.5% IPA sample was 16% slower per day than the rate for other IPA oils.

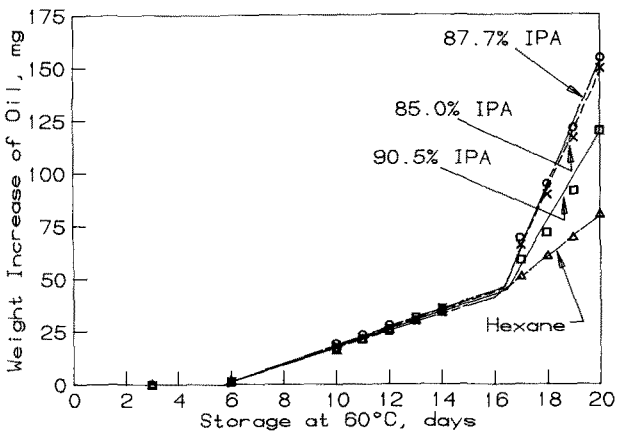

FIG. 1. Oxidation induction periods of hexane- and IPA-extracted soybean oils.

The initial flavor evaluation of the flours showed a score range of 7.0-7.6 with no significant difference among the 4 solvent extractions (Table III). The 87.7% and 90.5% IPA flours were scored consistently lower than the hexane flour. Flavor scores of these flours were lower than the typical 8.0 score for wheat flour but higher than the average commercial soy flour score of 5.9 (8). The 87.7% IPA flour and the 90.5% IPA flour both received slightly lower initial scores than the hexane or 85% IPA flours, probably because of higher intensity in toasted flavor. This problem is associated with heat treatment in the final processing phase and is independent of the solvent extraction. Aging of the flours for 1 mo at 49 C caused significant decreases in overall scores and increases in cereal/grain and toasted flavor intensities and in bitter taste intensity as compared to the initial flavors. No significant differences were noted between the scores of the initial and 3 mo, 37 C storage samples. The intensity of toasted flavors was higher for the 3 mo flours than for the unaged samples. Descriptions indicative of residual solvent were noted in the IPA-treated flours. These flavors include fruity, sweet and alcohol. No such descriptions were given for the hexane-treated sample. Levels of residual alcohol in these products were 70 ppm, 85% IPA; 59 ppm, 87.7% IPA, and 43 ppm, 90.5% IPA (6). Although these residual alcohol flavors were present, they did not adversely affect the overall scores.

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Linda Parrott conducted the sensory panel and oxidative stability analyses and Gary List and R.L. Brown refined and deodorized the oils.

TABLE III

Flavor Scores, Descriptions and Intensity Values of Soy Flours Extracted with Hexane and Aqueous Isopropyl Alcohol (IPA)

Storage	Descriptions	Solvent treatments			
		Hexane	85% IPA	87.7% IPA	90.5% IPA
0	Scores:[a]	7.6b	7.6b	7.0b	7.0b
	Cereal/grain	0.9	0.9	0.9	0.9
	Grassy/beany	0.1	0.2	0.2	0.3
	Toasted	0.1	0	0.5	0.6
	Other "off" flavors	0.3	0.3	0.6	0.2
1 Month 49 C	Scores:[a]	6.8c	6.8c	6.2c	6.2c
	Cereal/grain	0.8	1.1	1.3	1.1
	Grassy/beany	0.4	0.2	0.1	0.5
	Bitter	0.4	0.5	0.5	0.5
	Toasted	0.3	0.6	1.1	0.9
	Other "off" flavors	0.5	0.2	0.5	0.3
3 Months 37 C	Scores:[a]	7.0d	6.9d	6.7d	6.6d
	Cereal/grain	1.2	1.2	0.8	1.0
	Grassy/beany	0.1	0	0.3	0
	Bitter	0.1	0.2	0.2	0.2
	Toasted	0.9	1.0	0.8	1.2
	Other "off" flavors	0.3	0.1	0.3	0.3

[a]LSD (Least Significant Difference) = 0.8. Scores for each storage time with letters (b,c or d) in common are not significantly different.

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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%.