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☘Chia Seeds as a Source of Natural Lipid Antioxidants

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ABSTRACT

Chia (*Salvia* sp) seeds were investigated as a source of natural lipid antioxidants. Methanolic and aqueous extracts of defatted chia seeds possessed potent antioxidant activity. Analysis of 2 batches of chia-seed oils demonstrated marked difference in the fatty acid composition of the oils. In both batches, the oils had high concentrations of polyunsaturated fatty acids. The major antioxidant activity in the nonhydrolyzed extract was caused by flavonol glycosides, chlorogenic acid (7.1×10^{-4} mol/kg of seed) and caffeic acid (6.6×10^{-3} m/kg). Major antioxidants of the hydrolyzed extracts were flavonol aglycones/kaempferol (1.1×10^{-3} m/kg), quercetin (2.0×10^{-4} m/kg) and myricetin (3.1×10^{-3} m/kg); and caffeic acid (1.35×10^{-2} m/kg). Two methods were used to measure antioxidant activities. Both were based on measuring bleaching of β-carotene in the coupled oxidation of β-carotene and linoleic acid in the presence of added antioxidants.

INTRODUCTION

Many vegetable oils are protected by natural, endogenous polyphenolic antioxidants. These antioxidants exert a marked protective effect on plant lipids even though the natural oils contain high concentrations of polyunsaturated fatty acids (PUFA) (1) that are usually very susceptable to oxidation. Polyphenols responsible for this protective action are most commonly flavonoids and cinnamic acid derivatives that occur abundantly throughout the plant kingdom (2,3).

Seeds of the chia plant (*Salvia hispanica L.*, and other *Salvia* members of the family Labiatae) have long been components of foods of American Indians and Mexicans (4). The seeds yield between 25% (5) and 35% (6) extractable oil, which contain high concentrations of PUFAs. In fact, the fatty acid composition is such that the oil may range from a salad oil of excellent composition to a commercial drying oil (7). Because of the highly unsaturated nature of the oil, the seeds probably contain potent lipid antioxidants.

The current investigation was initiated to identify the major antioxidant principals of chia seeds and to ascertain their potential as food antioxidants.

MATERIAL AND METHODS

Extraction of Phenolic Compounds

Two different samples of chia seeds, purchased from a local health food store, were air dried at 40 C for 48 hr, then finely ground (1 mm screen) in a Wiley mill. One thousand grams were then extracted in a Soxhlet extractor with 3 L of petroleum ether (b.p., 35-60 C) for 24 hr, to remove the fats and other petroleum ether soluble components. The residue was air dried at room temperature for 12 hr and then reextracted with 5,000 mL methanol in a Soxhlet for 48 hr. The methanolic extract was concentrated in vacuo on a rotary evaporator at 40 C.

The dried methanolic extract was suspended in 1,000 mL of 1-butanol and transferred to a 4,000 mL separatory funnel. Water (ca. 100 mL) was added and the mixture was shaken vigorously. With the addition of 5 L of petroleum ether, the phenolic components (and other compounds of intermediate polarity) precipitated into the aqueous phase. The organic phase was discarded. The aqueous phase was freeze-dried and suspended in methanol. This fraction is referred to as the crude extract.

Total Phenolic Compounds

Total concentration of phenolics in the crude extract was determined by a modification of the method of Bray and Thorpe (8). Dried samples and standards were prepared in 60:40 acidified methanol/water (0.3% HCl). Test solutions (samples or standards) of 100 μL were added to 2.0 mL of 2% Na_2CO_3. After 2 min, 100 μL of 50% Folin-Ciocalteau reagent were added and allowed to stand at room temperature for 30 min. Absorbance was measured at 750 nm on a Beckman spectrophotometer, model 25. The blank consisted of all reagents and solvents without test compounds or standard. The standard was caffeic acid prepared in concentrations of 0.001 mg/mL to 1.0 mg/mL. The phenolic concentrations were determined by comparison with the standard calibration curve.

Hydrolytic Cleavage of Flavonol Glycosides and Cinnamic Acid Esters

Aliquots of the crude extract in HCl-methanol (2M) were heated in tightly capped 5 mL vials for 45 min at 100 C. After heating, 3 mL of water were added to the vials. The contents were transferred to a separatory funnel and extracted 3 times with 2 mL diethyl ether. The aqueous phase was discarded. The ether phases were combined and evaporated to dryness. The residue was redissolved in 0.5 mL spectral-grade methanol.

Completeness of hydrolysis was determined by spotting hydrolyzed and nonhydrolyzed extracts on 20 cm × 20 cm cellulose thin layer chromatographic (TLC) plates and developed in 3 different solvent systems: (a) 15% acetic acid, (b) chloroform/formic acid/water (10:9:1 v/v/v) and

(c) toluene/acetone/formic acid (3:6:1 v/v/v). Samples were compared with standards of flavanol aglycones (kaempferol, quercetin and myricetin) and cinnamic acid derivatives (caffeic and chlorogenic acids). Hydrolysis was considered complete with the disappearance of flavanol glycosides and chlorogenic acid.

Isolation and Identification of Phenolic Antioxidants

Aliquots of the hydrolyzed extract were streaked on cellulose plates (described earlier) and developed in 15% acetic acid. Bands were scraped from developed plates and eluted with methanol. Identification of phenolic antioxidants in hydrolyzed extracts and from eluted bands was achieved by thin layer chromatography (TLC) and gas liquid chromatography (GLC) and by UV spectral analyses (9).

TLC analyses were conducted on isolated bands using systems described above with an additional solvent system consisting of the upper phase of n-butanol/acetic acid/water (4:1:5 v/v/v). Isolated components were cochromatographed with known flavonols and cinnamic acid derivatives.

GLC analyses were conducted on trimethylsilyl (TMS) derivatives of separated components. Eluted bands were evaporated to dryness. The residue was treated with 0.05 mL bis(trimethylsilyl)trifluoroacetamide (BSTFA). Mixtures were tightly capped and heated for 30 min in a boiling water bath to facilitate derivatization. In some instances, heating was done in an autoclave at 121 C for 10 min. The gas chromatograph was a Toxichron GLC, model B-5800-1, equipped with a flame ionization detector (FID). TMS derivatives were separated on a 90 × 0.3 cm column of 3% SE 30 on 100/120 GCS. Carrier gas (nitrogen) flow rate was 40 mL/min. Analyses were conducted isothermally.

Evaluation of Antioxidant Effectiveness

Evaluation of antioxidant activity of nonhydrolyzed and hydrolyzed extracts and isolated phenols was based on coupled oxidation of β-carotene and linoleic acid. The technique developed by Marco (10) and modified by Miller (11) and by Pratt (2) consisted of measuring the bleaching of β-carotene resulting from oxidation by degradation products of linoleic acid. Another technique was a diffusion method developed by Araujo (12) in which the bleaching of β-carotene was observed visually.

Ca. 1.0 mg β-carotene was dissolved in 10 mL chloroform. The absorbance was tested after adding 0.2 mL of the solution to 5 mL of chloroform, then reading the absorbance of this solution at 470 nm using a Bausch-Lomb Spectronic 20. A reading between 0.6 and 0.9 indicated a workable concentration of β-carotene.

One mL of β-carotene chloroform solution was added with a pipette to a boiling flask that contained 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator at 40 C, 50 mL of oxygenated distilled water was slowly added to the flask with vigorous agitation to form an emulsion. Five mL of the emulsion was added to 0.2 mL of the antioxidant solution in spectrophotometer tubes. A blank consisting of 20 mg linoleic acid, 200 mg Tween 40 and 50 mL oxygenated water was used to bring the spectrophotometer to zero. Tubes were shaken and absorbance measurements made at 470 nm immediately after the addition of the emulsion to the antioxidant solution. The tubes were placed in an agitating water bath at 50 C. Absorbance measurements were made at 15 min intervals until the absorbance of the control read below 0.03. All determinations were made in triplicate.

The diffusion plates used to detect antioxidant activity were prepared as follows: 1.5 g of agar (Bacto-agar) was dissolved in 100 mL of water in a boiling water bath; the solution was cooled to 50 C and 2.0 mL of linoleic acid in ethanol (2.0 mg/mL) and 10 mL of β-carotene in acetone (0.5 mg/mL, prepared by refluxing for 30 min) were added. Agar was poured into petri plates and allowed to set for 30 min. Holes (ca. 40 μL capacity) were made in the agar using a small cork borer. Aliquots of methanolic extracts or compounds eluted from cellulose TLC plates (usually 20 μL) were dropped into the holes with a pipette while a control of 20 μL of solvent was dropped into a center hole in each plate. The plates were incubated at room temperature until background color bleached. Intensity and persistence of the carotene color were proportional to antioxidant activity.

Determination of Fatty Acid Composition of Chia-Seed Oils

Aliquots of fat (50 μg) were saponified with 1 mL of methanolic KOH (0.5 M) in tightly capped vials for 5 min at 100 C. To the hydrolyzed mixture, 400 μL of aqueous HCl/methanol (4:1 v/v) was added and the mixture was heated for 15 min at 100 C. Then the mixture was cooled and extracted twice with 3 mL of petroleum ether. The organic phase was dried with anhydrous Na_2SO_4 and concentrated to 500 μL. Resulting fatty acid methyl esters were determined by GLC analysis.

Methyl esters of fatty acids were analyzed on a Toxichron GLC, model B-5800-1, equipped with an FID. The compounds were separated on 180 × 0.3 cm column of DEGS on Chromosorb W. The carrier gas was nitrogen at a flow rate of 40 mL/min. Analyses were conducted isothermally at 185 C.

RESULTS AND DISCUSSION

Analyses of fatty acids of the 2 different samples of chia seed oils indicated potent antioxidant activity. The high degree of unsaturation of the oil would result in very rapid autoxidation if the oil were not adequately protected. The marked differences (Table I) in fatty acids of 2 different samples of seeds show that oils from different chia varieties may have diverse industrial uses. Because of the extreme differences in fatty acid composition, these 2 samples were considered to be representative of 2 varieties. One of the samples compared favorably with a high-quality cooking or salad oil, whereas the other appears to possess potential as a drying oil. In fact, the fatty acids are more polyunsaturated than the fatty acids of linseed oil.

Phenolic Compounds of Chia Seeds

The concentration of total phenolic compounds, calculated using caffeic acid as the standard, was 47 millimoles/1,000 g of chia seed.

TABLE I

Fatty Acid Composition of Chia-Seed Oil

Fatty acid	Sample	
	I	II
	Percentage	
16:0	9.9	5.2
16:1	Tr	Tr
18:0	16.2	2.9
18:1	21.3	7.6
18:2	46.3	15.3
18:3	6.3	69.0
20:0	Tr	Tr
Fat %	26.2	32.5

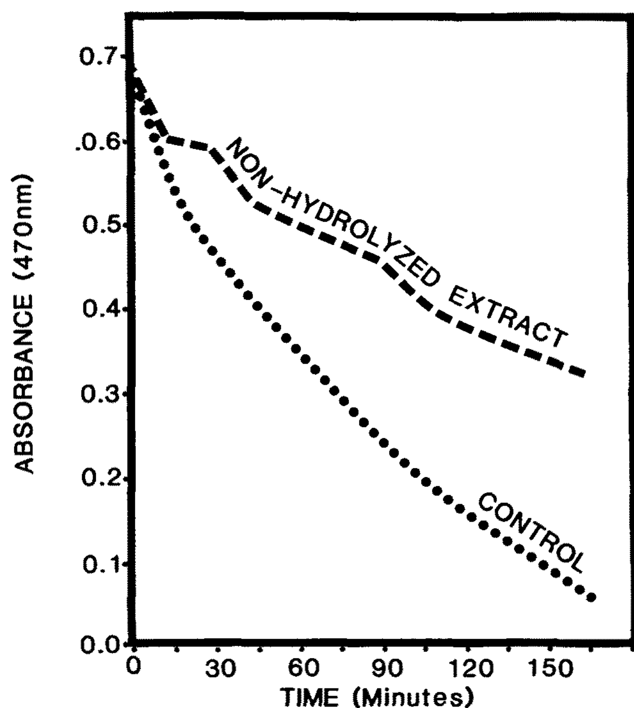

FIG. 1. Effect of aqueous extract of chia seeds on oxidation of linoleic acid at 50 C.

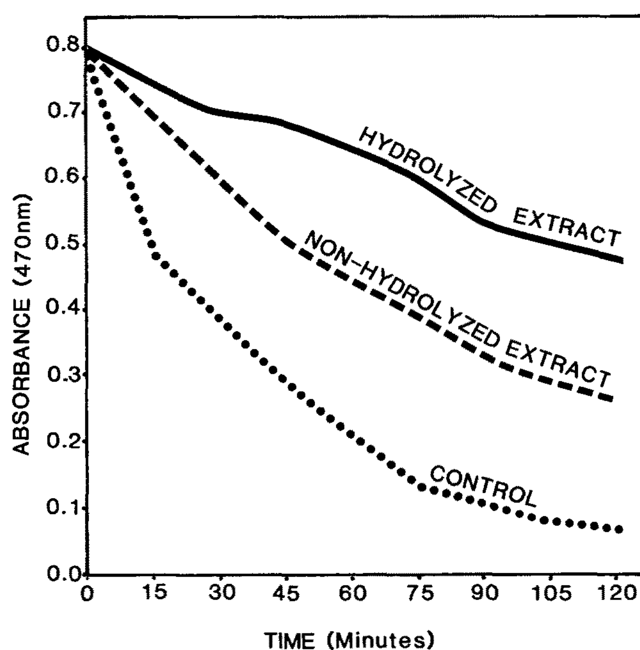

FIG. 2. Effect of methanolic extracts of chia seeds in oxidation of linoleic acid at 50 C.

Figures 1 and 2 show that aqueous and methanolic extracts of defatted chia seeds possess antioxidant activity. Nonhydrolyzed and hydrolyzed components of the methanolic extracts were both active. The phenolic concentration in extracts reported in Figures 1 and 2 was 23 μg/mL. This concentration would indicate that chia seeds possess lipid antioxidant in appreciable quantities. In fact, this concentration would compare favorably in antioxidant activity with many potent plant extracts. The difference in antioxidant activity between the nonhydrolyzed and hydrolyzed compounds is not unexpected. Hydrolysis of ether glycosides and acid esters makes hydroxyl groups available to serve as primary antioxidants.

TLC Analyses of Chia Seed Extracts

The nonhydrolyzed and hydrolyzed extracts streaked on cellulose plates and developed in 15% acetic acid yielded 4 and 5 distinct bands, respectively. The nonhydrolyzed extract had bands at the origin, at R_f values of 0.38 and 0.68 and at the solvent front. The hydrolyzed extract had bands at the origin and at R_f values of 0.38, 0.47, 0.69 and 0.87. Flavonols (both glycosides and aglycones) did not migrate to any extent in this solvent and were eluted with the origin. TLC analyses in 3 solvent systems and inspection under visible and UV light indicated, by cochromatography with known standards, that flavonols in the hydrolyzed extract were myricetin, quercetin and kaempferol.

Bands that migrated in 15% acetic acid on cellulose plates demonstrated several compounds. These compounds were cochromatographed with known standards. Further TLC analyses of these bands indicated that caffeic and chlorogenic acids were present in the nonhydrolyzed extracts. On acid hydrolysis, the band (or spot) that corresponded to chlorogenic acid disappeared and the band (or spot) that corresponded to caffeic acid increased in intensity. Such a conversion is not unexpected. On mild acid treatment, chlorogenic acid is readily hydrolyzed to caffeic and quinic acids. One unexpected result is that ferulic acid was not indicated in either nonhydrolyzed or hydrolyzed extracts. This cinnamic acid derivative is common in plant tissue, especially those containing quinic acid esters (13).

GLC characteristics of trimethylsilyl (TMS) derivatives demonstrated 3 flavonol aglycones in hydrolyzed extracts. Cochromatography with TMS derivatives with standard of myricetin, quercetin and kaempferol yields corresponding single peaks on the chromatogram. Based on TLC and GLC analyses, the flavonol aglycones were concluded to be myricetin, quercetin and kaempferol. The TMS derivative of genistein (5,7,4′-trihydroxyisoflavone) was used as an internal standard to calculate the concentration and relative retention times of the flavonols. Genistein was not found to be a component of the extracts.

GLC cochromatography confirmed the presence of chlorogenic and caffeic acid by comparison with known standards. In the hydrolyzed extract, the only cinnamic acid identified was caffeic acid. The concentration of flavanols and cinnamic acids are given in Table II.

TABLE II

Concentration of Antioxidants in Chia-Seed Extracts

Compound	Concentration (mol/kg of chia seeds)
Nonhydrolyzed	
Flavanols	–
Cinnamic acids	
Caffeic acid	6.6×10^{-3}
Chlorogenic acid	7.1×10^{-3}
Hydrolyzed	
Flavanols	
Myricetin	3.1×10^{-3}
Quercetin	0.2×10^{-3}
Kaempferol	1.1×10^{-3}
Cinnamic acids	
Caffeic acid	13.5×10^{-3}

Antioxidant Activity of Chia Seed Components

The antioxidant indices (AI) of bands from hydrolyzed and nonhydrolyzed extracts eluted from cellulose plates (developed in 15% acetic acid) are summarized in Table III. The antioxidant activity is expressed by the equation:

$$AI = \frac{\text{Bleaching time (hours) of } \beta\text{-carotene surrounding test spot}}{\text{Bleaching time (hours) of } \beta\text{-carotene surrounding control spot}}$$

As may be seen in Table III, the flavonol aglycones possessed the greatest antioxidant activity. Myricetin is primarily responsible for this activity. Myricetin possesses ca. 1.5 times the activity of quercetin and several times that of kaempferol (2,14). Since the ratio of myricetin to quercetin is 15:1, little doubt exists that myricetin is the primary flavonol antioxidant.

Caffeic acid also makes a significant contribution to the antioxidant activity of chia seed. In the hydrolyzed extract, caffeic acid concentration is ca. 4 times that of flavonols and must be considered as a major antioxidant source. Chlorogenic acid possesses about the same activity as caffeic acid in chia seed (as determined from the nonhydrolyzed extract). Concentrations of caffeic and chlorogenic acid are approximately equal. The caffeic acid moiety of chlorogenic acid is responsible for antioxidant activity.

Caffeic acid, perhaps, offers greater potential as an antioxidant from chia seeds than myricetin or other flavonols. Caffeic acid is easily derived and has not been shown to be a mutagen, as have myricetin and quercetin.

TABLE III

Antioxidant Indices of Components of Chia Seed

Band	AI	Antioxidant principal
		Hydrolyzed
I	6.5	Myricetin, quercetin, kaemferol
II	6.2	Caffeic acid
III	2.9	Not identified
IV	2.2	Not identified
V	1.1	None
		Nonhydrolyzed
I	6.0	Flavonol glycosides
II	4.2	Caffeic acid
III	4.8	Chlorogenic acid
IV	1.1	None

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Semiarid Legume Crops as Protein Resources

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ABSTRACT

Worldwide population pressures and accompanying increased demands on water supplies and tillable land has forced a reevaluation of traditional agricultural techniques and crops. Under-used semiarid lands are becoming candidates for crop production that uses stress-tolerant plants. Desert legume trees and shrubs, e.g., species of *Prosopis, Leucaena, Acacia, Geoffroea* and *Olneya,* fix nitrogen and could be sources of seed protein, forage or biomass. Seeds from desert legume perennials have a high potential as protein producers.

INTRODUCTION

The United States is currently facing the problem of overproduction of food, its main export commodity, and efforts to enhance agricultural production by cultivation of desert lands might appear illogical. However, long-term national objectives and international considerations combine to dictate that a high priority, intensive research effort be directed toward the goals of water and soil conservation as exemplified by minimum irrigation farming and development of crops and technologies to farm our arid and semiarid lands.

Today, ca. 1/3 of the earth's land mass is desert, compared with ca. 12% in 1882. Worldwide, 20% of the land is generally considered arid and 13% semiarid (Table I) (1,2) when arid zones or dry lands are defined by relating available precipitation to potential evapotransporation (3,4,5). These desert lands represent a diversity of soils and climates and are a home for a variety of cultures (6).

Ca. 8% of US land is arid and 22% semiarid. Three percent of North American land is arid and 11% semiarid. The Americas (North, Central and South) have ca. 14% of the world population, but 16% dry land. Of these countries, Mexico probably has the worst problem with 46% of its land dry and a population of ca. 70 million. Central America is generally tropical, but South America also has large desert areas.

Africa is 69% arid or semiarid. Egypt is essentially all desert, except for land along the Nile. Algeria, Tunisia, Niger and Ethiopia also have a severe lack of agricultural land.

To make a bad situation worse, deserts are expanding. In the Sudan, the desert is advancing at a rate of 5 km per year; in the Sahel 100,000 ha per year are lost; worldwide,