Characterization of Cold-Pressed Onion, Parsley, Cardamom, Mullein, Roasted Pumpkin, and Milk Thistle Seed Oils

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ABSTRACT: Cold-pressed onion, parsley, cardamom, mullein, roasted pumpkin, and milk thistle seed oils were characterized for their fatty acid (FA) composition, tocopherol content, carotenoid profile, total phenolic content (TPC), oxidative stability index (OSI), color, physical properties, and radical-scavenging capacities against peroxyl (oxygen radical-scavenging capacity) and stable DPPH (diphenylpicrylhydrazyl) radicals. Parsley seed oil had the highest oleic acid content, 81 g/100 g total FA, and the lowest saturated fat among the tested oils. Roasted pumpkin seed oil contained the highest level of total carotenoids, zeaxanthin, β-carotene, cryptoxanthin, and lutein at 71 μmol/kg and 28.5, 6.0, 4.9, and 0.3 mg/kg oil, respectively. Onion seed oil exhibited the highest levels of α- and total tocopherols under the experimental conditions. One of the parsley seed oils exhibited the strongest DPPH• scavenging capacity and the highest oxygen radical absorbance capacity (ORAC) value of 1098 μmol Trolox equiv/g oil. However, ORAC values of the tested seed oils were not necessarily correlated to their DPPH• scavenging capacities under the experimental conditions. The highest TPC of 3.4 mg gallic acid equiv/g oil was detected in one of the onion seed oils. The OSI values were 13.3, 16.9–31.4, 47.8, and 61.7 h for the milk thistle, onion, mullein, and roasted pumpkin seed oils, respectively. These data suggest that these seed oils may serve as dietary sources of special FA, tocopherols, carotenoids, phenolic compounds, and natural antioxidants.

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KEY WORDS: Antioxidant, carotenoid, fatty acid composition, OSI, phenolic, radical scavenging activity, seed oil, tocopherol.

Edible seed oils are a group of important food ingredients. Novel specialty seed oils rich in factors beneficial to health are in high demand because of consumers' interest in disease prevention and health promotion through improved diets. These beneficial factors include special FA compositions such as high content of monounsaturated FA (MUFA) or n-3 FA, tocopherols, carotenoids, and antioxidative phenolic compounds (1–4). A number of edible oils from herb, spice, and fruit seeds have been shown to contain special FA profiles (4). For instance, American ginseng seed oil contains about 87% oleic acid, and basil seed oil has 57–63% α-linolenic acid (18:3n-3), the essential n-3 FA (4). The cold-pressed edible seed oils may be preferred by consumers because the cold-pressing procedure involves neither heat nor chemicals, and may increase the retention of beneficial phytochemicals. Previous studies showed that cold-pressed carrot seed oil had about 82% oleic acid (18:1n-9), which has been associated with lowering the risk of cardiovascular disease (5). Cold-pressed edible hemp and berry fruit seed oils contain significant levels of α-linolenic acid (18:3n-3), which may be converted to the longer-chain n-3 PUFA, EPA (20:5n-3) and DHA (22:6n-3), *in vivo* through elongation and desaturation reactions. EPA and DHA potentially reduce the risk of cancer, heart disease, hypertension, and autoimmune disorders (6–8). These data suggest the possibility of developing novel edible seed oils with special FA composition for improving human nutrition.

Previous studies also showed that cold-pressed edible seed oils may contain significant levels of carotenoids and tocopherols (2). For instance, cold-pressed boysenberry seed oil exhibited a total carotenoid concentration of 30 μmol/kg oil (2). Cold-pressed blueberry, red raspberry, marionberry, and boysenberry seed oils also contained significant levels of α-, γ-, and δ-tocopherols at ranges of 21–151, 33–689, and 6–232 mg/kg oil, respectively (2). Tocopherols were also detected in raspberry, blackcurrant, and goldenberry seed oils (3,9,10). Carotenoids and tocopherols are well recognized for their potential health benefits. Characterization of carotenoid and tocopherol profiles in cold-pressed edible seed oils may provide a scientific basis to promote their consumption for improving human nutrition.

Additionally, previous studies detected significant levels of phenolic components in cold-pressed edible seed oils (2). Phenolic compounds have demonstrated powerful antioxidative potential and may reduce free radical-mediated cellular damage (2,3,11–13). The antioxidative activities of several cold-pressed edible seed oils have been studied (2,14). In 2005, Parry and co-workers (2) found considerable free radical-scavenging abilities in cold-pressed blueberry, red raspberry, marionberry, and boysenberry seed oil extracts against peroxyl and diphenylpicrylhydrazyl (DPPH) radicals. Boysenberry seed oil

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extract had an oxygen radical absorbance capacity (ORAC) of 77.9 Trolox equivalents, in micromoles, per gram of oil (μmol TE/g oil) (2). Cranberry seed oil has significant radical-scavenging activity against DPPH radicals (DPPH•) and 2,2′-azinobis(3-ethyl-benzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and also suppresses oxidation of human LDL (14). It is well accepted that antioxidants may protect important cellular components such as DNA and membrane lipids from oxidative damage and suppress the pathology of cancer, cardiovascular diseases, and other aging-associated health problems. Edible oils rich in natural antioxidants may play a role in reducing the risk of chronic diseases.

This research is part of our continuing efforts to search for novel cold-pressed edible seed oils rich in health-benefitting components. In the present study, cold-pressed extra virgin onion (*Allium cepa* L.), parsley (*Petroselinum crispum*), cardamom (*Elettaria cardamomum*), mullein (*Verbascum thapus*), roasted pumpkin (*Curcubita pepo*), and milk thistle (*Silibum marianum*) seed oils were investigated for their FA, tocopherol, and carotenoid compositions, total phenolic contents (TPC), antioxidant activities, oxidative stabilities, color, and physical properties. The oxidative stabilities, color, and physical properties are important characteristics related to the food utilization of these edible oils. The data obtained from this work will be used to promote the potential use of these oils in food products for improving human nutrition and health.

EXPERIMENTAL PROCEDURES

Materials. Cold-pressed extra virgin onion, parsley, cardamom, mullein, roasted pumpkin 'Triple Treat' variety, and milk thistle seed oils were gifts from the Badger Oil Company (Spooner, WI). All oil samples were freshly prepared and extractions were performed immediately after arrival. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO); β-cyclodextrin (RMCD) was purchased from Cyclolab R & D Ltd. (Budapest, Hungary), and 2,2′-azobis(2-amidino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). All other chemicals and solvents were of highest commercial grade and used without further purification.

Extraction and testing sample preparation. One gram of each cold-pressed edible seed oil was extracted with 3 mL of MeOH by vortexing at ambient temperature (2). The MeOH extracts were collected by centrifugation. The oil residues were re-extracted twice with MeOH (3 mL × 2). The three MeOH extracts were combined and the final volume was brought to 10 mL with MeOH to obtain the testing sample solutions. The solutions were kept in the dark under nitrogen until further analysis.

FA composition. FAME were prepared from oils according to the previously described method (2). The FAME samples were analyzed by GC–FID for FA compositions. GC analysis was conducted using a Shimadzu GC-2010 with a FID and a Shimadzu AOC-20i autosampler (Shimadzu, Columbia, MD). A Supelco (Bellefonte, PA) 2380 column, 30 m × 0.25 mm i.d. with a 0.20 μm film thickness, was used with helium as the carrier gas at a flow rate of 0.8 mL/min. Injection volume was 1 μL at a split ratio of 10:1. Initial oven temperature was 142ºC and was increased 6ºC/min to 184ºC and held for 3 min, then increased 6ºC/min to 244ºC. Identification of the individual FA was accomplished by comparing GC retention time with that of the authorized pure individual commercial standards.

Carotenoid composition. Concentrations of β-carotene, lutein, cryptoxanthin, and zeaxanthin were measured following a previously described method (2,15,16). Briefly, 1 mL of cold-pressed seed oil was dissolved in 160 mL of methanol/THF (1:1, vol/vol) and analyzed for carotenoid profile using HPLC-DAD-ESI-MSMS (high-performance liquid chromatography–diode array detector–electron spray ionization–tandem mass spectrometry). A TSQ quantum tandem mass spectrometer (Thermo-Finnigan, San Jose, CA) was equipped with an ESI interface, and an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) with a Zorbax SB C18 column, 50 mm × 1.0 mm i.d. with a 3.5-μm particle size (Agilent Technologies) was used to separate the carotenoid compounds. Identification of the individual components was accomplished by comparing HPLC retention time and selected reactant monitoring (SRM) analyses of the sample peaks with that of the authorized pure individual commercial compounds. Quantifications for the carotenoid and tocopherol compounds were conducted using the total ion counts with an external standard. Data were obtained using Xcalibur software system (Thermo-Finnigan).

Tocopherol profile. The methanol/THF solutions prepared for carotenoid composition were also used to quantify α-, δ-, and γ-tocopherol concentrations by a previously described method (2). HPLC with a Zorbax SB C18 column, 30 mm × 1.0 mm i.d with a 3.5-μm particle size (Agilent Technologies), was used to separate the tocopherols. The individual tocopherols were identified by peak retention time and SRM with those of the pure commercial compounds. Quantification was determined using the total ion counts with external standards of the individual compounds.

TPC. The Folin-Ciocalteu (FC) reagent was used to determine the TPC of the cold-pressed seed oils following a laboratory procedure previously described by Yu and others (17). Briefly, the reaction mixture contained 250 μL of fresh FC reagent, 750 μL of 20% Na_2CO_3, and 3 mL of pure H_2O to which 50 μL of oil extract or standard was added to start the reaction. Absorbance was determined at 765 nm after 2 h of reaction at ambient temperature and used to calculate the phenolic contents in oils. Gallic acid was used as the standard. The FC reagent was freshly prepared by refluxing a mixture of sodium molybdate, sodium tungstate, 85% phosphoric acid, and concentrated HCl for 10 h. This was followed by reaction with lithium sulfate, and oxidation with a few drops of bromine (18). The resulting solution was then filtered and ready for assay.

ORAC. ORAC was determined using the protocol previously described (18,19). Fluorescein was used as the fluorescent probe.

The complete assay mixture contained 0.067 M of fluorescein, 60 mM of AAPH, and 300 μL of oil extract or of MeOH for the reagent blank. The fluorescence of an assay mixture was recorded every minute, and the area under the curve of fluorescence vs. time was calculated and compared against a standard curve prepared with Trolox.

DPPH• scavenging activity. The DPPH• scavenging capacities of the cold-pressed seed oil extracts were analyzed following a previously described procedure using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH•) (17). A freshly prepared DPPH•/MeOH solution was mixed with seed oil extracts at concentrations of 10, 12.5, 16.7, and 25 mg oil equiv/mL to start the radical–antioxidant reaction. The final concentration was 100 μM for DPPH• and the final reaction volume was 2.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 0.67, 3, 6, 10, 20, 50, 80, and 1440 min of reaction and used to estimate the remaining radical levels according to the standard curve. The absorbance at 517 nm at 10 min of reaction was used to compare the DPPH• scavenging capacities of individual oil extracts at 40 mg oil equiv/mL. The dose and time dependencies of cold-pressed seed oil extracts and DPPH• reactions were demonstrated by plotting the percentage of DPPH• remaining against time for each level of the seed oil extract tested.

Oxidative stability index (OSI). The OSI of each cold-pressed edible seed oil was examined using a Rancimat instrument (Model 743; Metrohm Ltd., Herisau, Switzerland). Briefly, 4 mL of oil was placed in the reaction vessel. The oxidation reaction was performed at 80°C with an air flow of 7 L/h (1). The OSI was determined as the time for an oil sample to develop a measurable rancidity. The OSI of the cold-pressed seed oils were compared with those of commercial corn and soybean oils.

Determination of refractive index and density. The refractive indices of the cold-pressed seed oils were determined at 24°C according to the *Official and Tentative Methods of the American Oil Chemists' Society* procedure Cc 7-25 (20) using an Abbé Refractometer (American Optical Corporation, Buffalo, NY). The specific densities were determined at 24°C against pure H_2O at 4°C according to the *Official and Tentative Methods of the American Oil Chemists' Society* procedure To 1b-64 (21).

Color. Oil colors were evaluated using a HunterLab ColorFlex spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA). Fifteen milliliters of each oil was pipetted into a sample cup, and color value was obtained using a D65/10° (daylight 65 illuminant/10° observer) setting.

Statistical analysis. Data were reported as mean ± SD (n = 3). ANOVA and LSD tests (SPSS for Windows, Version Rel. 10.0.5, 1999; SPSS Inc., Chicago, IL) were conducted to identify differences among means, whereas a Pearson Correlation test was conducted to determine the correlations among means. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

FA composition. Cold-pressed parsley seed oils contained over 92% unsaturated FA, most of which was oleic acid (18:1n-9) at a level of 81 g per 100 g total FA (Table 1). The ratio of oleic to linoleic acid was about 7.4:1 which is a little higher than the 6.6:1 reported for parsley seed oil previously (22). Gunstone (22) previously reported parsley seed oil to contain 81.9% oleic acid, with an oleic to linoleic acid ratio of 6.6:1. The concentrations of oleic acid in the parsley seed oils were significantly higher than the concentrations commonly found in olive oil, which normally range between 68 and 73% (23). It has recently been observed that hamsters fed diets rich in oleic acid had reduced atherosclerotic development compared with hamsters fed diets rich in linolenic acid and that they also had reduced aortic accumulation of oxidized LDL, which may be positively associated with the formation of fatty streaks, the earliest identifiable lesions of atherosclerosis (24). The U.S. Food and Drug Administration recently approved a new qualified health claim for olive oil relating its potential to reduce the risk of coronary heart disease (25), and it is believed that the high concentration of MUFA may contribute to these beneficial effects (26). These

TABLE 1
FA Compositions of the Studied Cold-Pressed Seed Oils[a] (g/100 g oil)

	Onion1	Onion2	Parsley1	Parsley2	Cardamom	Mullein	Pumpkin	Milk thistle
14:0	TR	ND	TR	TR	1.5 ± 0.05	TR	0.1 ± 0.01	0.1 ± 0.00
16:0	6.4 ± 0.01	7.1 ± 0.01	3.1 ± 0.01	3.1 ± 0.00	26.4 ± 0.13	6.0 ± 0.02	8.9 ± 0.01	8.9 ± 0.02
16:1	0.2 ± 0.01	0.2 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	1.6 ± 0.02	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.01
18:0	2.4 ± 0.00	1.8 ± 0.00	4.2 ± 0.01	4.2 ± 0.02	2.3 ± 0.04	2.7 ± 0.01	6.4 ± 0.01	4.8 ± 0.01
18:1	24.8 ± 0.02	26.0 ± 0.01	81.0 ± 0.04	80.9 ± 0.03	49.2 ± 0.22	16.1 ± 0.02	36.3 ± 0.01	23.8 ± 0.03
18:2	65.2 ± 0.03	64.0 ± 0.02	11.0 ± 0.01	11.0 ± 0.02	15.2 ± 0.04	73.1 ± 0.04	47.2 ±0.01	60.8 ± 0.03
18:3	0.1 ± 0.03	0.3 ± 0.02	0.5 ± 0.01	0.5 ± 0.02	2.7 ± 0.01	1.0 ± 0.00	0.2 ± 0.01	0.2 ± 0.03
20:0	0.3 ± 0.00	0.3 ± 0.00	0.1 ± 0.00	0.1 ± 0.01	0.4 ± 0.02	0.7 ± 0.00	0.5 ± 0.01	ND
20:1	0.4 ± 0.01	0.4 ± 0.02	ND	ND	0.5 ± 0.02	0.2 ± 0.00	0.4 ± 0.01	1.2 ± 0.06
SAT	9.3	9.3	7.4	7.5	30.8	9.4	15.9	13.8
MUFA	25.4	26.6	81.1	81.0	51.3	16.4	36.7	25.2
PUFA	65.3	64.2	11.5	11.6	17.9	74.2	47.4	61.1

[a]FA composition was reported as mean ± SD (n = 3). TR, trace; ND, not detected; SAT, total saturated FA (g/100 g oil); MUFA, total monounsaturated FA (g/100 g oil); PUFA, total polyunsaturated FA (g/100 g oil).

results suggest that parsley seed oil may be an excellent choice for consumers who prefer a diet rich in MUFA.

None of the tested seed oils contained significant levels of α-linolenic acid (18:3n-3), the essential n-3 FA. Onion, mullein, and milk thistle seed oils had high PUFA contents (Table 1), with linoleic acid (18:2n-6) as the primary FA. The biomechanical functions of PUFA are currently under extensive research including their influence/impact on cellular signaling and membrane structure; gene expression and prostaglandin biosynthesis; and nervous, endocrine, and immune system mediations (27). The cold-pressed onion seed oil had 64–65% linoleic acid, which is much higher than the 45% found in Indian onion seed oil (28). The oleic acid level in onion seed oil was 25–26%, which is lower than the 34% reported by Rao (28). Also, the level of 16:0 was higher, but that of 18:0 was lower, than previously reported in onion seed oil. The major FA in the cold-pressed roasted pumpkin seed oil were linoleic, oleic, palmitic, and stearic acids at levels of 47.2, 36.3, 8.9, and 6.4%, respectively. These four FA were the primary FA reported in pumpkin seed oils with concentrations of 54.6, 27.6, 5.43, and 12.4% for linoleic, oleic, palmitic and stearic acids, respectively (29). The differences in the FA compositions may be partially due to the variety and growing conditions of the onion seed samples used in the two studies. In addition, the total unsaturated FA content were 69.2, 90.5, 84.2, and 86.1% in the cold-pressed cardamom, mullein, roasted pumpkin, and milk thistle seed oils, respectively, whereas the ratios of oleic to linoleic acid were 0.4, 3.2, 0.2, 0.8, and 0.4 for the onion, cardamom, mullein, roasted pumpkin, and milk thistle seed oils, respectively (Table 1).

Carotenoids and tocopherols. Significant levels of carotenoids (Table 2) and tocopherols (Table 3) were detected in the cold-pressed seed oils. Zeaxanthin was the primary carotenoid compound present, although its level varied by over 1000-fold among the samples. The cold-pressed roasted pumpkin seed oil had the highest total carotenoid concentration, followed by the parsley and mullein seed oils. Their levels were comparable with those observed in cold-pressed red raspberry, blueberry, marionberry, and boysenberry seed oils (12.5–30.0 μmol/kg) reported by Parry and co-workers (2). The roasted pumpkin seed oil also had the highest β-carotene content of 5958 μg/kg, or 5481 μg/L, among all the tested oil samples, which was much higher than that observed in cold-pressed boysenberry (2405 μg/kg), blueberry (1352 μg/kg), marionberry (443 μg/kg), and red raspberry (82 μg/kg) seed oils (2). This value was also much higher than that of corn (1200 μg/L), soybean (280 μg/L), and peanut (130 μg/L) oils (30). These data suggest that cold-pressed roasted pumpkin, parsley, and mullein seed oils may serve as dietary sources of carotenoids, especially zeaxanthin.

Concentrations of α-, γ-, δ-, and total tocopherols from the oil samples are shown in Table 3. The two cold-pressed onion seed oils contained significantly higher amounts of α-tocopherol ($P < 0.0001$) and more than double the total tocopherols found in the other tested seed oils. The concentrations of α-tocopherol at 498 and 682 mg/kg onion seed oil are equal to 460 and 634 mg/L, respectively. These levels are higher than or comparable with those reported for commercial extra virgin olive, peanut, corn, and sunflower seed oils (174–578 mg/L), and higher than soybean oil (89 mg/L) (30), but much lower than that of wheat germ oil (1330 mg/kg oil) (31). They are also higher than those detected in cold-pressed blueberry, red raspberry, marionberry, and boysenberry seed oils (21–151 mg/kg oil) (2), and comparable with hexane-extracted and cold-pressed red raspberry seed oil that contained 710 and 460 mg/kg oil, respectively (9). The highest total tocopherol content was also observed in the onion seed oil at a level of 1.8–2.0 mmol/kg oil, which was comparable to the cold-pressed red raspberry and boysenberry seed oils (2.1 and 2.3 mmol/kg), respectively (*2*). Interestingly, these seed oils differed in their tocopherol isomer compositions (Table 3). The parsley seed oils had the highest ratios of 7.4:1 and 10.7:1 for α- to γ- tocopherol isomers, whereas mullein and roasted pumpkin seed oils had relatively higher γ-tocopherol levels with a ratio of 1:10 for α- to γ-isomers. The ratio of α- to γ-tocopherols ranged from 2.4 to 4.5 for cardamom, onion, and milk thistle seed oils. Mullein seed oil contained the highest level of δ-tocopherol among all tested seed oils; and the lowest δ-tocopherol content, less than 2 mg per kg oil, was observed in parsley and cardamom seed oils. In summary, the cold-pressed onion seed oil is a preferred dietary source for total, α-, and γ-tocopherols with significant level of δ-tocopherol, whereas the cold-pressed mullein and roasted pumpkin seed oils may serve as dietary

TABLE 2
Carotenoid Contents in the Cold-Pressed Seed Oils[a]

	β-Carotene (μg/kg)	Lutein (μg/kg)	Zeaxanthin (mg/kg)	Cryptoxanthin (mg/kg)	Total carotenoids (μmol/kg)
Onion1	ND	17.3 ± 0.3^{e}	1.74 ± 0.014^{d}	0.51 ± 0.001^{f}	4.01
Onion2	ND	17.9 ± 0.2^{e}	1.22 ± 0.004^{f}	0.75 ± 0.007^{e}	3.52
Parsley1	783.8 ± 4.2^{d}	216.4 ± 2.4^{b}	20.40 ± 0.128^{b}	1.43 ± 0.019^{b}	40.28
Parsley2	989.1 ± 12.5^{c}	207.1 ± 1.8^{c}	20.55 ± 0.104^{b}	1.20 ± 0.019^{d}	40.49
Cardamom	ND	ND	0.03 ± 0.000^{g}	ND	0.05
Mullein	1121.0 ± 8.7^{b}	62.1 ± 1.0^{d}	6.40 ± 0.079^{c}	1.30 ± 0.025^{c}	15.80
Pumpkin	5957.6 ± 108.2^{a}	270.1 ± 1.1^{a}	28.52 ± 0.508^{a}	4.91 ± 0.008^{a}	70.59
Milk thistle	ND	ND	1.31 ± 0.001^{e}	ND	2.30

[a]Carotenoid content of each cold-pressed seed oil was reported as mean ± SD ($n = 3$). Different letters within columns represent significant difference ($P < 0.05$). For abbreviation see Table 1.

TABLE 3
Tocopherol Contents in the Cold-Pressed Seed Oils[a]

	α-Tocopherol (mg/kg)	γ-Tocopherol (mg/kg)	δ-Tocopherol (mg/kg)	Total tocopherols (μmol/kg)
Onion1	681.9 ± 18.4[a]	219.2 ± 4.6[a]	28.6 ± 0.1[b]	1973.8
Onion2	498.1 ± 27.2[b]	156.3 ± 4.0[b]	23.0 ± 0.0[c]	1762.6
Parsley1	29.5 ± 0.3[d]	4.0 ± 0.0[e]	1.2 ± 0.0[g]	81.0
Parsley2	29.9 ± 0.3[d]	2.8 ± 0.2[f]	0.9 ± 0.0[h]	78.5
Cardamom	10.4 ± 0.0[f]	4.3 ± 0.1[d]	1.6 ± 0.0[f]	38.4
Mullein	27.1 ± 0.2[e]	213.3 ± 1.9[a]	76.2 ± 0.2[a]	759.4
Pumpkin	26.8 ± 0.9[e]	216.3 ± 2.4[a]	19.2 ± 0.0[d]	625.6
Milk thistle	156.3 ± 0.9[c]	35.1 ± 0.4[c]	7.0 ± 0.0[e]	464.2

[a]Tocopherol contents were reported as mean ± SD (n = 3). Different letters within columns represent significant difference ($P < 0.05$).

sources for γ-tocopherol. The cold-pressed mullein seed oil may also provide dietary δ-tocopherol.

TPC and antioxidant activities. The TPC of the cold-pressed seed oils ranged from 0.98 to 3.35 mg gallic acid equivalents per gram of oil (mg GAE/g oil) (Fig. 1). The TPC values of milk thistle, onion, and cardamom seed oils were higher than those in cold-pressed boysenberry, blueberry, red raspberry, and marionberry seed oils (1.5–2.0 mg GAE/g) (2). The TPC values from this study were much higher than the TPC values of cold-pressed black raspberry seed oils (0.04–0.09 mg GAE/g) (11). This difference may be explained by the different extraction solvents used in the two studies. Methanol is a preferred solvent for antioxidant extraction compared with 50% acetone or 70–80% methanol owing to its better solubilization of lipophilic antioxidants. Significant differences in TPC were observed within the two pairs of onion and parsley seed oils ($P < 0.05$), indicating the possible influence of seed quality and processing conditions. It is well accepted that genotype, growing conditions such as soil and temperature, interactions between genotype and growing conditions, post-harvesting treatments including the mechanical grinding during oil processing, and storage may significantly alter the chemical composition of selected botanical materials. Additional research is required to fully understand and explain the different TPC values between the two onion and parsley seed oils. The different TPC values may partially explain the relative radical scavenging capacities and oxidative stabilities of these cold-pressed edible seed oils.

ORAC. ORAC is a widely accepted measurement of free radical-scavenging capacity. ORAC values of the cold-pressed seed oil extracts are shown in Figures 2a and 2b. Parsley, cardamom, and milk thistle seed oils exhibited ORAC values over 100 μmol TE/g oil, whereas mullein, onion, and roasted pumpkin seed oils had ORAC values less than 30 μmol TE/g. Parsley seed oil had the highest ORAC value and was approximately 1000 times higher than that for roasted pumpkin seed oil. These

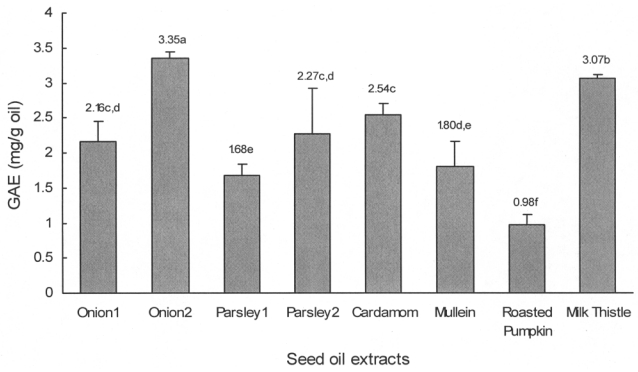

FIG. 1. Total phenolic contents of the cold-pressed seed oils. GAE, gallic acid equivalents. Error bars represent the SD (n = 3). Different letters indicate significant difference ($P < 0.05$).

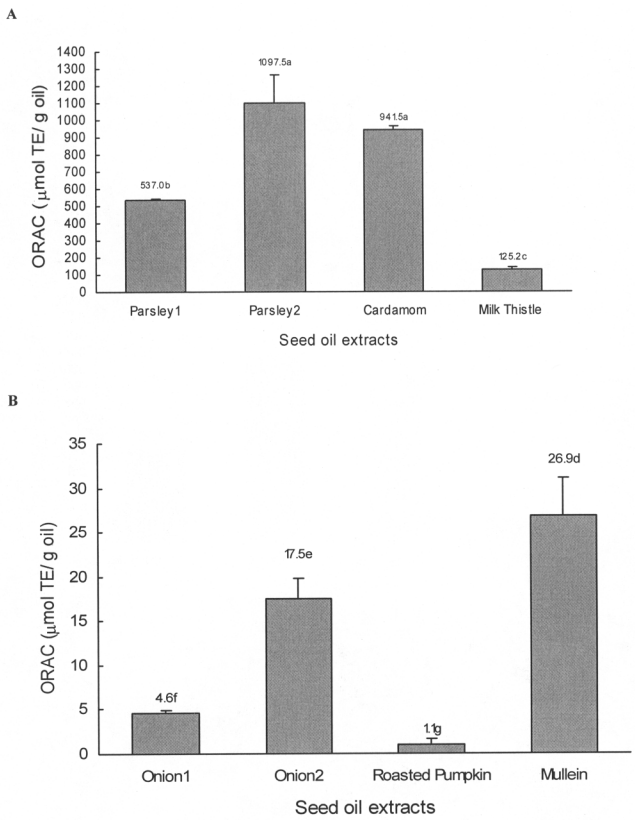

FIG. 2. Oxygen radical absorbance capacity (ORAC) values of the cold-pressed seed oils. TE, Trolox equivalents. Error bars represent the SD (n = 3). Different letters indicate significant difference ($P < 0.05$).

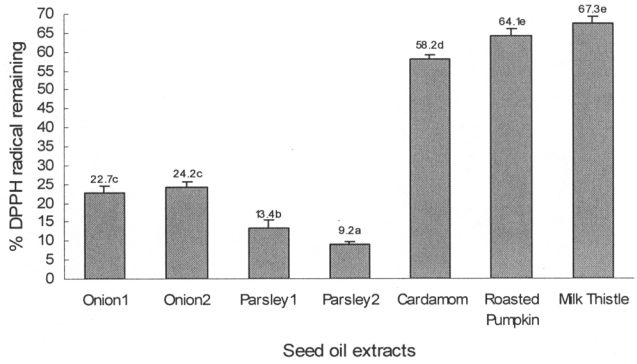

FIG. 3. Diphenylpicrylhydrazyl radical (DPPH•)-scavenging properties of the cold-pressed seed oils. The initial DPPH• concentration was 100 μM in all reaction mixtures, whereas the final concentration in the cold-pressed seed oil extracts was 40 mg oil equiv/mL. Measurements were taken at 10 min of reaction. Error bars represent the SD ($n = 3$). Different letters represent a significant difference ($P < 0.05$).

ORAC values were higher than those for cold-pressed boysenberry, red raspberry, blueberry, and marionberry seed oils (78, 49, 36, and 17 μmol TE/g oil, respectively) (2). The ORAC values were also higher than those of wheat grain (51 μmol TE/g) (18) and wheat bran (107–136 μmol TE/g) (18,32), common vegetables (19–154 μmol TE/g on a dry weight basis) (33), and dry fruits (13–154 μmol TE/g) (34,35). These data suggest that cold-pressed parsley, cardamom, and milk thistle seed oils contain higher levels of peroxyl radical-scavenging components.

DPPH• scavenging capacity. The cold-pressed seed oils differed in their DPPH• scavenging abilities, although all showed the capacity to react directly with and quench free DPPH• in the reaction mixtures (Fig. 3). Parsley seed oils exhibited the strongest DPPH• scavenging activity, and quenched 87–91% of the radicals in the reaction mixtures in 10 min (Fig. 3). Onion seed oil extract had the next strongest DPPH• scavenging activity, followed by cardamom, roasted pumpkin, and milk thistle seed oil extracts. Also, all the tested cold-pressed seed oils were able to react with DPPH• in a dose- and time-dependent manner. The time and dose effects of the onion1 seed oil extract against DPPH• are shown in Figure 4A, and those of the roasted pumpkin seed oil extract are presented in Figure 4B. In contrast to the previous observations by Parry and coworkers (2), in the present study a strong ORAC of a seed oil extract might not guarantee a higher DPPH• scavenging capacity. For instance, the methanol extracts of onion seed oils had the second-strongest DPPH• scavenging capacity, but the second-lowest ORAC among the seed oils tested (Fig. 2). This observation reflects the potential influence(s) of the free radical system on antioxidant activity estimation, because different chemical mechanisms may be involved in the individual radical–antioxidant reactions. In the ORAC assay, antioxidants compete with the fluorescent probe for the peroxyl radical generated from the radical initiator (AAPH), and the prevention of fluorescence decay is measured and used to calculate the relative radical-scavenging capacity of potential antioxidative samples. Chemicals capable of directly interacting with the radical initiator and fluorescent probe may alter the ORAC values in either direction. On the other hand, the DPPH• scavenging capacity assay measures the disappearance of the radical in the assay mixture. In addition to the radical-scavenging agents, color background from the potential antioxidative chemicals or

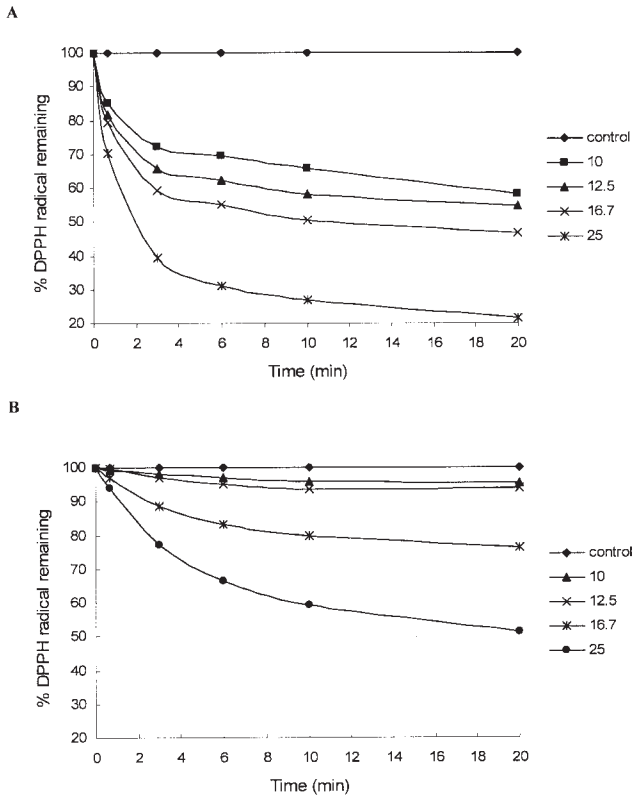

FIG.4. Dose and time effects of the oil antioxidants–DPPH• reactions. (A) Dose and time effects of 100% MeOH extract of cold-pressed onion seed oil and DPPH• reactions; (B) dose and time effects of 100% MeOH extract of cold-pressed roasted pumpkin seed oil and DPPH• reactions. The numbers 0, 10, 12.5, 16.7, and 25 represent the final concentrations of the seed oil extracts at 0, 10, 12.5, 16.7, and 25 mg oil equivalents per mL in the antioxidant–radical reaction mixtures, respectively. The initial DPPH• concentration was 100 μM in all reaction mixtures. For abbreviation see Figure 3.

TABLE 4
Oxidative Stability Index (OSI), Refractive Index, and Density of the Studied Cold-Pressed Seed Oils[a]

	OSI (h)	Refractive index n^{25}_D	Density (g/mL)
Onion1	31.4 ± 1.41[c]	1.4752	0.930
Onion2	16.9 ± 0.37[d]	1.4752	0.923
Parsley1	>369.4	1.4858	0.985
Parsley2	>148.4	1.4862	0.981
Cardamom	>63.5	1.4666	0.954
Mullein	47.8 ± 1.1[b]	1.4753	0.933
Pumpkin	61.7 ± 2.1[a]	1.4721	0.920
Milk thistle	13.3 ± 0.3[e]	1.4335	0.921
Soybean oil	46.8 ± 0.4	NA	NA
Corn oil	66.0 ± 0.4	NA	NA

[a]Data were reported as mean ± SD ($n = 3$). Different letters within columns represent significant difference ($P < 0.05$). NA, not available.

TABLE 5
HunterLab Color Measurements[a*]

	L value	a value	b value
Onion1	3.50 ± 0.02[c]	–1.38 ± 0.43[b]	3.90 ± 0.26[c]
Onion2	3.17 ± 0.07[d]	–1.26 ± 0.37[b,c]	2.96 ± 0.12[d]
Parsley1	1.96 ± 0.08[e]	2.88 ± 0.22[a]	1.92 ± 0.15[e]
Parsley2	1.22 ± 0.04[f]	2.70 ± 0.25[a]	1.44 ± 0.14[f]
Cardamom	6.03 ± 0.15[b]	–0.63 ± 0.27[c]	7.44 ± 0.31[b]
Mullein	2.07 ± 0.05[e]	2.30 ± 0.16[a]	3.05 ± 0.12[d]
Pumpkin	0.70 ± 0.11[g]	2.60 ± 0.16[a]	0.78 ± 0.10[g]
Milk thistle	10.64 ± 0.19[a]	–1.73 ± 0.85[b]	11.60 ± 0.32[a]

[a]Data were reported as mean ± SD (n = 3). Color measurement parameters: D65/10° illuminant/observer. "L," measure of lightness, increasing from 0 (dark) to 100 (light); "a," measure of red (+) to green (–); "b," measure of yellow (+) to blue (–).

the products generated from the antioxidant–radical reaction may alter the DPPH• scavenging capacity estimation. It is also well accepted that antioxidant compounds with different chemical structures may have different reactivities against different free radicals owing to their electronic and steric interactions with different free radicals. To fully understand why the estimated antioxidant capacities may vary between different radical systems such as ORAC and DPPH•, it may be necessary to identify the individual compounds that are significantly involved in the different radical/antioxidant reactions.

OSI and physicochemical properties. OSI determines the oxidative stability of an oil or fat sample, and higher OSI values are associated with a longer shelf life. OSI values for cold-pressed parsley and cardamom seed oils were not measurable under these experimental conditions, possibly owing to very high concentrations of volatile components. The other tested cold-pressed seed oils differed in their OSI values (Table 4). Cold-pressed roasted pumpkin seed oil had the highest measurable OSI value of 61.7 h, followed by mullein and onion seed oils (Table 4). The OSI value of roasted pumpkin seed oil was comparable with that of commercial corn oil, whereas the OSI value of mullein seed oil was similar to that of commercial soybean oil (Table 4). Cold-pressed milk thistle seed oil had the lowest OSI value, 13.3 h. Interestingly, the OSI value of onion1 oil was nearly double the value of onion2 oil (Table 4), suggesting a possible influence of seed quality on oil OSI.

Refractive index and density values of the cold-pressed seed oils are shown in Table 4. The refractive index values ranged from 1.4335^{25}_{D} to 1.4862^{25}_{D}. The two onion seed oils and the two parsley seed oils exhibited the same or similar refractive index values. The density of the seed oil samples ranged 0.920 to 0.985 g/mL.

Color is another important characteristic for determining visual acceptance of oil. The Hunter L-, a-, and b-values of the cold-pressed seed oils are shown in Table 5. The L value is the "lightness" of a sample from 0 to 100 with 100 being pure white; the a-value describes red (+) to green (–); the b value represents yellow (+) to blue (–); and zero values for "a" and "b" represent gray. The tested seed oils differed in their colors. The cold-pressed milk thistle seed oil was the lightest within the group and had the highest yellowness.

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