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Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils

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Abstract

Cold-pressed black caraway, carrot, cranberry, and hemp seed oils were extracted with methanol and evaluated for radical-scavenging activities against $ABTS^{\cdot+}$ and $DPPH^{\cdot}$, chelating activity, oxygen radical absorbing capacity (ORAC), and total phenolic contents (TPC). All the oil extracts had significant antioxidant activities. The ORAC value ranged from 28 to 220 μmol TE/g oil for the cold-pressed hemp, carrot, and black caraway seed oils, whereas the $ABTS^{\cdot+}$ – scavenging capacity ranged 8.9–30.8 μmol TE/g oil for the four cold-pressed edible seed oils. The greatest TPC, 3.53 mg gallic acid equivalent (GE) per gramme of oil, was detected in the cold-pressed black caraway seed oil extract, while the lowest TPC, 0.44 mg GE/g, was observed in the cold-pressed hemp seed oil extract. In addition, methanol extracts of the cold-pressed black caraway and cranberry seed oils were evaluated for their inhibitory capacities on human LDL oxidation by measuring the reduction of the thiobarbituric acid-reactive substance production (TBARS). Both oil extracts significantly suppressed the lipid peroxidation in human LDL, with TBARS reductions of 2.84 and 3.77 mg/g for cranberry and black caraway seed oil extracts, respectively. These results suggest that cold-pressed black caraway, cranberry, carrot and hemp seed oils may serve as dietary sources of natural antioxidants for health promotion and disease prevention, and the cold-pressed black caraway seed oil may be used as a natural antioxidative food additive for improving food quality and stability.

Keywords: Antioxidant; Free radical scavenging; LDL oxidation, Cold-pressed edible oil, Black caraway seed oil; Cranberry seed oil, Carrot seed oil, Hemp seed oil

1. Introduction

The pathology of numerous chronic diseases, including cancer and heart disease, involves oxidative damage to cellular components. Reactive oxygen species (ROS), capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Cadenas & Davies, 2000; Marnett, 2000; Uchida, 2000). Minimizing oxidative damage may well be one of the most important approaches to the primary prevention of these aging-associated diseases and health problems, since antioxidants terminate direct ROS attacks and radical-mediated oxidative reactions, and appear to be of primary importance in the prevention of these diseases and health problems. Antioxidants have been detected in a number of food and agricultural products, including cereal grains, vegetables, fruits, and oil seeds (Burits & Bucar, 2000; Kalt, Forney, Martin, & Prior, 1999; Yu et al., 2002a; Yu, Perret, Davy, Wilson, & Melby, 2002d). Recently, cold-pressed edible seed oils, including black caraway, carrot, hemp, and cranberry seed oils, have become commercially available. Cold-pressed seed oils may retain more natural beneficial components of the seeds, including natural antioxidants, and are free of chemical contamination.

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The cold-pressing procedure, involves neither heat nor chemical treatments, and is becoming a more interesting substitute for conventional practices because of consumers' desire for natural and safe food products.

Cold-pressed black caraway, carrot, hemp, cranberry, and black raspberry seed oils have been investigated for their fatty acid composition, oxidative stability and colour characteristics (Parker, Adams, Zhou, Harris, & Yu, 2003; Parry & Yu, 2004). α-Linolenic acid accounts for 35%, 22.3% and 19.3% of total fatty acids in the cold-pressed black raspberry, cranberry, and hemp seed oils, respectively, whereas the cold-pressed carrot seed oil contains about 80% oleic acid and is low in total saturated fatty acids (Parker et al., 2003; Parry & Yu, 2004). These facts show the potential health benefits from consuming cold-pressed edible seed oils. Fifty percent acetone extracts of the black raspberry oils exhibit radical-scavenging activities against stable DPPH$^{\cdot}$ and cation radical ABTS$^{\cdot+}$, and contain 35–93 ppm total phenolic components on a weight basis (Parry & Yu, 2004). In addition, the cold-pressed black caraway seed oil shows excellent oxidative stability compared to commercial soybean and corn oils, suggesting the possible presence of natural antioxidants in the cold-pressed seed oils (Parker et al., 2003). Antioxidants are well recognized for their potential in health promotion and prevention of aging-related diseases, including cancer and heart disease (Yu et al., 2002a). Novel dietary sources of natural antioxidants are desired by consumers and food manufacturers to benefit human health through improving nutrition. Therefore, the present study was conducted to evaluate the antioxidant properties of cold-pressed black caraway, cranberry, carrot, and hemp seed oils to determine their potential application in health promotion and disease prevention against radical mediated oxidative damages.

2. Materials and methods

2.1. Materials

Cold-pressed, 'extra virgin', unrefined black caraway, cranberry, carrot, and hemp seed oils were provided by Badger Oil Company (Spooner, WI). Fluorescein (FL), 2,2′-bipyridyl and 2,2-diphenyl-1-picryhydrazyl radical (DPPH$^{\cdot}$), 2,2′-bipyridyl, disodium ethylenediaminetetraacetate (EDTA), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were purchased from Sigma–Aldrich (St. Louis, MO), while 2,2′-azobis (2-amino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). β-Cyclodextrin was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). A total antioxidant status kit was purchased from Randox Laboratories Ltd. (San Francisco, CA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Preparation of antioxidant extract

One gram of each seed oil was extracted with 3×2 ml of methanol at ambient temperature. The methanol extract was flushed with nitrogen, and kept in the dark until further analysis. In order to prepare dimethyl sulfoxide (DMSO) solution, methanol was removed under reduced pressure from a known volume of the methanol extract, and the residue was quantitatively re-dissolved in DMSO. The resulting DMSO solution was also kept in the dark after nitrogen flushing until further analysis.

2.3. Oxygen radical absorbing capacity assay

Oxygen radical absorbing capacity (ORAC) assay was performed using FL as the fluorescent probe, following a previously described protocol (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002). The DMSO stock solution was diluted with the 7% β-cyclodextrin in acetone/water (1:1, v/v) to obtain the assay sample. The final assay mixture contained 0.067 μM of FL, 60 mM of AAPH, 300 μl of the assay sample or 7% β-cyclodextrin containing DMSO for a reagent blank. The total volume was 3000 μl for each reaction mixture. The fluorescence of an assay mixture was determined and recorded every minute using a Turner Quantech™ Fluorometer (Dubuque, IA). Trolox was used to prepare the standard curve to calculate the trolox equivalents for cold-pressed black caraway, cranberry, carrot and hemp seed oils.

2.4. Radical cation ABTS$^{\cdot+}$ scavenging activity

The ABTS$^{\cdot+}$ scavenging activity was determined using a commercial kit from Randox Laboratories Ltd. (San Francisco, CA). The DMSO solution was used in assay without further dilution. Trolox was used to prepare the standard curve and calculate the trolox equivalents of each cold-pressed seed oil (Yu et al., 2002a). Tests were conducted in triplicate.

2.5. Radical DPPH scavenging activity

DPPH$^{\cdot}$ scavenging capacity was estimated according to the previously described procedure (Yu et al., 2002a). Freshly prepared DPPH$^{\cdot}$ solution was mixed into the antioxidant extract to start the radical-antioxidant reaction. The DPPH$^{\cdot}$ -antioxidant reactions were examined at seven antioxidant concentrations for each oil extract. The final concentration was 100 μM for DPPH$^{\cdot}$, and the total volume was 2000 μl for each reaction mixture. The absorbance at 517 nm was determined against a blank of

pure methanol at 0, 0.5, 1, 2, 5, 10, 20, 40 and 80 min of reaction and used to estimate the remaining radical levels according to the standard curve. The dose- and time-dependencies of the oil extracts and DPPH· reactions were demonstrated by plotting the percent of DPPH· remaining against time for each level of the oil extract tested. Triplicate reactions were carried out.

2.6. Chelating activity

Fe^{2+} chelating capacity was estimated using a 2,2′-bipyridyl competition assay, following the previously described procedure except on a reduced scale (Yu, Haley, Perret, & Harris, 2002b). The reaction mixture contained 100 μl of 1.8 mM $FeSO_4$ solution, 100 μl of 2% SDS solution, 200 μl of the DMSO solution of the oil extract, 800 μl of Tris–HCl buffer (pH 7.4), 320 μl of 10% hydroxylamine–HCl, 800 μl of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), and 200 μl of pure water. The absorbance at 522 nm was measured against a solvent blank containing no antioxidant and used to calculate Fe^{2+}-chelating capacity using a standard curve prepared with EDTA.

2.7. Inhibition of LDL oxidation

Commercial human LDL–EDTA solution, from Sigma–Aldrich (St. Louis, MO), was dialyzed in a 100-fold volume of nitrogen-saturated 0.01 M phosphate buffer solution (PBS), pH 7.4, containing 0.16 M NaCl (Esterbauer, Streigl, Puhl, & Rotheneder, 1989; Fernandes, Filipe, Freitas, & Manso, 1996; Ohta, Semboku, Kuchii, Egashira, & Sanada, 1997). The buffer was changed four times during a 24-h period. Protein content of the EDTA-free LDL solution was measured by Bradford assay (Bradford, 1976), and a stock solution of 200 μg protein per ml was prepared with nitrogen-saturated PBS, and stored under nitrogen at 4 °C in the dark for the LDL oxidation assay within 24 h.

Oxidation of LDL was initiated by the addition of a freshly prepared copper chloride solution in the LDL oxidation assay mixture. The assay mixture contained 1% SDS and 20 μl of the DMSO solution of cold-pressed seed oil extract or cranberry seed oil extracts (Fernandes et al., 1996; Ohta et al., 1997). The control, containing no antioxidant, was performed using 20 μl of DMSO. The final $CuCl_2$ concentration was 15 μM, and final LDL content was 100 μg of protein per ml in the assay mixture. The total volume of each assay mixture was 1.0 ml. The oxidation reaction was carried out at ambient temperature for 60 min. The level of lipid oxidation in the assay mixture was evaluated by measuring the thiobarbituric acid-reactive substances (TBARS). TBARS were determined by addition of 1 ml of TBA reagent to each mixture and heating the resulting solution in a boiling water bath for 30 min (Tarladgis, Pearson, & Dugan, 1964; Yu, Scanlin, Wilson, & Schemidt, 2002c). After cooling to ambient temperature, absorbance at 532 nm was determined and used to calculate the TBARS using a standard curve prepared with 1,1,3,3-tetraethoxypropane.

2.8. Total phenolic contents

The total phenolic contents (TPC) of the cold-pressed edible seed oil extracts were determined using the Folin–Ciocalteu reagent (Yu et al., 2002a). The reaction mixture contained 100 μl of the seed oil extract in DMSO, 500 μl of the Folin–Ciocalteu reagent, and 1.5 ml of 20% sodium carbonate. The final volume was made up to 10 ml with pure water. After 2 h of reaction at ambient temperature, absorbance at 765 nm was measured and used to calculate the phenolic contents using a standard curve prepared with gallic acid. Triplicate reactions were performed.

2.9. Statistical analysis

Data were obtained as means ± standard deviations (SD). Analysis of variance and least significant difference tests were conducted to identify differences among means. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

3.1. General

Food rich in bioactive factors has become an important approach for more consumers, to achieve their desires to reduce the risk of a specific disease or a health problem, and to treat minor illnesses (Sloan, 2000). Development and characterization of the bioactive factors in novel food and agricultural products are required to provide scientific evidence for improving quality and nutritional value of the human diet. These are also important for improved utilization of food and agricultural products. The present study demonstrated that the cold-pressed cranberry, black caraway, hemp, and carrot seed oils contained significant levels of natural antioxidants. These natural antioxidants might directly react with and quench stable DPPH· and cation $ABTS^{\cdot+}$ radicals, form chelating complex with transition metals, absorb oxygen radicals generated by AAPH (ORAC), and prevent lipid peroxidation in human LDL and provide health benefits to the consumers.

3.2. Oxygen radical absorbance capacity

ORAC measures the capacity of antioxidants to protect a fluorescing protein from peroxyl radical attacks,

and is a widely used assay for estimating antioxidant activity (Huang et al., 2002). Cold-pressed black caraway seed oil had the greatest ORAC value of 220 μmol trolox equivalents (TE) per gram of the oil, and the lowest ORAC value of 28 μmol TE/g oil was detected in the cold-pressed hemp seed oil (Table 1). This range is comparable to that detected in other botanical materials, such as cucumber, cabbage and spinach (0.5–179 μmol TE/g dry vegetable; Cao, Sofic, & Prior, 1996), apple, orange and banana (5.0–15.9 μmol TE/g fresh fruit; Wang, Cao, & Prior, 1996), and highbush blueberry (15.9 μmol TE/g fresh fruit; Ehlenfeldt & Prior, 2001). These data suggest that cold-pressed black caraway and carrot seed oils may serve as excellent dietary sources of natural antioxidants for protection of important protein molecules from radical-mediated damage. No ORAC value was available for the cold-pressed cranberry seed oil due to the limited sample availability.

3.3. Radical cation $ABTS^{\cdot+}$ scavenging activity

Radical $ABTS^{\cdot+}$-scavenging activity is a widely used measurement of radical-scavenging capacity. The $ABTS^{\cdot+}$ may be generated through an enzymatic or a chemical oxidation reaction (Miller & Rice-Evans, 1997). In the present study, the four cold-pressed seed oils were evaluated for their scavenging activity against the $ABTS^{\cdot+}$ generated by enzymatic means. All oil extracts directly reacted with and quenched $ABTS^{\cdot+}$, with a TE range of 11.4–30.8 μmol/g oil (Table 1), whereas the greatest $ABTS^{\cdot+}$-scavenging capacity was observed in the cold-pressed black caraway seed oil. This range is greater than that detected in ready-to-eat cereal products (2.3–3.2 μmol TE/g cereal; Yu et al., 2002d) and in hard winter wheat grain (1.1–1.9 μmol TE/g grain; Yu et al., 2002a) and comparable to that detected in bran samples of Akron wheat grown at different locations (28–33 μmol TE/g bran; Yu, Perret, Harris, Wilson, & Haley, 2003) under the same experimental conditions. These data suggest that cold-pressed black caraway, cranberry, carrot and hemp seed oils may have the potential to protect biologically important components, such as membrane lipid, DNA and proteins, from free radical attacks and radical-mediated oxidative damage.

Lipid peroxidation in food products shares the same chemical mechanism, the free radical-mediated oxidative chain reaction. The products from lipid peroxidation contribute to the rancidity and ''off-flavour'' of food and consequently cause deterioration in food quality. Some of these products, such as aldehydes, are highly reactive and may also raise concerns of food safety. Antioxidants are added to food to prevent oxidative deterioration during processing and storage. The effectiveness of a selected antioxidant is greatly affected by its physicochemical properties and other components in the food (Frankel, Huang, Prior, & Aeschbach, 1996). Novel natural antioxidants, with desired physicochemical properties, are highly demanded for food applications to replace synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), because of consumer preference. Therefore, cold-pressed edible seed oils and their antioxidant extracts may also have the potential to be further developed as food additives for improving food quality, stability and safety.

3.4. $DPPH^{\cdot}$-scavenging activity

The radical-scavenging activity of antioxidants may be influenced by the radical system and other testing conditions. Two or more radical systems are needed to better study a selected antioxidant for its radical-scavenging properties. $DPPH^{\cdot}$ is a stable radical, and has been used to estimate the radical-scavenging capacities of antioxidants and to evaluate the kinetics and thermodynamic properties of radical-antioxidant reactions (Yu et al., 2002a). In this study, all extracts of the cold-pressed edible seed oils directly reacted with and quenched DPPH radicals (Fig. 1). The cold-pressed cranberry seed oil, at a concentration of 11.3 mg oil equivalents per ml of reaction mixture, had the strongest $DPPH^{\cdot}$-scavenging capacity among the four tested oil extracts, followed by the black caraway (2.7 mg oil equivalent/ml), carrot (10.9 mg oil equiva-

Table 1
Antioxidant properties of oil extracts[a]

Oils/tests	ORAC (μmol TE/g)	$ABTS^{\cdot+}$ (μmol TE/g)	Chelating (EDTAE mg/g)	TPC (mg GE/g)
Caraway	220 ± 19.2c	30.8 ± 3.58d	12.6 ± 0.26b	3.53 ± 0.11c
Carrot	160 ± 14.9b	8.90 ± 0.39a	25.5 ± 1.21c	1.98 ± 0.66b
Cranberry	NA	22.5 ± 1.22c	NA	1.61 ± 0.14b
Hemp	28.2 ± 6.19a	11.4 ± 2.08b	10.5 ± 0.83a	0.44 ± 0.01a

[a] Data are expressed as means ± SD ($n = 3$). ORAC stands for the oxygen radical absorbance capacity, and greater ORAC value is associated with a stronger capacity of the antioxidant to protect against protein oxidation. The $ABTS^{\cdot+}$ was generated by an enzymatic method; $DPPH^{\cdot}$ is a stable free radical. A greater $ABTS^{\cdot+}$ value represents a stronger radical scavenger. EDTA equivalent represents the chelating capacity of an antioxidant, with a EDTA equivalent positively correlated to the chelating activity. TPC means total phenolic content. NA means data were not available due to limited sample availability.

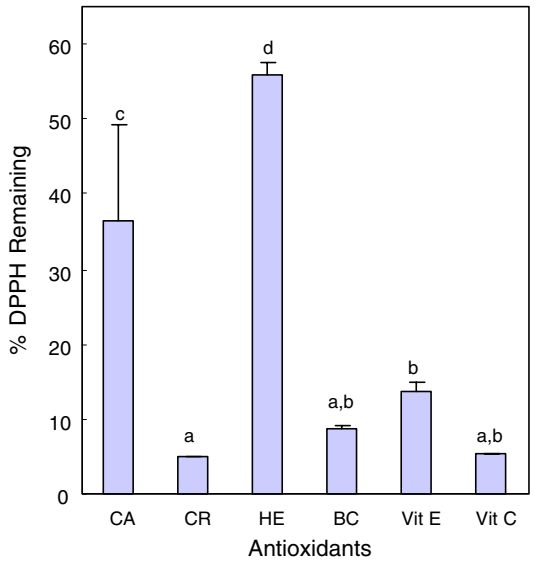

Fig. 1. *Comparison of DPPH· scavenging activities.* The radical DPPH-scavenging capacities of the cold-pressed carrot (CA), cranberry (CR), hemp (HE) and black caraway (BC) seed oil extracts are compared with that of 50 mM α-tocopherol (Vit E) and 50 mM ascorbic acid (Vit C). The final concentration of oil antioxidants was 10.9, 11.3, 10.5 or 2.7 mg oil equivalent/ml in the reaction mixtures for carrot, cranberry, hemp, or black caraway seed oils, respectively. The final concentration for DPPH· was 100 μM in all reaction mixtures, and the total volume was 2.0 ml for each reaction mixture. The absorbance at 517 nm of each reaction was measured at minute 40 of the reaction and used to calculate the percent DPPH· remaining. All tests were conducted in triplicate and the means were used. The vertical bars represent the SD for each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

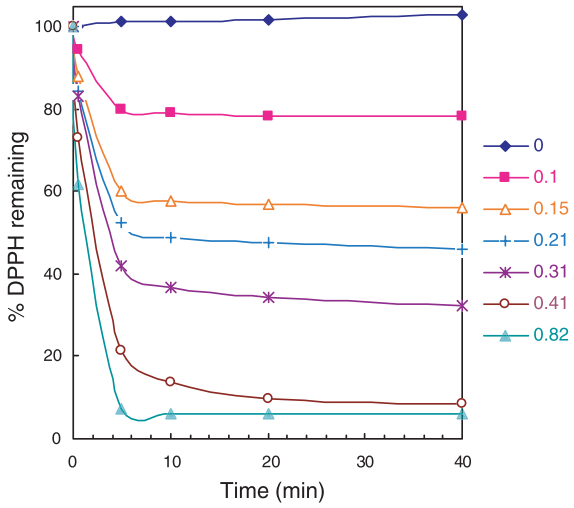

Fig. 2. *Kinetics of the cold-pressed black caraway seed oil extract — DPPH· reaction.* 0, 0.1, 0.15, 0.21, 0.31, 0.41 and 0.82 represent the final antioxidant extract concentrations of 0, 0.1, 0.15, 0.21, 0.31, 0.41 and 0.82 mg/ml in the reaction mixtures. The final DPPH· concentration was 100 μM in all reaction mixtures. All tests were conducted in triplicate and the means were used.

lent/ml), and hemp (10.5 mg oil equivalent/ml) seed oils, respectively. The extract of the cold-pressed cranberry seed oil, at 11.3 mg oil equivalent/ml, quenched 95.0% of the DPPH· in the reaction mixture (Fig. 1), which is comparable to vitamin C at 8.8 mg/ml (50 mM, quenched 94.7% DPPH·) and is stronger than α-tocopherol at 21.5 mg/ml (50 mM, quenched 86.3% DPPH·). The extract of the cold-pressed black caraway seed oil at 2.7 mg oil equivalent/ml quenched 86% of the DPPH·, suggesting that the cold-pressed black caraway seed oil had comparable scavenging activity against DPPH· to vitamin C and α-tocopherol. This is consistent with the previous observation that *Nigella sativa* seeds contained radical-scavenging agents that could directly react with and quench stable diphenylpicrylhydrazyl (DPPH) radicals and hydroxyl radicals (HO·) (Burits & Bucar, 2000). The seeds of black caraway (*N. sativa*) have been used as a condiment in bread and a spice in cooking for many years, particularly in Italy and Southern France (Burits & Bucar, 2000; Nergiz & Ötles, 1993). The seeds have also been used to treat fever, headache, cough, asthma, bronchitis, rheumatism, influenza and eczema (Burits & Bucar, 2000; Kumara & Huat, 2001) and have been investigated for their hypoglycemic, antimicrobial, and hypotensive activities (Al-Hader, Aqel, & Hasan, 1993; Hanafy & Hatern, 1991). Furthermore, the dose and time effects of the oil extracts-DPPH radical reactions were also investigated using seven levels of each oil extract. The DPPH radical-scavenging activities were both dose- and time-dependent for all four tested cold-pressed edible seed oils. Fig. 2 represents the reaction kinetics of the cold-pressed black caraway seed oil extract with DPPH radicals, whereas Fig. 3 represents that of the cold-pressed cranberry seed oil extract.

3.5. Chelating activity

Transition metals may act as catalysts that promote the generation of the first few radicals, which initiate the oxidative chain reactions (Nawar, 1996). Chelating agents may reduce the availability of transition metals and inhibit the radical-mediated oxidative chain reactions in biological or food systems, and consequently improve human health, and food quality, stability, and safety. The present study detected significant chelating activity against Fe^{2+} in the cold-pressed black caraway, cranberry and hemp seed oils (Table 1), but data for the potential chelating capacity of the cold-pressed cranberry seed oil were not available due to the limited sample availability. Cranberry seed oil extract showed the strongest chelating capacity, which is 2-fold greater than that detected in the cold-pressed black caraway seed oil extract, on a per oil weight basis. These results suggest the potential of cold-pressed cranberry, black caraway, and hemp seed oils to prevent oxidative damage from free radical mediated oxidation.

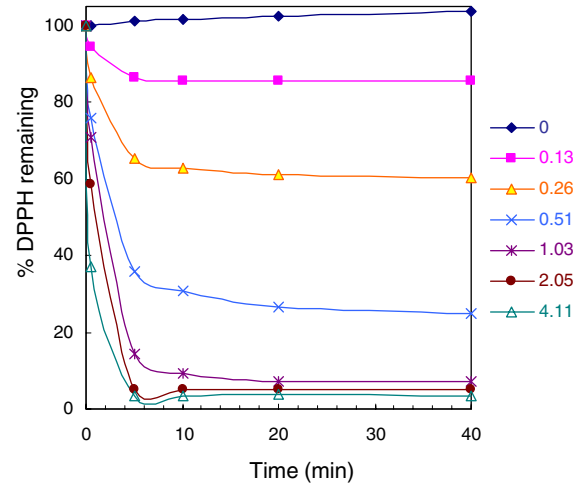

Fig. 3. *Kinetics of the cold-pressed cranberry seed oil e extract — DPPH· reaction.* 0, 0.13, 0.26, 0.51, 1.03, 2.05 and 4.11 represent the final antioxidant extract concentrations of 0, 0.13, 0.26, 0.51, 1.03, 2.05 and 4.11 mg/ml in the reaction mixtures. The final DPPH· concentration was 100 μM in all reaction mixtures. All tests were conducted in triplicate and the means were used.

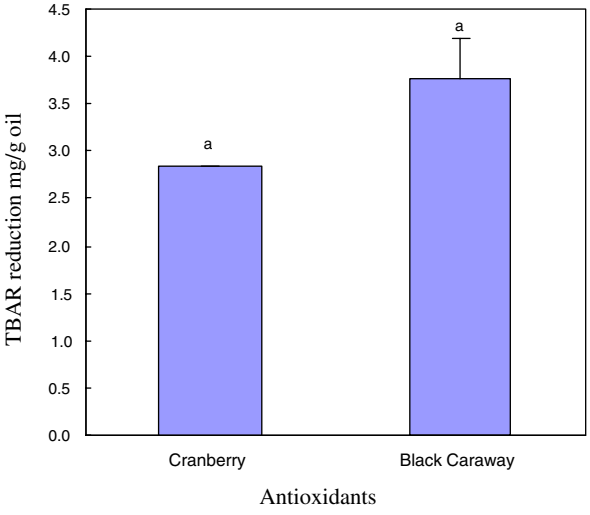

Fig. 4. *Inhibition of human LDL oxidation.* Cold-pressed cranberry and black caraway seed oil extracts were evaluated for their potential inhibitory effects on lipid peroxidation in human LDL. The TBARS stands for thiobarbituric acid-reactive substances. Greater TBARS reduction is correlated with a stronger prevention of LDL oxidation. All tests were conducted in triplicate and the means were used. The vertical bars represent the SD for each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

3.6. Inhibition of LDL oxidation

Growing evidence suggests that LDL oxidation may play a role in the pathogenesis of atherosclerotic complications, including coronary heart disease (CHD) (Ohta et al., 1997; Pearson et al., 1997). The radical-mediated oxidative chain reaction is a possible mechanism involved in LDL oxidation. LDL oxidation is believed to be a complex and multi-step process involving both lipid and protein fractions through different mechanisms (Pearson, Frankel, Aeschbach, & German, 1997). Antioxidants, including vitamins C and E, flavonoids, and other plant phenolics, have been shown to suppress LDL oxidation and delay the development of heart diseases (Naidu & Thippeswamy, 2002; Pearson et al., 1997; Steer et al., 2002). In the present study, the antioxidant extracts of the cold-pressed cranberry and black caraway seed oils were evaluated for their potential suppressing of lipid peroxidation in human LDL, measured as the reduction in TBARS production. TBARS reflect the level of the secondary products from lipid peroxidation, with a positive association with lipid peroxidation. The results showed that both cold-pressed cranberry and black caraway seed oils significantly protected human LDL from Cu^{2+}-induced oxidation, with reductions in TBARS of 2.8 and 3.8 mg/g oil, respectively, for the cold-pressed cranberry and black caraway seed oils (Fig. 4). These results suggest the potential benefits of cold-pressed black caraway and cranberry seed oils in protection of atherosclerotic complications including CHD, although further animal and human studies are required to confirm their in vivo activities and effective doses. The cold-pressed hemp and carrot seed oils were not evaluated for their potential in prevention of human LDL peroxidation in this research.

3.7. Total phenolic contents

Because phenolic compounds may contribute to overall antioxidant activities, the present study measured the TPC in the cold-pressed black caraway, cranberry, hemp and carrot seed oil extracts. TPC was determined using the Folin–Ciocalteu reagent. The TPC was 0.44–3.53 mg gallic acid equivalents (GE) in each gram of the cold-pressed edible seed oils (Table 1). The greatest TPC, 3.53 mg GE/g oil, was detected in the cold-pressed black caraway seed oil. This level is greater than that detected in Akron wheat bran (2.29–3.24 mg GE/g bran) (Yu et al., 2003), suggesting that cold-pressed black caraway seed oil may serve as a dietary source of phenolic substances, which may act as antioxidants for disease prevention and/or general health promotion through improved nutrition.

4. Conclusion

Cold-pressed black caraway, cranberry, hemp and carrot seed oils contain significant levels of antioxidants and may serve as dietary sources of natural antioxidants for disease prevention and health promotion. The cold-pressed black caraway seed oil may also be used as natural antioxidative additive to improve the quality, stability and safety of food products, including edible

oil blends. In addition to phenolic compounds, other chemicals retained during the cold-pressing procedure may contribute to the overall antioxidant properties of these cold-pressed edible seed oils. Further research is needed to investigate the other chemical compounds present in this cold-pressed oil and their potential biological activities.

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References

Al-Hader, A., Aqel, M., & Hasan, Z. (1993). Hypoglycemic effects of the volatile oil of *Nigella sativa* seeds. *International journal of Pharmacognosy, 31*, 96–100.

Bradford, M. M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry, 72*, 248–254.

Burits, M., & Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research, 14*, 323–328.

Cadenas, E., & Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine, 29*, 222–230.

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*, 3426–3431.

Ehlenfeldt, M. K., & Prior, R. L. (2001). Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *Journal of Agricultural and Food Chemistry, 49*, 2222–2227.

Esterbauer, H., Streigl, G., Puhl, H., & Rotheneder, M. (1989). Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radical Research Communications, 6*, 67–75.

Fernandes, A. C., Filipe, P. M., Freitas, J., & Manso, C. F. (1996). Different effects of thiol and nonthiol ace inhibitors on copper-induced lipid and protein oxidative modification. *Free Radical Biology and Medicine, 20*, 507–514.

Frankel, E., Huang, S., Prior, E., & Aeschbach, R. (1996). Evaluation of antioxidant activity of rosemary extracts, carnosol, and carnisic acid in bulk vegetable oils, and fish oil and their emulsions. *Journal of the Science of Food and Agriculture, 72*, 201–208.

Hanafy, M. S. M., & Hatern, M. E. (1991). Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *Journal of Ethnopharmacology, 34*, 275–278.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry, 50*, 1815–1821.

Kalt, W., Forney, C., Martin, A., & Prior, R. L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry, 47*, 4638–4644.

Kumara, S. S. M., & Huat, B. T. K. (2001). Extraction, isolation and characterization of antitumor principle, α-hederin, from the seeds of *Nigella sativa*. *Planta Medica, 67*, 29–32.

Marnett, L. (2000). Oxyradicals and DNA damage. *Carcinogenesis, 21*, 361–370.

Miller, N. J., & Rice-Evans, C. A. (1997). Factors influencing the antioxidant activity determination by the $ABTS^{\bullet+}$ radical cation assay. *Free Radical Research, 26*, 195–199.

Naidu, K. A., & Thippeswamy, N. B. (2002). Inhibition of human low density lipoprotein oxidation by active principles from spices. *Molecular and Cellular Biochemistry, 229*, 19–23.

Nawar, W. W. (1996). Lipids. In O. R. Fennema (Ed.), *Food chemistry* (pp. 225–313). New York: Marcel Dekker, Inc..

Nergiz, C., & Ötles, S. (1993). Chemical composition of *Nigella sativa* L. seeds. *Food Chemistry, 48*, 259–261.

Ohta, T., Semboku, N., Kuchii, A., Egashira, Y., & Sanada, H. (1997). Antioxidant activity of corn bran cell-wall fragments in the LDL oxidation system. *Journal of Agricultural and Food Chemistry, 45*, 1644–1648.

Parker, T. D., Adams, D. A., Zhou, K., Harris, M., & Yu, L. (2003). Fatty acid composition and oxidative stability of cold-pressed edible seed oils. *Journal of Food Science, 68*, 1240–1243.

Parry, J., & Yu, L. (2004). Fatty acid content and antioxidant properties of cold-pressed black raspberry seed oil and meal. *Journal of Food Science, 69*, 189–193.

Pearson, D., Frankel, E. N., Aeschbach, R., & German, J. B. (1997). Inhibition of endothelial cell-mediated oxidation of low-density lipoprotein by rosemary and plant phenolics. *Journal of Agricultural and Food Chemistry, 45*, 578–582.

Sloan, A. E. (2000). The top ten functional food trends. *Food Technology, 54*, 33–62.

Steer, P., Millgard, J., Sarabi, D. M., Basu, S., Vessby, B., Kahan, T., et al. (2002). Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. *Lipids, 37*, 231–236.

Tarladgis, B. G., Pearson, A. M., & Dugan, L. Jr., (1964). Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in Foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. *Journal of the Science of Food and Agriculture, 15*, 602–607.

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biology and Medicine, 28*, 1685–1696.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*, 701–705.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., & Qian, M. (2002a). Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry, 50*, 1619–1624.

Yu, L., Haley, S., Perret, J., & Harris, M. (2002b). Antioxidant properties of hard winter wheat extracts. *Food Chemistry, 78*, 457–461.

Yu, L., Scanlin, L., Wilson, J., & Schemidt, G. (2002c). Rosemary extracts as inhibitors of lipid oxidation and color change in cooked turkey products during refrigerated storage. *Journal of Food Science, 67*, 582–585.

Yu, L., Perret, J., Davy, B., Wilson, J., & Melby, C. L. (2002d). Antioxidant properties of cereal products. *Journal of Food Science, 67*, 2600–2603.

Yu, L., Perret, J., Harris, M., Wilson, J., & Haley, S. (2003). Antioxidnat properties of bran extracts from ''Akron'' wheat grown at different locations. *Journal of Agricultural and Food Chemistry, 51*, 1566–1570.