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Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds

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Abstract

Chia seeds from two different regions in the states of Jalisco and Sinaloa were analyzed for soluble and insoluble fibre and antioxidant activity of phenolic compounds. The soluble and insoluble fibre content of the Sinaloa and Jalisco seeds was similar. The major compounds identified in hydrolyzed and crude extracts were quercetin and kaempferol, while caffeic and chlorogenic acids were present in low concentrations. Screening of antioxidant activity using 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical cation ($ABTS^{\cdot+}$), β-carotene linoleic acid model system (β-CLAMS) and *in vitro* liposome peroxidation system assays, showed that the crude extract of the Jalisco seed has an antioxidant activity comparable to the commercial antioxidant Trolox® used as a reference. Different concentrations of the hydrolyzed and crude extracts of the seeds from both regions showed antioxidant effect when tested in a model water-in-oil food emulsion.

Keywords: *Salvia hispanica*; Dietary fibre; Phenolic compounds; Antioxidant activity; Lipid oxidation

1. Introduction

The *Salvia* genus consists of about 900 species in the family Lamiaceae, with some of them cultivated and used worldwide in flavouring and folk medicines (Lu & Foo, 2002). This genus has been the object of numerous chemical studies that describe the isolation of diterpenoids, tanshinones and polyphenols from different parts of the plants (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003; Gu & Weng, 2001; Lu, Foo, & Wong, 1999; Lu & Foo, 2002; Miliauskas, Venskutonis, & van Beek, 2004; Tepe, Sokmen, Akpulat, & Sokmen, 2006).

The seeds of the species *Salvia hispanica* L. commonly known as "chia", "chia sage" and "Spanish sage" were an important staple food, oil source and medicine for Mesoamericans in pre-Columbian times. The roasted and grounded seeds (chiapinolli) were eaten as a gruel and the oil was used as a body emollient and pottery lacquer. The curative properties of the seeds were also appreciated, for example, for treating eye obstructions, infections and respiratory malaises. The seeds soaked in water or fruit juice were and still are consumed in some regions as a refreshing drink (Cahill, 2003). The oil content of the seeds ranges from 25% to 35% and contains high concentrations of polyunsaturated fatty acids (Taga, Miller, & Pratt, 1984). The presence of cinnamic, chlorogenic and caffeic acids together with the flavonoids, myrcetin, quercetin and kaempferol in methanolic hydrolyzed extracts has also been reported (Taga et al., 1984).

The total dietary fibre (TDF) has become an important component in the daily diet. Intake of TDF has health beneficial effects. Some of them include reduction of cholesterolaemia, modification of the glycemic and insulinaemic responses, changes in intestinal function and antioxidant activity. TDF has also technological functionalities as a fat-binding, gel-forming, chelating and texturizing agent (Abdul-Hamid & Luan, 2000; Borderías, Sanchez-Alonso, & Pérez-Mateos, 2005; Chau, Chen, & Lee, 2004; Esposito et al., 2005; Guillon & Champ, 2000;

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Saura-Calixto, 1998; Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997).

The residues of the oil-extracting process of oilseeds contain phenolic compounds with antioxidant activity that makes them suitable as natural antioxidants for fats and oils (Matthäus, 2002). The antioxidant activity of different plant extracts is particular to the type and concentration of the phenolic compounds they contain. Therefore, the activity of a given antioxidant has to be examined under particular conditions over a sufficient number of systems if one wants to get valuable information on the possible use of a given extract. During storage of oils, fats and other fat-containing foods, lipid oxidation is still a major cause of food quality deterioration in spite of the wide use of several antioxidants. Addition of antioxidants to fats, oils or to foods in which they are present is effectively helpful in retarding lipid oxidation. The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are quite volatile and decompose easily at high temperatures (Branen, 1975). There are serious concerns about the safety and toxicity of BHA, BHT and *tert*-butylhydroxyquinone (TBHQ) related to their metabolism and possible absorption and accumulation in body organs and tissues (Lindenschmidt, Tryka, Goad, & Witschi, 1986; Tappel, 1995). Therefore, the use of plant extracts containing antioxidant compounds instead of commercial antioxidants like BHT, BHA and TBHQ has been examined some years ago (Abdalla & Roozen, 1999; Duh, 1999).

The purpose of this study was to determine the dietary fibre content of chia seeds obtained from two different regions of Mexico and investigate the antioxidant activity of seed extracts in order to assess them as potential functional ingredients in food systems. $ABTS^{\cdot+}$ radical scavenging together with β-carotene-linoleic acid and peroxidation of phospholipid liposomes were used as indicators of antioxidant activity. The antioxidant effectiveness of chia seed extracts was also tested in a model water-in-oil (w/o) food emulsion.

2. Materials and methods

2.1. Seeds

The chia seeds (*S. hispanica* L.) from western regions located in the states of Jalisco and Sinaloa, México were purchased in a local market (Central de Abasto) in Mexico City. Extraneous matter (dust, vain seeds, and straw from threshed seeds) was separated manually. The cleansed seeds were grounded in a laboratory impact mill (IKA MF10, IKA Works, Inc Wilmington, CA, USA) and passed through a 0.600 mm sieve. Defatting with *n*-hexane was immediately carried out in a Soxhlet apparatus. The resulting flour was stored in dark-tightly-closed bottles under a nitrogen atmosphere until further analysis.

2.2. Total, soluble and insoluble fibre

Total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were determined using the enzymatic-gravimetric AOAC method (Prosky, Asp, Schweizer, De Vries, & Furda, 1988) modified as described by Mañas, Bravo, and Saura-Calixto (1994). The modification consisted of using dialysis instead of ethanolic precipitation to avoid losses of SDF. An additional modification consisted of using a sample weight of 0.1 g owing to the presence of mucilage in the samples. The neutral sugars (NS) and uronic acids (UA) were determined in the SDF and IDF fractions, respectively, by spectrophotometric procedures (Scott, 1979; Southgate, 1976). The IDF fraction was hydrolyzed with sulphuric acid and the remaining residue gravimetrically quantified as Klason Lignin (KL) (Mañas et al., 1994).

2.3. Extraction of phenolic compounds

Many authors have proposed different extraction methods, solvents and conditions to obtain a greater yield of phenolic compounds from natural sources and evaluate the effectiveness of each method (Kim, Tsao, Yang, & Cui, 2006; Koşar, Dorman, & Hiltunen, 2005; Naczk & Shahidi, 2004). We used two extraction procedures.

2.3.1. Extraction with hydrochloric acid in ethanol

A mass of 10 g of deffated flour was mixed with 100 mL of 1.2 M HCl in ethanol and the mixture kept at 90 °C for 3 h under reflux conditions. Once cooled to ambient temperature, 50 mL of ethanol were added and the resulting mixture centrifuged at 2500*g* for 15 min. The supernatant was evaporated at 40 °C in a rotary vacuum evaporator (Büchi Rotavapor R-200-205, Flawil, Switzerland). The remaining moisture was removed by blowing nitrogen gas into the flask. The dry residues were re-extracted with 15 mL of diethyl ether and washed five times with 5 mL of distilled water, recovering the organic phase. When the organic phase was clear, it was concentrated again under vacuum and re-dissolved in 15 mL of ethanol to obtain the hydrolyzed extract.

2.3.2. Extraction with ethanol

A mass of 10 g of deffated flour was extracted with 100 mL of ethanol at room temperature for 48 h under mechanical shaking. The mixture was centrifuged at 2500*g* for 15 min. The supernatant was evaporated at 40 °C in a rotary vacuum evaporator. The remaining moisture was removed by blowing nitrogen gas into the flask. The dry residues were re-extracted with 15 mL ethanol and filtered to recover the crude extract.

2.4. Total phenolics assay

Total phenolics were determined as gallic acid equivalents (GAE) (Matthäus, 2002). Two millilitres of the crude

extract were mixed with 3 mL of 0.3% HCl. An aliquot of 100 μL was mixed with 2 mL of 2% Na_2CO_3, stirred and mixed with an equal volume of water-diluted (1:1, v/v) Folin-Ciocalteau reagent. After 30 min incubation at ambient temperature (≈25 °C) the absorbance at 750 nm was measured and compared to a previously prepared gallic acid calibration curve.

2.5. Determination of phenolic compounds

We used the method developed by Tsao and Yang (2003) for fruits and related products for the simultaneous determination of the five major groups of phenolic compounds: single-ring phenolic acids, procyanidins, anthocyanidins, flavonols and dihydrochalcones. Quantification and identification of the various phenolics in the crude and hydrolized extracts were carried out using an HPLC Waters 600 controller equipped with a quaternary pump, a degasser and a diode array detector (DAD Waters 996). Phenolic compounds were separated using a Symmetry® C18 column, 250 mm × 4.6 mm i.d.; particle No. WAT 054275, Waters (Waters, S.A. de C.V., Mexico City, Mexico). The binary mobile phase consisted of 6% acetic acid in 2 mM sodium acetate (final pH 2.55, v/v, solvent A) and acetonitrile (solvent B). Solvent A was prepared by mixing a freshly prepared 2 mM sodium acetate solution with acetic acid at a ratio of 94:6 by volume. All solvents were filtered through a 0.45 μm membrane prior to analysis. The flow rate was kept constant at 1.0 mL/min for a total run time of 80 min. The system was run with a gradient program: 0–15% solvent B in 45 min, 15–30% solvent B in 15 min, 30–100% solvent B in 10 min. The column was equilibrated by post running during 10 min under the initial conditions. The injection volume for chia seeds extracts and standards was 10 μL. All standards were dissolved in ethanol (caffeic acid = 0.025 mg/mL; chlorogenic acid = 0.114 mg/mL; ferulic acid = 0.1 mg/mL; *p*-coumaric acid = 0.029 mg/mL; 7-hydroxycumarin = 0.025 mg/mL; chatecol = 0.114 mg/mL; quercetin = 0.114 mg/mL; quercetin-3-glucoside = 0.114 mg/mL and kaempferol = 0.085 mg/mL). The detector was set at 280, 320, 360, and 520 nm for simultaneous monitoring of the different phenolics. Compounds were identified by comparing their retention times and UV–Vis spectra with those of the standards.

2.6. Antioxidant activity of the extracts

The activity of antioxidants in foods and biological systems depends on a multitude of factors. A reliable protocol demands measuring more than one property relevant to either foods or biological systems. The antioxidant capacity of phenolic extracts from the chia seeds was determined through four complementary assay procedures.

2.6.1. ABTS radical scavenging assay

This method measures the capacity of different components to scavenge the 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical cation ($ABTS^{\cdot+}$) (Arnao, Cano, & Acosta, 2001). The antioxidant activity was measured in a reaction mixture containing 0.5 mL of 15 μM H_2O_2, 0.5 mL of 2 mM ABTS (Sigma–Aldrich Química S.A. de C.V., Toluca, Mexico) and 0.5 mL of horseradish peroxidase (1 mg/mL) in 50 mM Na-phosphate buffer, pH 7.5; the donor was hydrogen-peroxideoxidoreductase; EC 1.11.1.7, 50,000 U, P-8250 (Sigma–Aldrich Química S.A. de C.V., Toluca, Mexico). The reaction was monitored at 730 nm, until a stable absorbance was reached. Then, 0.5 mL of the extract (0.2 mg of phenolics/mL of extract) was added to the reaction medium and the decrease in absorbance was recorded during 12 min. The scavenging ability was calculated using Eq. (1), where A_0 is absorbance at $t = 0$ min and A_{12} is absorbance at $t = 12$ min.

$$SA_{ABTS^+} = \left(\frac{A_0 - A_{12}}{A_0}\right)100 \quad (1)$$

2.6.2. β-Carotene-linoleic acid model system (β-CLAMS) assay

Twenty milligrams of linoleic acid and 200 mg of Tween 80 were transferred to a flask and 1 mL of a 0.2 mg/mL solution of β-carotene (Sigma–Aldrich Química S.A. de C.V., Toluca, Mexico) in chloroform was added. Chloroform was vacuum evaporated at 40 °C. Then, 50 mL of distilled water were slowly added to the residue and the solution was vigorously agitated to form a stable emulsion. To an aliquot of 5 mL of this emulsion, 0.2 mL of antioxidant solution was added, and the absorbance was immediately measured at 470 nm ($t = 0$) against a blank consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the absorbance measured (Matthäus, 2002). The antioxidant activity (AA) of the extracts was calculated from the absorbance at the end of 2 h.

2.6.3. Peroxidation of phospholipid liposomes

Liposomes were prepared according to the literature (Siddhuraju, Mohan, & Becker, 2002; Tsuda, Ohshima, Kawakishi, & Osawa, 1994; Yen & Chuang, 2000). Egg lecithin (5 g) was dispersed in sodium phosphate buffer (500 mL, 20 mM, pH 7.4) and sonicated (Ultrasonic cleaner, CAVITATOR Mettler Electronics, Bruce Medica International S.A. de C.V., Mexico City, Mexico) for 30 min under nitrogen gas atmosphere in an ice-cold water bath. Crude extracts with 0.2 mg of phenolics/mL of extract were tested for lipid peroxidation activities with the following mixture. The extract (0.5 mL) was mixed with liposomes (2 mL), 25 mM $FeCl_3$ (0.1 mL), 25 mM H_2O_2 (0.1 mL), 25 mM ascorbic acid (0.1 mL) and 0.2 M phosphate buffer (1.2 mL, pH 7.4). The reaction mixture was incubated at 37 °C for 4 h. At the end of the incubation, 1 mL of BHA (20 mg/mL in methanol) was added to stop the oxidation reaction. The extent of oxidation of liposomes was subsequently determined by measuring the thiobarbituric acid-reactive substances (TBARS). One milliliter

of 1% thiobarbituric acid (TBA) and 1 mL of 10% HCl were added to the reaction mixture and then the mixture heated in a water bath at 100 °C for 30 min. After cooling the mixture in an ice bath for 15 min, 5 mL of chloroform were added, and the mixture centrifuged at 3000*g* for 20 min. The absorbance of the supernatant was measured at 532 nm. The percent inhibition of TBARS formation was calculated using the absorbance of the control, the blank and the sample added. Trolox® was assayed for comparison of the results.

2.7. Effect of the concentration of chia extracts on the oxidation of a model w/o food emulsion

Model w/o food emulsions were prepared by mixing 64 mL of chia oil, 11.3 mL of vinegar, 0.1 g of xanthan, 10 mL of crude or hydrolyzed extracts of the Jalisco seed with four different concentrations of phenolics (0.02, 0.05, 0.07 and 0.1 g/100 mL). A blank (B) was prepared under the same conditions, without antioxidant or extract, and also a reference sample with 0.02% of TBHQ. The rate of autoxidation of the oil in the emulsion was estimated according to the increase of its peroxide value (PV), which was determined by the method of Crowe & White (2001). The changes in the induction period (IP) of oil after addition of each extract, as a function of its concentration in the oil, were determined. The IP was the time in hours for which a change in the slope of the PV of the sample together with the start of the propagation period in the oxidation kinetics were observed. Values of the protection or antioxidant factor (PF) for chia oil and antioxidant activities (AA) of the extracts were calculated using Eqs. (2) and (3), respectively, where IP_{inh} is the induction period (h) of the sample with antioxidant, IP_0 is the induction period (h) of the sample without antioxidant and IP_{TBHQ} is the induction period (h) of the sample with synthetic antioxidant TBHQ (Bandonienė, Pukalskas, Ventskutonis, & Gruzdienė, 2000; Yanishlieva & Marinova, 1992).

$$PF = \frac{IP_{inh}}{IP_0} \quad (2)$$

$$AA = \frac{IP_{inh} - IP_0}{IP_{TBHQ} - IP_0} \quad (3)$$

2.8. Statistical analysis

All experiments were carried out in triplicate. Statistics on a completely randomized design were determined with the SPSS 10.0 for windows procedure. Differences were considered to be statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Content and composition of TDF, SDF and IDF

The Jalisco and Sinaloa seeds were rich in TDF and their TDF, SDF and IDF content was not significantly different (see Table 1). When we used the AOAC method (Prosky et al., 1988), complete enzyme digestion was not possible because of the presence of mucilage. For this reason the method of Mañas et al. (1994) was modified using a sample weight of 0.1 g. Based on this method, the SDF and IDF content of Jalisco seeds were 6.84 and 34.9 g/100 g, respectively, while in Sinaloa seeds, it was 6.16 and 32.87 g/100 g, respectively. The ratio between IDF and SDF gives important information on nutritional and physiological effects in consumers. The American Dietetic Association recommends fibre intakes for adults of 25–30 g/day with an IDF/SDF ratio of 3–1 (Borderías et al., 2005).

The IDF was the predominant fraction in both seeds (see Table 1). The main component of IDF is Klason lignin (KL), which makes up to 39–41% of TDF. This component is thought to protect the unsaturated fats in chia seeds by building a strong and resistant structure and also by the antioxidant compounds it contains. The content of neutral sugars in IDF (NS_{IDF}), 13.79–14.97%, show the presence of cellulose and hemicellulose in contrast with a

Table 1
Dietary fibre content and fibre fractions of chia seeds on a dry weight basis (g/100 g seed)

Seed	TDF[A]	TDF[B]	SDF		IDF			UDF/SDF
Jalisco	39.94 ± 0.5[a]	41.41 ± 0.2[a]	6.84 ± 0.9[a]		34.90 ± 0.9[a]			5.10
			NS_{SDF}	UA_{SDF}	NS_{IDF}	UA_{IDF}	KL	
			5.12 ± 0.2[a]	1.72 ± 0.5[a]	14.97 ± 0.4[a]	3.60 ± 0.2[a]	16.33 ± 0.4[a]	
			SDF		IDF			
Sinaloa	36.97 ± 0.7[a]	38.79 ± 0.3[a]	6.16 ± 0.3[a]		32.87 ± 0.1[a]			5.34
			NS_{SDF}	UA_{SDF}	NS_{IDF}	UA_{IDF}	KL	
			4.69 ± 0.3[a]	1.47 ± 0.4[a]	13.79 ± 0.3[a]	3.05 ± 0.9[a]	16.03 ± 0.7[a]	

Values in a column with different letters are significantly different at $p < 0.5$. Data means ± standard deviation ($n = 3$). NS = neutral sugars; UA = uronic acids, KL = Klason lignin.
[A] Prosky et al. (1988).
[B] Mañas et al. (1994).

lower content of uronic acids (UA_{IDF}), 3.05–3.60%, that represent the amount of glucuronic acid residues associated with insoluble hemicellulose. Due to its capacity to absorb bile acids, lignin is responsible for the hypocholesterolemic effect associated with fibre intake. These results indicate that the dietary fibre present in the Jalisco and Sinaloa seeds may be beneficial from a nutritional and health standpoint. The SDF represents about 6% of chia seeds. In this natural product the presence of SDF is also evident from the mucilaginous capsule formed when the seeds are soaked in water. The SDF is mainly composed of neutral sugars (NS_{SDF}), which indicates the presence of diverse carbohydrates that form the structure of the mucilage. The low amount of uronic acids (UA_{SDF}) suggests that there is no pectin associated with the mucilage and that the amount of UA_{SDF} comes from the uronic acid residues present in the polysaccharide chains of the mucilage.

3.2. Total phenolics content

Following the suggestion of others (Kim et al., 2006) we used two different methods of extraction, in order to obtain more information about the concentration and composition of phenolic compounds in chia seeds. The phenolics content (mg/g of chia seed extract, expressed as gallic acid equivalents, GAE) in Jalisco seeds (0.9211 ± 0.040) and Sinaloa seeds (0.8800 ± 0.008) was not significantly different. Also, no significant difference between both hydrolyzed extracts was observed (Jalisco seeds: 0.8899 ± 0.02 and Sinaloa seeds: 0.8800 ± 0.008). The mean concentration, with this assay, was about 0.88 mg per gram of chia seed extract. Hydrolysis was chosen in order to obtain a maximum yield of polyphenolic acids as in the case of cereal grains (Kim et al., 2006). In terms of phenolics content, we did not find an increase of GAE as quantified by the Folin-Ciocalteau method.

3.3. Phenolics profile of hydrolyzed and crude extracts

According to Tsao & Yang (2003), quantification of phenolic compounds is based on their retention time and their UV–Vis spectrum. The λ_{max} for the single-ring phenolic acids other than the hydroxybenzoic acids was near 320 nm; for the hydroxybenzoic acids, flavan-3-ols (including dimers), and dihydrochalcones λ_{max} was near 280 nm; for the flavonols λ_{max} was 360 nm; and for the anthocyanins λ_{max} was near 520 nm. Each standard (caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid, 7-hydroxycumarin; chatecol, quercetin, quercetin-3-glucoside and kaempferol) was injected to the HPLC unit and the UV–Vis spectrum obtained by using the diode array

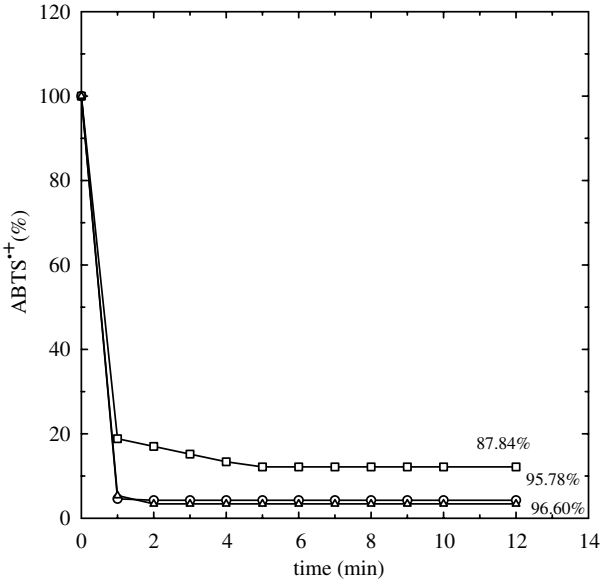

Fig. 1. Inhibition assay on $ABTS^{\cdot+}$ absorption for 200 ppm crude extracts of Jalisco seed (circles), Sinaloa seed (squares) and Trolox® (triangles). Values at $t = 12$ min indicate the percentage of radical scavenging.

Table 2
Concentration of phenolic compounds in crude and hydrolyzed extracts of Jalisco and Sinaloa chia seeds

Concentration	Phenolic glycoside-Q	Phenolic glycoside-K	Chlorogenic acid	Caffeic acid	Quercetin	Kaempferol	Total phenols
Jalisco seed: crude extracts							
mg/mL	0.124	0.202	0.0513	0.0015	ND	ND	0.379
mg/g Seed	0.248	0.403	0.102	0.0030	ND	ND	0.757
Jalisco seed: hydrolyzed extracts							
mg/mL	ND	ND	ND	ND	0.125	0.301	0.427
mg/g Seed	ND	ND	ND	ND	0.150	0.360	0.511
Sinaloa seed: crude extracts							
mg/mL	0.2174	0.337	0.0307	0.00450	ND	ND	0.590
mg/g Seed	0.3248	0.503	0.0459	0.00680	ND	ND	0.881
Sinaloa seeds: hydrolyzed extracts							
mg/mL	ND	ND	ND	ND	0.225	0.426	0.650
mg/g Seed	ND	ND	ND	ND	0.268	0.509	0.777

Phenolic glycosides-Q: probably a mixture of quercetin glycosides also include their methyl ethers. Phenolic glycosides-K: probably a mixture of kaempferol glycosides also include mono to triglycosides. ND: not detected.

detector (chromatograms not shown). The total concentrations of each group of phenolic compounds in the crude and hydrolyzed extracts of the Jalisco and Sinaloa seeds are listed in Table 2. Using this method in combination with the diode array detector, a simplified estimation of concentrations of similar compounds as a group can be obtained. The extracts from the chia seeds analyzed here did not contain the anthocyanins group.

In both chia seeds, the flavonols group was present in the highest amount. Sinaloa seeds contain the greatest amount, 0.590 mg/mL crude extract and 0.650 mg/mL hydrolyzed extract, while the Jalisco seed contained 0.379 mg/mL crude extract and 0.427 mg/mL hydrolyzed extract. These differences could be explained by the fact that the phenolics content is affected by a number of external factors, such as weather and post harvest conditions. It is likely that the main phenolic components of crude extracts are a mixture of glycosides of quercetin and kaempferol, respectively. In contrast, significant amounts of quercetin and kaempferol in aglycon forms are present in the hydrolyzed extracts. Low amounts of chlorogenic and caffeic acids were detected only in the crude extracts; probably these acids were sensitive to hydrolysis conditions.

Table 3
Antioxidant activity (% AA) of chia seeds extracts

Extract	β-CLAMS	Inhibition of lipid peroxidation
Jalisco	79.3 ± 0.13^{a}	89.4 ± 0.35^{a}
Sinaloa	73.5 ± 0.19^{a}	88.26 ± 0.47^{a}
Trolox®	88.3 ± 0.10^{a}	94.58 ± 0.41^{a}

Values in a column with different letters are significantly different to $p < 0.5$. Data are means ± standard deviation ($n = 3$).

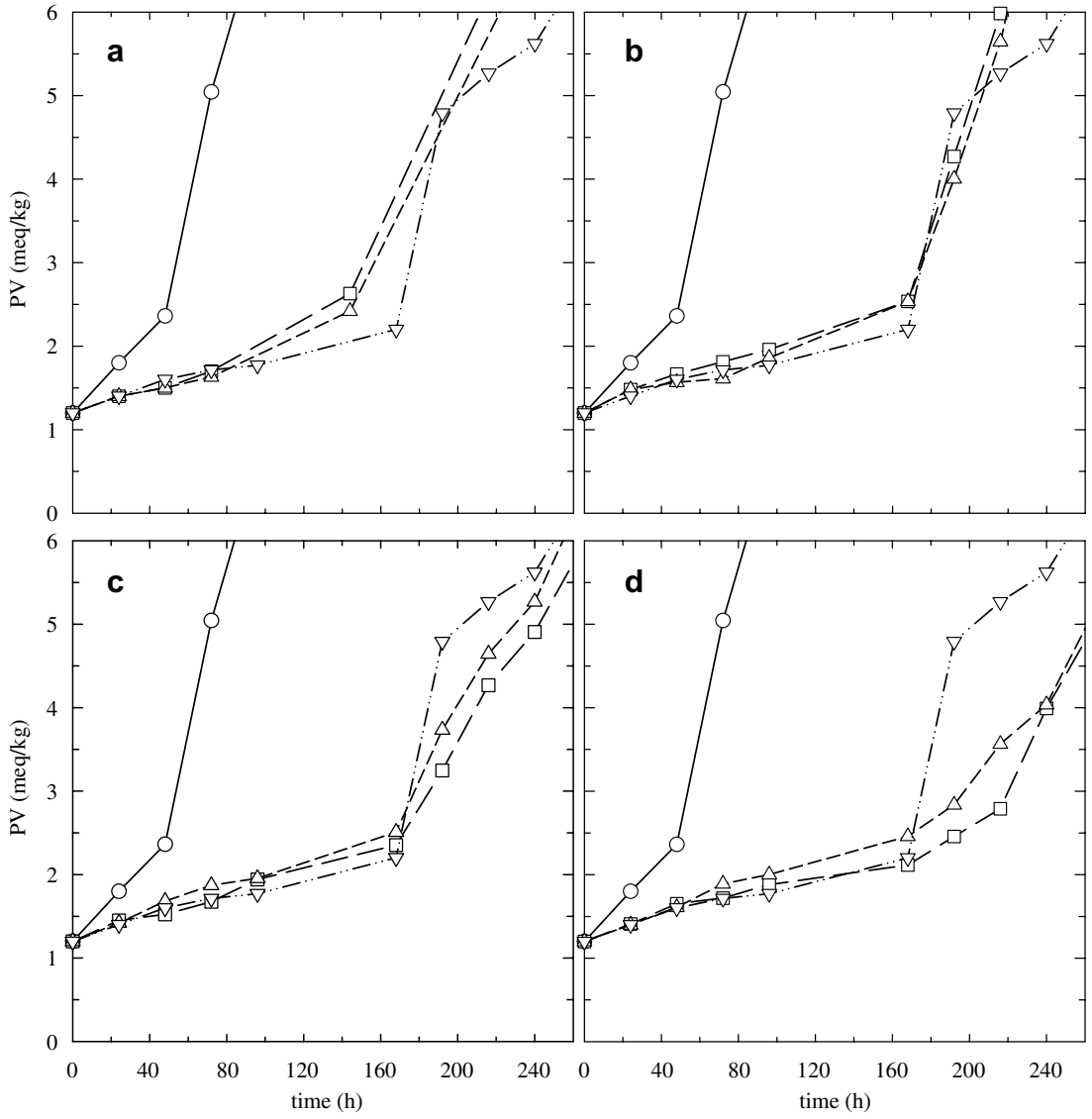

Fig. 2. Kinetics of peroxide accumulation during lipid oxidation of the w/o food emulsion at 37 °C in the presence of crude (squares) and hydrolyzed (triangles) chia seed extracts at 200 (a), 500 (b), 750 (c) and 1000 ppm (d). Blank (circles) and TBHQ at 200 ppm (inverted triangles).

3.4. Antioxidant activity

Four *in vitro* assays for antioxidant activity were examined to assess the action of crude extracts at 200 ppm polyphenols (GAE).

3.4.1. ABTS cationic radical scavenging activity

The action of the extract of chia seed on $ABTS^{\cdot+}$ is shown in Fig. 1. There was no significant difference between the two seeds. However, the extract of the Jalisco seed seemed to have a faster radical-scavenging activity than the Sinaloa seed, but essentially the same as that of Trolox®.

3.4.2. Inhibition of β-carotene linoleic acid model system (β-CLAMS)

In this system one of the hydrogen atoms of one of the methylene groups of linoleic acid is withdrawn leaving the free radical of the acid ready to attack β-carotene molecules. They loose their double bond and therefore the characteristic orange colour degrades. This oxidative destruction of β-carotene by the radicals of linoleic acid degradation is measured by the decrease in absorbance at 470 nm. Table 3 shows the percentages of antioxidant activity of chia seed extracts on β-carotene. The chia extracts of Jalisco and Sinaloa, showed higher antioxidant activity than other *Salvia* species; *Salvia caespitosa* (55.9%), *Salvia candidissima* (62.3%), *Salvia hypargeia* (62.9%), *Salvia euphratica* (59.1%), *Salvia sclarea* (63.5%) and *Salvia aethiopis* (29.0%) (Tepe et al., 2006). This assay reveals the chia extracts ability to stabilize the reactive oxygen species and some lipidic radicals responsible of the oxidation of linolenic acid.

3.4.3. Inhibition of lipid peroxidation

In this assay system it has been proven that singlet oxygen is the precursor of hydrogen peroxide and hydroxyl radical formation (Siddhuraju et al., 2000). Examination of the extracts shows a comparable activity with Trolox® (Table 3). The antioxidant activity between the crude extract of the Sinaloa and Jalisco seeds is not significantly different, suggesting that the phenols in both extracts have an important activity as oxygen singlet quenchers. Polyphenolic substances have more than one mechanism of action for free radicals and are able to suppress free reactions. These compounds are also able to act as antioxidants by the hydrogen-donating capacity of their phenolic groups. In addition, the metal-chelating potential may also play a role in the protection against iron- and copper-induced free radical reactions.

3.4.4. Antioxidant activity on the model w/o food emulsion

The crude and hydrolyzed extracts of the Jalisco seed were tested in the model w/o food emulsion. In Fig. 2, the kinetics of each assay is shown. Fig. 2(d) illustrates the kinetics in the blank and the reference. The IP of the blank breaks earlier (48 h) than the reference (about 160 h). All the concentrations of hydrolyzed and crude extracts were found to be good natural antioxidants, retarding the oxidation of the emulsion. There is no significant difference between the crude and hydrolyzed extracts for the same concentration.

Table 4
Antioxidant activity of chia extracts in different concentrations and their effect on the stability of oil in a model water-in-oil food emulsion

Additive	Concentration (%)	Protection factor (PF)[a]	Antioxidant activity (AA)[b]
Blank	–	1.00	–
TBHQ	0.020	3.33	–
Crude extract	0.020	1.67	0.29
Crude extract	0.050	2.08	0.46
Crude extract	0.075	3.13	0.91
Crude extract	0.100	3.75	1.18
Hydrolyzed extract	0.020	1.88	0.38
Hydrolyzed extract	0.050	2.08	0.46
Hydrolyzed extract	0.075	2.92	0.82
Hydrolyzed extract	0.100	3.54	1.09

[a] PF is the ratio of the IP of the sample with additive to the IP of the sample without additive.
[b] AA was calculated in comparison with TBHQ at 0.02%.

The PF's and AA's of the extracts are shown in Table 4. The effectiveness of the antioxidants was compared on the basis of their stability values and protection factors. The effectiveness of the antioxidants, under the conditions tested, in decreasing order is: Crude extract 0.1% > hydrolyzed extract 0.1% > TBHQ 0.02% > crude extract 0.075% > hydrolyzed extract 0.075% > crude extract 0.05% > hydrolyzed extract 0.05% > hydrolyzed extract 0.02% > crude extract 0.02% > blank. It is observed that for a concentration of 0.1% both extracts exhibit a "very high" (PF > 3) antioxidant activity comparable to the commercially used TBHQ used here as reference. A linear correlation was found between the PF and concentration for both extracts.

4. Conclusions

The Jalisco and Sinaloa chia seeds are an important source of dietary fibre with nutritional and technological potential as antioxidants in food products because of the non-negligible activity of the associated phenolic compounds. The results of this study show consistent antioxidant activity comparable to Trolox® in all of the assay model systems examined. The isolation and preparation of bioactive compounds from chia seeds could be used to produce potent natural antioxidants or functional ingredients with commercial applications.

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