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Radical Scavenging Activity of Black Cumin (*Nigella sativa* L.), Coriander (*Coriandrum sativum* L.), and Niger (*Guizotia abyssinica* Cass.) Crude Seed Oils and Oil Fractions

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Crude vegetable oils are usually oxidatively more stable than the corresponding refined oils. Tocopherols, phospholipids (PL), phytosterols, and phenols are the most important natural antioxidants in crude oils. Processing of vegetable oils, moreover, could induce the formation of antioxidants. Black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.), and niger (*Guizotia abyssinica* Cass.) crude seed oils were extracted with *n*-hexane and the oils were further fractionated into neutral lipids (NL), glycolipids (GL), and PL. Crude oils and their fractions were investigated for their radical scavenging activity (RSA) toward the stable galvinoxyl radical by electron spin resonance (ESR) spectrometry and toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by spectrophotometric method. Coriander seed oil and its fractions exhibited the strongest RSA compared to black cumin and niger seed oils. The data correlated well with the total content of polyunsaturated fatty acids, unsaponifiables, and PL, as well as the initial peroxide values of crude oils. In overall ranking, RSA of oil fractions showed similar patterns wherein the PL exhibited greater activity to scavenge both free radicals followed by GL and NL, respectively. The positive relationship observed between the RSA of crude oils and their color intensity suggests the Maillard reaction products may have contributed to the RSA of seed oils and their polar fractions. The results demonstrate the importance of minor components in crude seed oils on their oxidative stability, which will reflect on their food value and shelf life. As part of the effort to assess the potential of these seed oils, the information is also of importance in processing and utilizing the crude oils and their byproducts.

KEYWORDS: Black cumin; niger seed; coriander; crude seed oil; oil fractions; radical scavenging activity; ESR; antioxidants; DPPH; galvinoxyl; Maillard reaction products

INTRODUCTION

Nonconventional oilseeds are being considered because their constituents have unique chemical properties and may augment the supply of edible oils. Interest in newer sources of edible oils has recently grown. Among the various seed oils, black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.), and niger (*Guizotia abyssinica* Cass.) are of particular interest because they may be utilized for the production of formulations containing phytochemicals with significant antioxidant properties and health benefits. The safety of synthetic antioxidants has been questioned in recent years (*1*). A trend toward the use of natural additives in foods has been apparent for quite some time as a result of consumer demand. Recent research has focused on isolation and characterization of effective natural antioxidants (*2*). Reports have described antioxidants and compounds with radical scavenging activity (RSA) present in olive oil (*3*−*6*) and some commercial vegetable oils (*7*). Very little information, however, is currently available on the bioactive constituents present in crude seed oils that are responsible for their antioxidant properties. Natural antioxidants may function (a) as reducing agents, (b) as free radical scavengers, (c) as complexers of pro-oxidant metals, and (d) as quenchers of the formation of singlet oxygen. They can be used in the food industry and there is evidence that they may exert their antioxidant effects within the human body (*8*, *9*). Crude oils are consumed in their natural state, thus conserving a number of minor substances, which are usually removed from other vegetable oils at various stages of refining (*8*, *10*). These minor constituents can have either pro-oxidative (free fatty acids and hydroperoxides) or antioxidative (tocopherols, phenols, and phospholipids "PL") effects (*7*, *8*). The nutritionally important antioxidants such as tocopherols and carotenes improve the stability of the oils (*11*, *12*). Phytosterols contained in vegetable oils are hypocholesterolemic and their antioxidant activity has been attributed to the formation of an allylic free radical and

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its isomerization to other relatively stable free radicals (*13*, *14*). Phenolics have a great effect on the stability, sensory, and nutritional characteristics of the product and may prevent deterioration through the quenching of radical reactions responsible for lipid rancidity (*15*, *16*). Moreover, a part of the higher stability of crude oils is due to PL. The literature is replete with references to the antioxidant properties of PL (*12*, *17*−*23*). They have usually been considered as free radical scavengers, an antioxidant synergist, and an extender for the action of primary antioxidants. The emulsifying action of PL, furthermore, can play an important role or synergy by increasing the contact between the antioxidant and the oxidizing fat. On the other hand, antioxidants in foods may be formed from reactions during processing (*2*). Nonenzymatic browning of aminophospholipids has early been recognized in dried egg (*24*). Formation of melanophospholipids (Melano-PL) in hexane miscella of soybean was also reported (*25*). More recently, aminophospholipids-linked Maillard products have been definitely established in foodstuffs such as spray-dried egg yolk and lecithin (*26*). King et al. (*27*) postulated a positive relationship between the antioxidant properties of PL and the formation of Maillard reaction products (MRP) in a salmon oil model system. As products formed during solvent extraction, it is hard to find investigations characterizing MRP and/or Melano-PL in other crude oils.

The consumption of foodstuffs rich in antioxidants provides protection against cancer and cardio- and cerebrovascular diseases. This protection can be explained by the capacity of these antioxidants to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins, and nucleic acids (*28*). Black cumin, coriander, and niger seed oils have been introduced to the market relatively recently and therefore data on these oils are rather limited. Although the chemical composition of the mentioned seed oils has been reported (*29*−*37*), no data about their RSA and their antioxidant properties are yet available. The objectives of this study were the following: (a) to compare the RSA of the mentioned crude oils and their fractions, (b) to study the effect of minor constituents in oils, especially polar lipids, on their RSA, (c) to characterize MRP in crude oils, and (d) to report on the relationship between color intensity of seed oils and their antioxidant properties. The described arrangement for the experiments uses the addition of stable radicals galvinoxyl and 1,1-diphenyl-2-picrylhydrazyl (DPPH), which will be decomposed by components having antioxidant properties to crude seed oils and their fractions. These radicals were assayed by means of the Electron Spin Resonance (ESR) spectroscopy and spectrophotometric processes of quenching.

EXPERIMENTAL PROCEDURES

Oilseeds and Chemicals. Mature black cumin [*Nigella sativa* L., from Turkey], coriander [*Coriandrum sativum* L., from Hungary] and niger [*Guizotia abyssinica* Cass., from India] seeds were obtained from Alfred Galke GmbH (Gittelde, Germany) and stored at 4 °C until extraction. 1,1-Diphenyl-2-picrylhydrazyl (DPPH, approximately 90%) was from Sigma (St. Louis, Mo, USA). Galvinoxyl, free radical were from Aldrich Chemical Co. (Milwaukee, WI). The Folin-Ciocalteu reagent was from Merck (Darmstadt, Germany). Toluene of HPLC grade was used throughout the experiments. All solvents and reagents from various suppliers were of the highest purity needed for each application and used without further purification.

Extraction, Fractionation, and Compositional Analysis of Crude Seed Oils. Oilseeds were finely ground to 1−2 mm particle size in a mill (Analysenmühle A10, Janke & Kunkel GmbH, Staufen Br., Germany) and the extraction process (8 h at 70 °C) was performed in a Soxhlet extractor with use of 20 g of seed and 200 mL of *n*-hexane. Recovered oils in chloroform were then fractionated into the different classes by elution with different polar solvents over a glass column (20 mm diameter × 30 cm length) packed with a slurry of activated silicic acid (70 to 230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, w/v) according to Rouser et al. (*38*). Neutral lipids (NL) were eluted with 3-times the column volume of chloroform. The major portion of glycolipids (GL) was eluted with 5-times the column volume of acetone and that of PL with 4-times the column volume of methanol. Solvents were evaporated by using a rotary evaporator and the residues were dissolved in chloroform then stored at −20 °C as oil fractions. In accordance with our previous contributions (*32*−*37*), the following parameters were determined: fatty acids, tocopherols, sterols, and β-carotene. HPLC and GLC techniques were performed to assay the oil fractions and their fatty acid profile. The initial peroxide value (PV) was determined according to AOAC methods (*39*).

Extraction and Purification of the Phenolic Compounds. Aliquots of oil (2 g) were dissolved in *n*-hexane (5 mL) and mixed with 10 mL of methanol−water (80:20, v/v) in a glass tube for 2 min in a vortex. After centrifugation at 3000 rpm for 10 min, the hydroalcoholic extracts were separated from the lipid phase by using a Pasteur pipet then combined and concentrated in vacuo at 30 °C until a syrup consistency was reached. The lipidic residue was redissolved in 10 mL of methanol−water (80:20, v/v) and the extraction was repeated twice. Hydroalcoholic extracts were redissolved in acetonitrile (15 mL) and the mixture was washed three times with *n*-hexane (15 mL each). Purified phenols in acetonitrile were concentrated in vacuo at 30 °C then dissolved in methanol for further analysis. Due to the low amounts of phenolics in niger seed oil, 10 more times niger seed oil was extracted and subjected to the Folin-Ciocalteu test.

Quantification and Characterization of Phenolic Compounds. Aliquots of phenolic extracts were evaporated to dryness under nitrogen. According to Koski et al. (*8*) the residue was redissolved in 0.2 mL of water and diluted (1:30) Folin-Ciocalteu's phenol reagent (1 mL) was added. After 3 min, 7.5% sodium carbonate (0.8 mL) was added. After a further 30 min, the absorbance was measured at 765 nm on a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Caffeic acid was used for the calibration and the results of triplicate analyses are expressed as parts per million of caffeic acid. Ultraviolet (UV) spectra of methanolic solutions of purified phenolic fractions (final concentration 1 mg in 2 mL of methanol) were recorded with a Shimadzu UV-260 spectrophotometer (Kyoto, Japan).

Radical Scavenging Activity (RSA) of Crude Seed Oils and Oil Fractions. Different solvents were used to assay the RSA of seed oils and their fractions, whereas the best results were achieved with toluene, which was able to dissolve completely the hydrophobic and the hydrophilic compounds of the different oils under study. Therefore, the RSA of the crude seed oils and their fractions was assayed with DPPH and galvinoxyl radicals previously dissolved in toluene.

Radical Scavenging Activity toward DPPH Radical (Spectrophotometric Assay). RSA and the presence of hydrogen donors in crude seed oils and oil fractions were examined by reduction of DPPH in toluene. A toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M. The radical, in the absence of antioxidant compounds, was stable for more than 2 h of normal kinetic assay. For evaluation, 10 mg of crude seed oils or their fractions (in 100 μL of toluene) was mixed with 390 μL of toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 1, 30, and 60 min of mixing, using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control) and the inhibition percent was calculated according to Lee et al. (*9*) from the following equation:

$$\%\ \text{inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample})/\text{absorbance of control}] \times 100$$

Radical Scavenging Activity toward Galvinoxyl Radical (Spectrometric Assay). A miniscope MS 100 ESR spectrometer (Magnettech

GmbH; Berlin, Germany) was used throughout the analysis. Experimental conditions were as follows: measurement at room temperature; microwave power, 6 db; centerfield, 3397 G, sweep width, 83 G;, receiver gain, 10; and modulation amplitude, 2000 mG. Ten milligrams of crude seed oils or their fractions (in 100 μL of toluene) was allowed to react with 100 μL of a toluenic solution of galvioxyl (0.125 mM). The mixture was stirred on a vortex stirrer for 20 s then transferred into a 50-μL micropipet (Hirschmann Laborgeräte GmbH, Ederstadt, Germany) for ESR analysis. The amount of galvinoxyl radical inhibited was measured exactly 60 s after the addition of the galvinoxyl radical solution. The galvinoxyl signal intensities were evaluated by the peak height of signals against a control. Further ESR spectra have been recorded in intervals of 90 s for a total incubation time of 60 min. A quantitative estimation of the radical concentration was obtained by evaluating the decrease of the ESR signals in arbitrary units between 1 and 60 min incubation, using the KinetikShow 1.06 Software program (Magnettech GmbH; Berlin, Germany).

Characterization of Maillard Reaction Products (MRP). The influence of extraction conditions on forming MRP was evaluated spectrophotometrically. Samples of 20 mg from hot-extracted (8 h at 70 °C) and cold-extracted (8 h at room temperature) seed oils and their fractions were dissolved in 3 mL of chloroform for spectrophotometric measurements of color changes. According to King et al. (*27*) color intensity, as an indicator for formation of MRP, was measured at 430 nm with a Shimadzu recording spectrophotometer UV-260 (Kyoto, Japan).

Experiments were always performed on freshly made up solutions, wherein all tests were conducted in triplicate and averaged. No statistically significant difference ($P > 0.05$) was found among the experiments.

RESULTS AND DISCUSSION

Natural antioxidants allow food processors to produce stable products with clean labels and tout all-natural ingredients. The tests expressing antioxidant potency can be categorized into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions. However, the model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods (*40*). Pervious study on radical scavenging properties of commercial refined seed oils had used different solvents to dissolve the oil fractions and the free radicals (*7*). Hence, the results were difficult to compare because the reactions occurred under different conditions. Different solvents may have caused differences in the antioxidant pattern between the groups of assays, since it has been shown that the solvent may affect the hydrogen-donating ability of the antioxidant. Moreover, if the test was performed in polar medium the effectiveness of polar antioxidants can be different, as described by the "polar paradox", which states that lipophilic antioxidants are more effective in polar media, while polar antioxidants are more active in lipophilic media (*40*). In contrast, our experiment has been performed with the same solvent (toluene) to dissolve the oil samples and the free radicals. This allowed us to characterize and compare the RSA of all samples under the same conditions.

Radical Scavenging Activity (RSA) of Crude Seed Oils. Crude vegetable oils are usually oxidatively more stable than their processed counterparts. A part of their oxidative stability depends on the fatty acid composition, the presence of minor fat-soluble bioactives, and the initial amount of hydroperoxides. Antiradical properties of the crude oils under study were compared by using two different stable free radicals (DPPH and galvinoxyl). Both ESR and spectrophotometric assays showed similar trends in the quenching of free radicals. **Figures 1** and **2** show that coriander seed oil has the highest RSA followed by black cumin and niger seed oils, respectively. After 1 h of incubation, 35% of DPPH radicals was quenched by coriander seed oil, while black cumin and niger seed oils were able to quench 25.1% and 14.0%, respectively. ESR measurements showed also the same pattern, when coriander, black cumin, and niger crude seed oils quenched 32.4%, 23.3%, and 12.8% of galvinoxyl radical, respectively. Statistics regarding the composition of crude seed oils (mean and standard deviation SD) are given in **Table 1**. The three seed oils have different fatty acid pattern. Petrocelinic acid (C18:1$n-12$) is the fatty acid marker of coriander seed oil (67% of total fatty acids), while linoleic (C18:2$n-6$) and oleic (C18:1$n-9$) are the major fatty acids in black cumin and niger seed oils. Niger seed oil contains a significant amount of polyunsaturated fatty acids (PUFA) and black cumin seed oil is characterized by a relatively high content of the monounsaturated oleic acid (*33*, *34*, *37*). The ratio of monosaturated to polyunsaturated fatty acids was 4.10, 0.40, and 0.17 for coriander, black cumin, and niger seed oils, respectively. Generally, it is accepted that the higher the degree of unsaturation of an oil, the more susceptible it is to oxidative deterioration. Moreover, it was reported that oleic acid oxidizes at a rate 50 times slower than linoleic acid (*22*). Thus, the lowest RSA of niger seed oil could be partly explained by the fact that niger seed oil has the highest level of PUFA. Aside from fatty acid profile, factors such as oxygen concentration, metal contaminants, lipid hydroxy compounds, enzymes, and light may also influence the RSA of the oils. Since hydoperoxides are the primary products of lipid oxidation, the peroxide value (PV) provides a clear indication of the oxidative state of vegetable oils. On the basis of PV, the oxidative stability of several oils varied significantly, with the oil having the lowest initial PV being the most stable. Results of our investigation are in agreement with this phenomenon, wherein levels of primary oxidative products in crude oils correlated well with their RSA.

Among the substances with antioxidant properties, coriander seed oil contains as much as 21.8 g/kg of unsaponifiables

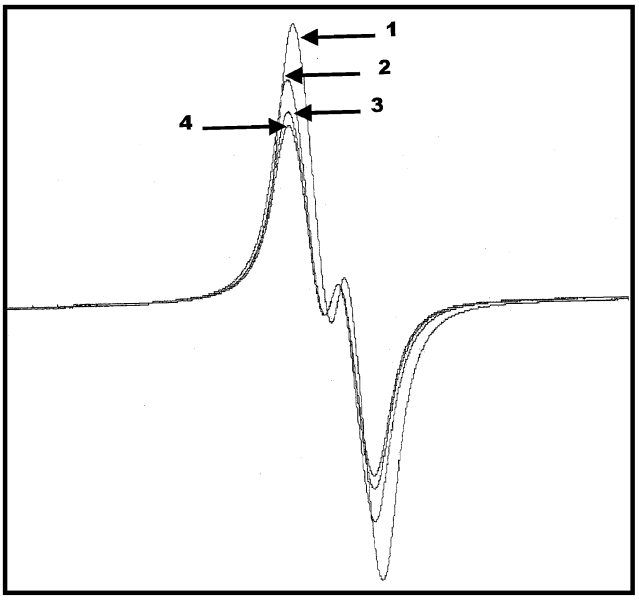

Figure 1. Electron spin resonance (ESR) spectra recorded with galvinoxyl radicals after 1 h of incubation. ESR conditions are described in the text. The scanning was started at 60 min after the mixing of samples with galvinoxyl in toluene. (1) ESR spectrum of galvinoxyl radical without oil sample. (2) ESR spectrum of galvinoxyl radical with niger seed oil. (3) ESR spectrum of galvinoxyl radical with black cumin seed oil. (4) ESR spectrum of galvinoxyl radical with coriander seed oil.

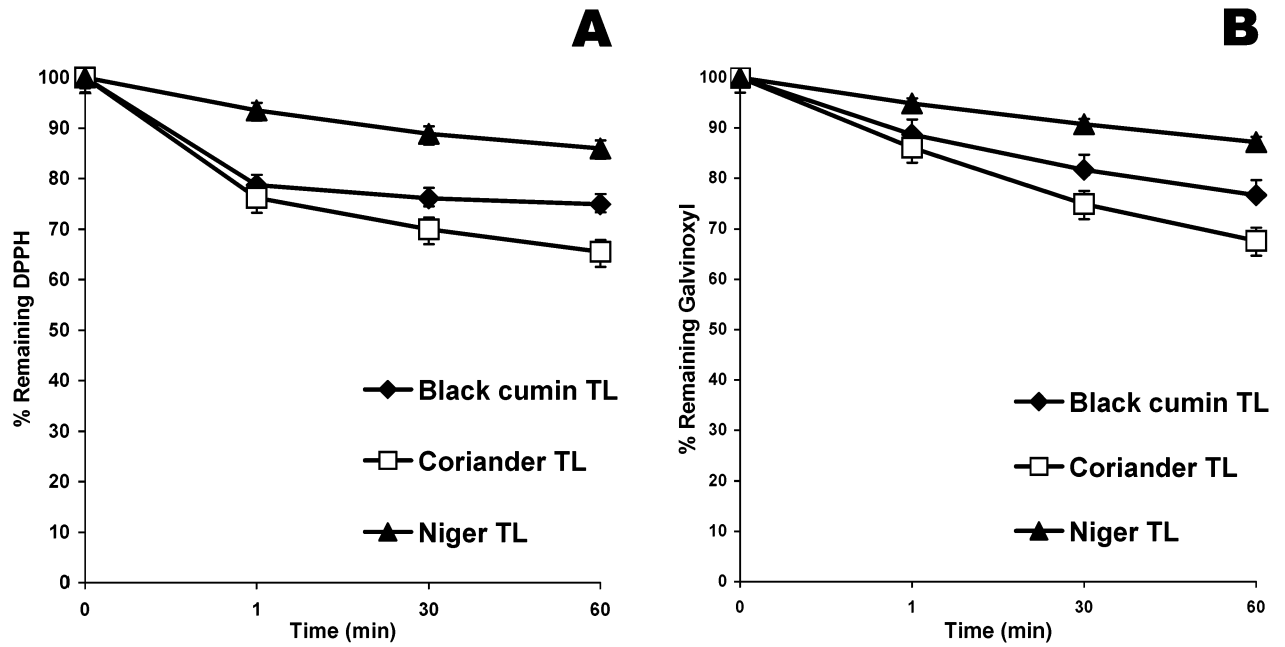

Figure 2. Scavenging effect at different incubation times of crude seed oils on (A) DPPH radical as measured by changes in absorbance values at 515 nm and on (B) galvinoxyl radical as recorded by ESR. Error bars show the variations of three determinations in terms of standard deviation.

Table 1. Analysis of Fatty Acids (As a Percentage of Total Fatty Acids), Initial Peroxide Values (PV), Unsaponifiables, and Total Phenolics of Crude Seed Oils[a]

	black cumin seed oil	coriander seed oil	niger seed oil		black cumin seed oil	coriander seed oil	niger seed oil
C16:0	13.0 ± 0.03	5.54 ± 0.02	17.0 ± 0.37	PV (mequiv/kg)	17.8 ± 0.12	2.68 ± 0.05	17.9 ± 0.10
C18:0	3.16 ± 0.01	1.36 ± 0.01	6.52 ± 0.08	α-tocopherol (g/kg)	0.284 ± 0.01	0.086 ± 0.01	0.861 ± 0.02
C18:1*n*–12	nd[b]	67.0 ± 0.98	nd	β-tocopherol (g/kg)	0.040 ± 0.01	0.672 ± 0.02	0.331 ± 0.01
C18:1*n*–9	24.1 ± 0.03	7.86 ± 0.13	11.2 ± 0.26	γ-tocopherol (g/kg)	0.225 ± 0.02	0.162 ± 0.01	0.570 ± 0.04
C18:2*n*–6	57.3 ± 0.04	15.9 ± 0.02	63.0 ± 0.15	δ-tocopherol (g/kg)	0.048 ± 0.01	0.347 ± 0.02	0.185 ± 0.02
C18:3*n*–6	nd	1.00	nd	β-carotene (g/kg)	0.593 ± 0.03	0.892 ± 0.05	0.702 ± 0.03
C20:2*n*–6	2.44 ± 0.01	nd	nd	ergosterol (g/kg)	nd[b]	0.186 ± 0.01	nd[b]
C22:0	nd	nd	0.52 ± 0.01	campesterol (g/kg)	0.226 ± 0.01	0.735 ± 0.03	0.713 ± 0.06
C22:1*n*–9	nd	0.77 ± 0.01	nd	stigmasterol (g/kg)	0.314 ± 0.02	1.512 ± 0.07	0.667 ± 0.02
C20:5*n*–3	nd	nd	1.72 ± 0.02	lanosterol (g/kg)	0.106 ± 0.01	0.152 ± 0.01	0.113 ± 0.02
C22:6*n*–3	nd	0.57 ± 0.01	nd	β-sitosterol (g/kg)	1.182 ± 0.05	1.553 ± 0.05	2.035 ± 0.08
∑SFA[c]	16.1 ± 0.02	6.90 ± 0.05	24.0 ± 0.08	Δ5-avenasterol (g/kg)	1.025 ± 0.04	1.466 ± 0.03	0.530 ± 0.06
∑MUFA[d]	24.1 ± 0.03	74.8 ± 0.25	11.2 ± 0.06	Δ7-avenasterol (g/kg)	0.809 ± 0.02	0.365 ± 0.02	0.164 ± 0.01
∑PUFA[e]	59.7 ± 0.31	18.2 ± 0.07	64.7 ± 0.11	total unsaponifiables (g/kg)	14.9 ± 0.09	21.8 ± 0.15	10.1 ± 0.07
S/P[f]	0.269 ± 0.01	0.379 ± 0.01	0.370 ± 0.01	total phenolics (ppm caffeic acid)	24 ± 0.11	11 ± 0.06	5 ± 0.03

[a] Values given are the mean of three replicates ± standard deviation. [b] nd = not detected. [c] Total saturated fatty acids. [d] Total monounsaturated fatty acids. [e] Total polyunsaturated fatty acids. [f] The ratio of saturated to polyunsaturated fatty acids.

followed by black cumin seed oil (14.9 g/kg), whereas niger seed oil comprised the lowest amount (10.1 g/kg). Amounts of unsaponifiable matter in crude oils correlate with their RSA. Preliminary screening of the tocopherols in seed oils showed that the α-isomer followed by the γ-isomer were the predominant tocopherols in niger seed oil and a similar distribution of tocopherols was determined in black cumin seed oil (*32*). The amounts of tocopherols in niger seed oil were 3.28- and 1.54-fold higher than those of black cumin and coriander seed oils, respectively. Surprisingly, no correlation was noted between RSA and the levels of tocopherols in oils. Although tocopherols, traditional antioxidants in oils, have been highly effective as active free-radical destroyers, such effectiveness has been known to be greatly influenced by the type and concentration of the fat (*41*). The vitamin E scavenging effect is probably overwhelmed by the amount of radical formed from PUFA, which is reflected in the highest initial PV of niger seed oil. α-Tocopherol was not as good an antioxidant for the prevention of hydroperoxide formation as the other phenolic compounds. In addition, α-tocopherol at high concentrations showed a prooxidant effect, a fact already reported by other investigators in bulk oil and in free fatty acid systems (*42*, *43*). β-Carotene was detected in approximately equal amounts in the crude oils. It is accepted that carotenoids can act as primary antioxidants by trapping free radicals or as secondary antioxidants by quenching singlet oxygen. Moreover, combinations of carotenoids and tocopherols act synergistically (*2*, *44*). On the other side, phytosterols accounted for 5.97, 4.22, and 3.66 g/kg of coriander, niger, and black cumin seed oils, respectively. A proportional correlation was found between sterol levels in seed oils and their RSA. Sterols have been documented to have antioxidant activity by interaction with oil surfaces and inhibit oxidation. Moreover, they may oxidized at oil surfaces and inhibit propagation by acting as hydrogen donors (*2*).

Another finding to be noted is the polar lipid profile of seed oils. Crude seed oils were reported to be constantly more stable

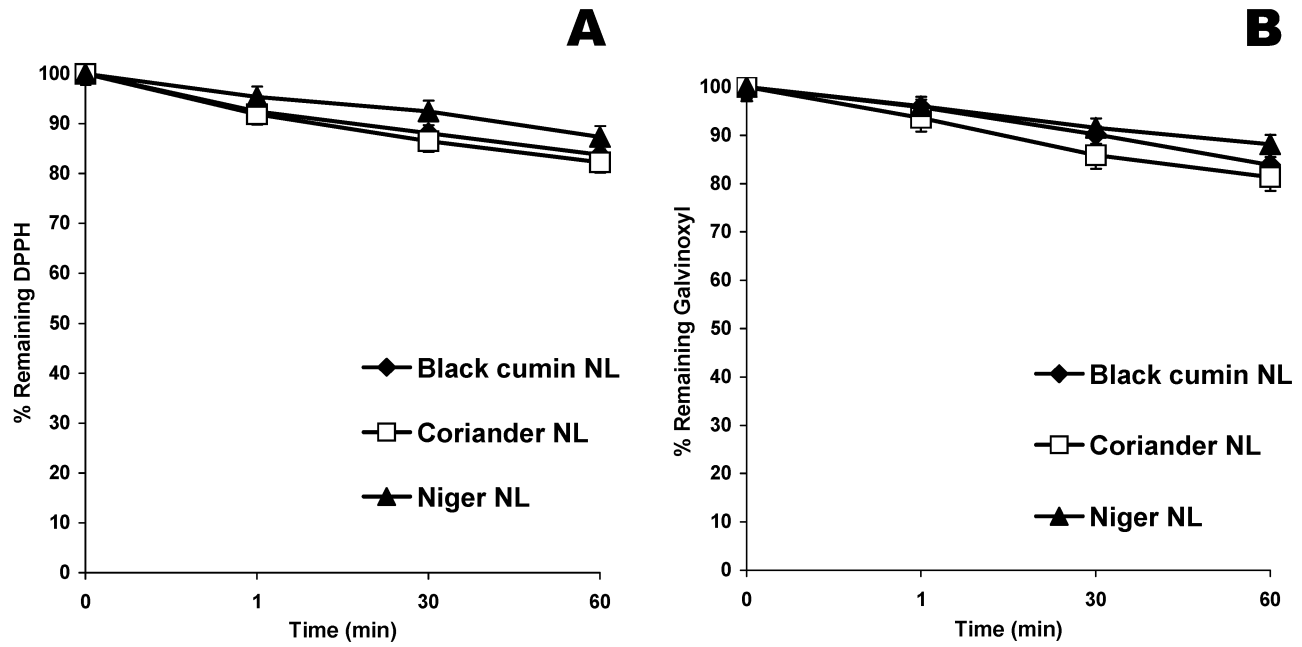

Figure 3. Scavenging effect at different incubation times of neutral lipids on (A) DPPH radical as measured by changes in absorbance values at 515 nm and on (B) galvinoxyl radical as recorded by ESR. Error bars show the variations of three determinations in terms of standard deviation.

than processed oils due to the presence of phosphorus-containing compounds (*45*). A positive correlation was found between the RSA of seed oils and the total PL content. Crude oils were characterized by a considerable amount of polar lipids, wherein GL was the main polar lipid fraction followed by PL (*33*−*35*, *37*). Polar lipids were found in their highest level in coriander seed oil followed by black cumin and niger seed oils, respectively. The amine group of phosphatidylethanolamine and phosphatidylcholine as well as the reducing sugar of phosphatidylinositol can apparently all facilitate hydrogen or electron donation to tocopherols. Hence, PL could extend the effectiveness of tocopherols by delaying the irreversible oxidation of tocopherols to tocopheryl quinone, thereby delaying the oxidation (*17*, *46*). The higher RSA of coriander and black cumin seed oils also can be attributed to their high amount of polar lipids, which could act synergistically toward tocopherols, enhancing their activity. Therefore, the level of polar lipids, unsaponifiables, and the initial PV might be the major factors affecting the RSA of crude oils. Phenols make up a part of the "polar fraction" of vegetable oils. Since crude seed oils are not refined, the phenolics are partly preserved and these compounds are reportedly responsible for their RSA. The total phenolics level of black cumin seed oil was 5-fold higher than that of niger seed oil and 2-fold higher than that of coriander seed oil (**Table 1**). The antiradical action of black cumin seed oil, which comprised the highest level of phenols, was considerably higher than that of the niger seed oil. However, the RSA of the different seed oils do not correlate directly with the amounts of phenolics. It was mentioned that oil stability is correlated not only with the total amount of phenolics, but also with the presence of selected phenols (*16*). To assist in characterizing phenolic compounds, absorption ranges were scanned between 200 and 400 nm. The UV spectra of methanolic solutions of coriander phenolics exhibited two absorption maxima (282 and 320 nm), whereas those of black cumin and niger seeds displayed one maximum at (280 nm). The absorption maximum at the longer wavelength (320 nm) may be due to the presence of phenolic acids, while the absorption maximum at the shorter wavelength (280 nm) may be due to the presence of *p*-hydroxybenzoic acid and flavone/flavonol derivatives (*4*, *22*). Last, it could be said

Table 2. Levels of Oil Fractions (g/kg) and Their Ratios of Saturated to Polyunsaturated Fatty Acids (S/P)[a]

oil fraction	black cumin seed oil	coriander seed oil	niger seed oil
neutral lipids	972 ± 3.03	960 ± 2.55	970 ± 3.22
S/P	0.246 ± 0.01	0.264 ± 0.02	0.359 ± 0.01
glycolipids	21.8 ± 0.39	23.9 ± 0.27	19.0 ± 0.25
S/P	0.383 ± 0.01	0.506 ± 0.01	0.496 ± 0.02
phospholipids	3.20 ± 0.04	8.50 ± 0.05	2.80 ± 0.01
S/P	0.571 ± 0.01	0.714 ± 0.03	0.477 ± 0.01

[a] Values given are the mean of three replicates ± standard deviation.

that the RSA of crude seed oils can be interpreted as the combined action of different endogenous antioxidants. However, when polar fractions, which contain mainly polar lipids and in low level phenolics, are found in high levels, strong RSA of these components can be expected as well as synergistic activity with primary antioxidants. The significantly stronger RSA of coriander seed oil compared to black cumin and niger oils may be due to (i) the differences in content and composition of polar lipids and unsaponifiables, (ii) the diversity in structural characteristics of potential phenolic antioxidants present in crude oil fractions, (iii) a synergism of polar lipids with other components present in each fraction, and (iv) different kinetic behaviors of potential antioxidants. All these factors may contribute to the radical quenching efficiency of crude seed oils.

Radical Scavenging Activity (RSA) of Seed Oil Fractions. Crude seed oils were sequentially fractionated with chloroform to recover NL, followed by acetone to recover GL then methanol to recover PL. The importance of studying oil fractions is reflected in the utilization of each fraction in the industry. As expected in seed oils, NLs (constituted mainly of triacylglycerols) were the major oil fraction followed by GL and PL, respectively (**Table 2**). In coriander and niger seed oils, glucocerebroside, steryl glucoside, and acylated steryl glucoside were the GL subfractions. In addition to the later subfractions, diglucosyldiacylglycerol, monoglucosyldiacylglycero,l and sulfoquinovosyldiacylglycerol were detected in black cumin seed oil (*35*). The PL pattern was similar in the three seed oils,

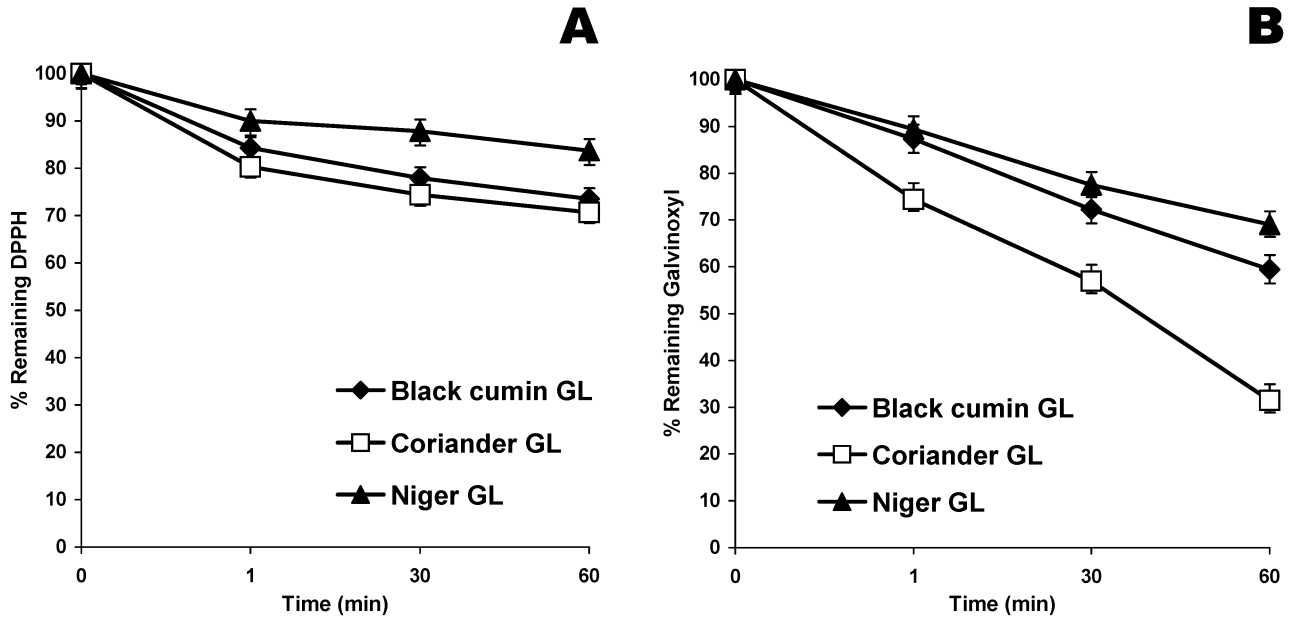

Figure 4. Scavenging effect at different incubation times of glycolipids on (A) DPPH radical as measured by changes in absorbance values at 515 nm and on (B) galvinoxyl radical as recorded by ESR. Error bars show the variations of three determinations in terms of standard deviation.

wherein phosphatidylcholine (45−50% of total PL) followed by phosphatidylethanolamine (22−25% of total PL) comprised the major components. Phosphatidylinositol and phosphatidylserine were estimated in relatively equal amounts (*33*, *34*, *37*). The ratio of saturated to polyunsaturated fatty acids (S/P) in the seed oil fractions is summarized in **Table 2**. The fractions had a rather similar S/P pattern wherein the ratio increased with the increase of the polarity of the oil fraction. It is also worthy to mention that the S/P ratio recorded the highest level in the polar fractions (GL and PL) of coriander seed oil. The oil fractions acquired with different solvents showed increased RSA when the polarity of solvent applied increased. Among the three oils, PL fractions exhibited the lowest yield and the highest RSA. Both radical scavenging assays exhibited the same results (**Figures 3**, **4**, and **5**), wherein PL possess the highest RSA followed by GL and NL, respectively. Inhibition of the DPPH radical was 28.6%, 26.5%, and 16.2% when PL, GL, and NL fractions from black cumin seed oils were assayed. **Figure 3** shows the RSA of NL measured toward DPPH galvinoxyl radicals. The results showed again that the RSA of NL seems to be affected by the level of PUFA and the initial PV. In general, in all oil fractions niger had a much weaker RSA compared to black cumin and coriander. For example, after 60 min, the PL fraction of coriander seed oil quenched 98.8% of the galvinoxyl radical, while PL fractions of black cumin and niger quenched 83.7% and 62.2%, respectively.

Polar lipids, namely GL and PL, occur normally at low levels in freshly extracted edible oils and their partial removal by degumming is usually regarded as an essential first step in refining. Numerous studies have been focused on the antioxidant properties of PL (*8*, *19*−*21*, *27*, *47*). The radical quenching property of GL is so far not reported in the published literature. Hence, we may have for the first time definitively established the antioxidant properties of GL in crude seed oils. It may be expected that the reducing sugars in all GL subfractions and the sterol moiety in steryl glucoside enhance the RSA of GL (**Figure 4**). Moreover, less polar phenolic compounds that have been extracted with GL may be responsible for the strong antiradical action of these acetone fractions. In comparison, **Figure 5** shows the effect of PL fractions on the stable radicals. It is clear that PL fractions possess superior RSA compared with GL and NL fractions. The fatty acid composition of individual PL subfractions may play an important role in the RSA of PL. It was observed that the RSA of PL was highly correlated with the degree of fatty acid saturation, wherein the higher the S/P ratio the stronger the RSA (**Table 2**). Recently, Boyd (*47*) reported that the ability of PL to stabilize lipids is affected by the chain length and degree of saturation of the fatty acids on the PL. Those PL with longer chain length and PL containing more saturated fatty acid are the most effective antioxidants. It is likely that antioxidant activity differs among the various PL fractions as a result of the wide variance in functional groups, structures, and fatty acid composition (*48*). Though the exact mechanism of action of PL is still not fully established, four postulates have been proposed to explain their antioxidant activity: (i) synergism between PL and tocopherols (*49*); (ii) chelation of pro-oxidant metals by phosphate groups (*50*); (iii) formation of Maillard-type products between PL and oxidation products (*51*); and (iv) action as an oxygen barrier between oil/air interfaces (*52*).

Influence of Extraction Conditions on the Color Intensity of Seed Oils and Their Fractions. Maillard reaction products (MRP) are an excellent example of natural process-induced oxidation inhibitors that arise as a result of thermal treatment (*53*). They are produced from the reaction of amines and reducing sugars. Lipids, vitamins, and other food constituents containing amino groups also participate in Maillard reactions. Fluorescence in lipophosphatidic materials has been found to be directly proportional to the amount of brown color formed by heating. It was suggested that the brown color and fluorescence are similar to those found in egg lipids (*24*) and they probably result from amine carbonyl reactions between phosphatidylethanolamine and carbonyl groups in sugars or oxidized fatty acids. The brownish yellow color was considered evidence for Maillard-type browning reactions or a polymer retaining the structure of the original lecithin, wherein the carbonyl groups of oxidized fatty acids condensed in an aldol reaction catalyzed by the phosphorylcholine group of phosphatidylcholine (*54*). MRP have been shown to have antioxidant activity in model systems as well as in some fat-containing foods (*2*). Because of the conflicting views present in the literature, it is difficult to state conclusively which of the numerous MRP are actually

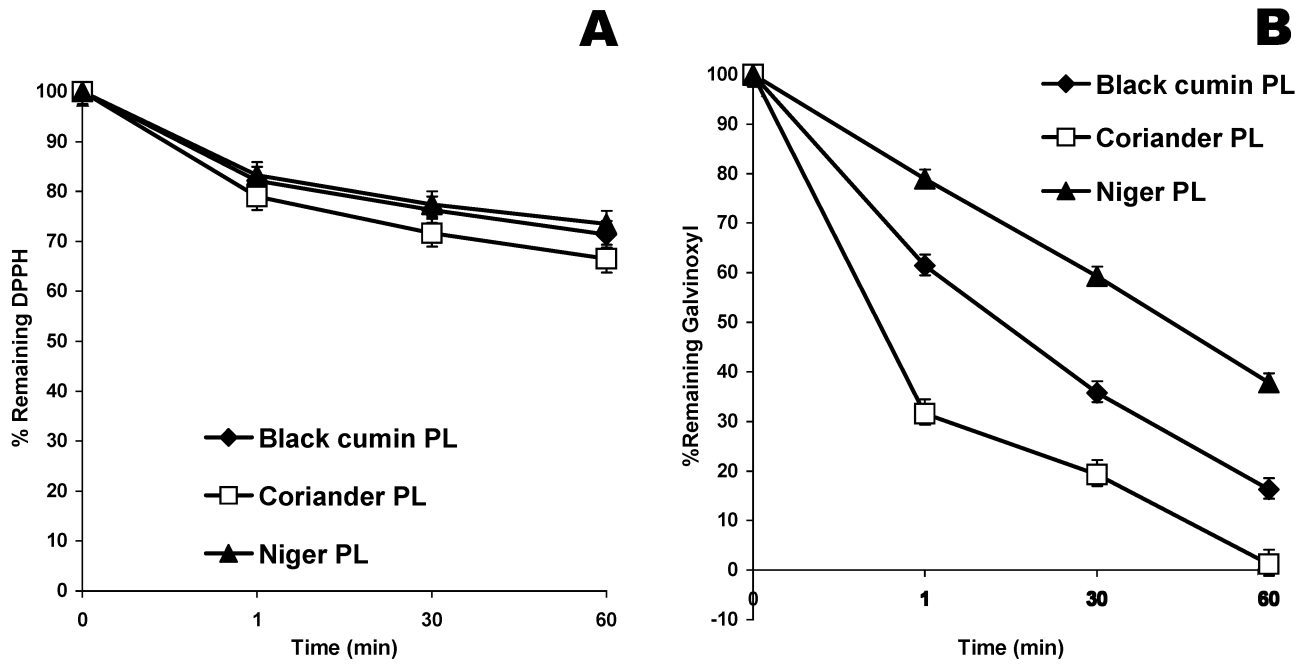

Figure 5. Scavenging effect at different incubation times of phospholipids on (A) DPPH radical as measured by changes in absorbance values at 515 nm and on (B) galvinoxyl radical as recorded by ESR. Error bars show the variations of three determinations in terms of standard deviation.

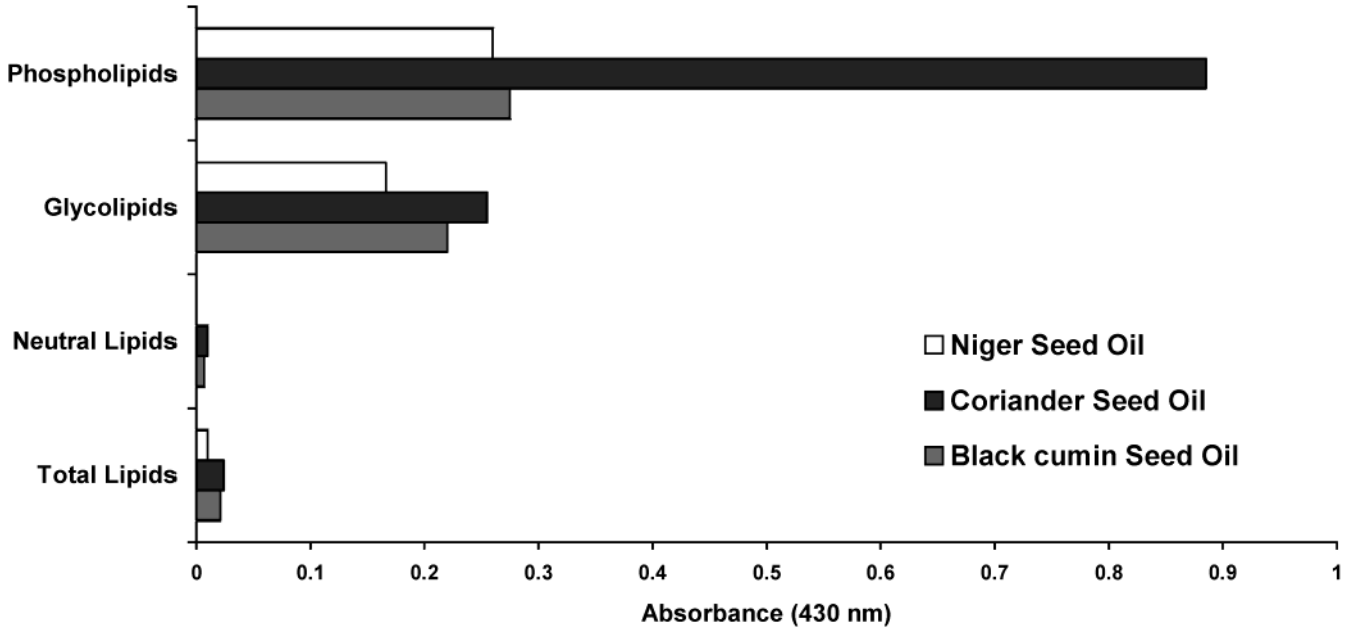

Figure 6. Absorbance values measured at 430 nm of crude seed oils [extracted with *n*-hexane (70 °C for 8 h)] and their fractions.

responsible for antioxidant activity. It is even more difficult to attempt to describe the mechanism of action of these suspected antioxidants. Theories on the mechanism of antioxidant activity of MRP conflict as well. Kawakishi et al. (*55*) hypothesized that the protective effects of melanoidins against autoxidation were likely to depend on their ability to chelate metals.

Since King et al. (*27*) have reported on the relationship between the antioxidant properties of PL and the formation of MRP, this study investigated the relationship between color intensity of crude seed oils and their RSA. Although the formation of Melano-PL in a hexane miscella of soybean was reported (*25*), no further studies have been performed in this field. It could be said that the temperature (70 °C) and extended incubation time (8 h) used throughout the solvent extraction may favor the formation of MRP. On the other hand, cold extraction (8 h at room temperature) did not demonstrate the presence of MRP. Under thermal conditions, furthermore, the formation of MRP was time dependent (data not shown). The color intensity showed a high positive correlation with RSA of seed oils, indicative of the formation of MRP during hot extraction. These colored compounds have been reported to form Melano-PL, which has the ability to inactivate hydroperoxides formed during oxidation (*27*, *51*, *56*). Ultraviolet characterization at 430 nm of crude seed oils and their fractions demonstrates that the hot extraction induced a high level of MRP in the polar lipid fraction (**Figure 6**). It was clear that PL possess the highest level of MRP followed by GL, while in NL it was hard to characterize any. Moreover, the increasing color intensity of seed oils appears to have been dependent on the polar lipid concentrations. These results indicate that an increase in the

formation of MRP was contributing to increased RSA. Since formation of MRP most likely depends on the processing of crude vegetable oils and the total content of polar lipids, the color intensity of crude seed oil may be used as a quality criterion.

CONCLUSION

Although black cumin, coriander, and niger seed oils have been part of a supplemental diet in many parts of the world and their consumption is also becoming increasingly popular in the nonproducer countries, information on the phytochemicals in these oils is limited. Yet these phytochemicals may bring nutraceutical and functional benefits to food systems. RSA of the crude seed oils has not been studied so far. Here we report, for the first time, on the RSA of black cumin, coriander, and niger seed oils. The RSA of crude seed oil can be interpreted as the combined action of endogenous antioxidants. However, any individual parameter could not alone explain the differences in the antioxidant properties. It could be said that RSA of crude oils is affect by the level of PUFA, the initial PV, and significantly the levels of unsaponifiables as well as polar lipids in seed oils. The preliminary finding of a higher RSA of coriander, in comparison with other oils, indicates that crude polar lipids are a potent source of antioxidant compounds. These qualities project the potential of polar fractions from seed oils as a natural antioxidant for use in lipid-containing foods. The double benefit derived from crude PL offers food manufacturers an alternative to adding synthetic antioxidants to formulations that need emulsification characteristics. However, it should be noted that the bleached PL (commercial lecithin) may have an elevated PV and could possibly act as a pro-oxidant in systems that do not contain any other antioxidant. Thus, commercial lecithin would not be expected to perform as effectively as the crude PL employed in these studies. The color intensity and the related MRP formed during extraction appears to be a good secondary index of the RSA of crude seed oils. In light of this evidence, these bioactive substance could have extranutritional properties and a novel role in diet−disease relationships. Additional studies are necessary to show the RSA under physiological conditions and to determine whether there is any link between their antiradical properties and their biological effects.

ABBREVIATIONS USED

RSA, radical scavenging activity; NL, neutral lipids; GL, glycolipids; PL, phospholipids; ESR, electron spin resonance; DPPH, 1,1-diphenyl-2-picrylhydrazyl; PV, peroxide value; PUFA, polyunsaturated fatty acids; Melano-PL, melanophospholipids; MRP, Maillard reaction products.

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