The unique fatty acid and antioxidant composition of ostrich fern (*Matteuccia struthiopteris*) fiddleheads

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DeLong, J. M., Hodges, D. M., Prange, R. K., Forney, C. F., Toivenon, P. M. A., Bishop, M. C., Elliot, M. L. and Jordan, M. A. 2011. **The unique fatty acid and antioxidant composition of ostrich fern (*Matteuccia struthiopteris*) fiddleheads**. Can. J. Plant Sci. **91**: 919–930. The purpose of this study was to investigate the health-promoting composition of ostrich fern (*Matteuccia struthiopteris*) fiddlehead tissue by focussing on its fatty acid and antioxidant content and antioxidant activity. The curled crosiers (fiddleheads) were harvested following emergence and before 10 cm growth from eight or nine sites in eastern Canada during 2008 and 2009. The crosiers were then refrigerated or kept on ice until cleaned, subsequently frozen in liquid nitrogen, and then stored at -85°C. All tissue samples (except those used for ascorbate analysis) were freeze-dried, ground in a ball mill and stored at -80°C until analyzed. The current study showed that fiddlehead tissue had an unusual fatty acid composition including γ-linolenic, dihomo-γ-linolenic, arachidonic and eicosapentanoeic acids. The concentration of the antioxidant compounds ascorbic acid [3.0 μmol g^{-1} dry weight (DW)], α- and γ-tocopherol (314 and 80.8 μg g^{-1} DW, respectively) and α- and β-carotene (43.8 and 122 μg g^{-1} DW, respectively) and the xanthophyll pigments violaxanthin (225 μg g^{-1} DW), zeaxanthin (127 μg g^{-1} DW) and lutein (238 μg g^{-1} DW), ranged from high to very high for green plant tissue. The phenolic compound content (51.6 mg gallic acid equiv. g^{-1} DW) was also high compared with other fruits and vegetables and was likely responsible for the elevated antioxidant activity (1529 μmol trolox equiv. g^{-1} DW; oxygen radical absorbing capacity assay) values recorded. Site differences were apparent for several of these measurements. Ostrich fern fiddlehead tissue appears to be a rich and unique source of antioxidant compounds, xanthophyll pigments and essential fatty acids.

Key words: Antheraxanthin, ascorbate, carotene, lutein, oxygen radical absorbing capacity, phenolic, tocopherol, violaxanthin, zeaxanthin

DeLong, J. M., Hodges, D. M., Prange, R. K., Forney, C. F., Toivenon, P. M. A., Bishop, M. C., Elliot, M. L. et Jordan, M. A. 2011. **Composition particulière en acides gras et en antioxydants des crosses de la fougère d'Allemagne (*Matteuccia struthiopteris*)**. Can. J. Plant Sci. **91**: 919–930. L'étude devait préciser la composition en nutriments bénéfiques à la santé des crosses de la fougère d'Allemagne (*Matteuccia struthiopteris*), plus particulièrement sa teneur en acides gras et en antioxydants et le degré d'activité des antioxydants. Les crosses de fougère ont été récoltées après la levée mais avant qu'elles atteignent 10 cm, à huit ou neuf sites, dans l'est du Canada, en 2008 et 2009. Elles ont ensuite été réfrigérées ou refroidies avec de la glace, puis congelées dans de l'azote liquide et stockées à -85°C. Les échantillons de tissus (sauf ceux utilisés pour doser l'ascorbate) ont été séchés à froid, moulus dans un broyeur à billes et entreposés à -80°C jusqu'à l'analyse. L'étude révèle que le tissu des crosses de fougère se démarque par la composition unique de ses acides gras, parmi lesquels figurent retrouve les acides γ-linolénique, dihomo-γ-linolénique, arachidonique et eicosapentanoéique. La concentration des antioxydants que sont l'acide ascorbique [3,0 μmol par g de poids sec (PS)], l'α- et le γ- tocophérol (314 et 80,8μg par g de PS, respectivement) et l'α- et le β-carotène (43,8 et 122μg par g de PS, respectivement) ainsi que celle des pigments de la xanthophylle, soit la violaxanthine (225 μg par g de PS), la zéaxanthine (127 μg par g de PS) et la lutéine (238 μg par g de PS), varient d'élevée à très élevée dans les tissus verts. La teneur en composés phénoliques (51,6 mg d'équivalent d'acide gallique par g de PS) était également élevée, comparativement à celle des autres fruits et légumes, et explique sans doute la forte activité des antioxydants (1 529 μmol d'équivalent de trolox par g de PS; dosage ORAC) observée. Plusieurs mesures variaient avec le site. Les tissus des crosses de la fougère d'Allemagne semblent être une source aussi riche qu'unique d'antioxydants, de pigments de la xanthophylle et d'acides gras essentiels.

Mots clés: Anthéraxanthine, ascorbate, carotène, lutéine, ORAC, phénolique, tocophérol, violaxanthine, zéaxanthine

The goal of contemporary plant bioprospecting is to identify flora that possess medical, pharmaceutical or to a lesser extent, health-promoting (e.g., nutraceutical) compounds that can be extracted for commercial purposes (Soejarto et al. 2005). While much research effort has focussed on tropical or sub-tropical plants as sources of new bioactive compounds, temperate zone species have also shown potential as sources of new

Abbreviations: **DW**, dry weight; **FAME**, fatty acid methyl ester; **HPLC**, high performance liquid chromatography; **ORAC**, oxygen radical absorbing capacity

medicines or health-promoting compounds (Heinreich and Leimkugel 1999; Spoor et al. 2006).

The results of nutritional research over the past 15–20 yr has encouraged the consumption of foods having a high content of antioxidant compounds (i.e., activity) and foods with a high degree of polyunsaturated fatty acids, which have an omega 6:omega 3 ratio of 4:1 or less (Simopoulos 2008; Sartorelli et al. 2010). Data from numerous studies have demonstrated the potential health benefits from ingestion of such foods and/or supplements, including: reduction in inflammatory mediators (Kelley 2001; Fetterman and Zdanowicz 2009), cardioprotection (Marik and Varon 2009; Shargorodsky et al. 2010), and reduced morbidity in allergic diseases (Biltagi et al. 2009; Birch et al. 2010), dermatology, epilepsy, depression and mood disorders, rheumatoid arthritis (Freeman et al. 2010; McClusker and Grant-Kels 2010; Rondanelli et al. 2010; Taha et al. 2010) as well as neuroprotection benefits (Devore et al. 2010; Palacios-Pelaez et al. 2010; Zhang and Bazan 2010). It is thus desirable to identify foods that ideally have both a high antioxidant titre and a high content of omega-3 fatty acids.

In African, Asian, Middle-Eastern, Central and South American and Oceanic societies, ferns and lichens have been used for food and medicines for hundreds of years (Chhabra et al. 1987; Bourdy and Walter 1992; Nwosu 2002; Glew et al. 2005; Marc et al. 2008; Nonato et al. 2009). In the modern West, ferns and lichens are not commonly utilised as a source of human foods or medicines. Nonetheless, the immature fronds or fiddleheads of the ostrich fern (*Matteuccia struthiopteris* L. Todaro) have been consumed as a spring vegetable for generations by native Aboriginal populations and European immigrants, particularly in New England and in eastern Canada (von Aderkas 1984; DeLong and Prange 2008). A report of three decades past indicates that the fiddlehead fern is potentially a good source of human nutrition (Gellerman et al. 1972). However, the profile of endogenous bioactive compounds in this early study was limited or non-existent. Hence, the goal of this work was to investigate the health-promoting composition of ostrich fern fiddlehead tissue by focussing on the fatty acid and antioxidant content and antioxidant activity.

MATERIALS AND METHODS

Following emergence and before 10 cm growth, the curled crosiers (fiddleheads) of *Mattueccia struthiopteris* were harvested from eight or nine sites in eastern Canada during 2008 and 2009, including: Kings County, Nova Scotia (NS1, NS2, NS3); Hants County, Nova Scotia (NS4, NS5); Restigouche County, New Brunswick (NB1); Queens County, New Brunswick (NB2, NB3); and the Asbestos Regional County Municipality, Province of Quebec (PQ). The crosiers were either refrigerated or kept on ice until cleaned, frozen in liquid nitrogen and then stored at -85°C. All tissue samples (except those used for ascorbate analysis) were freeze-dried, ground in a ball mill (Fritsch Planetary Ball Mill, Pulverisette 5, Idar-Oberstein, Germany) and stored at -80°C until analyzed. As a fatty acid comparison plant, purslane [*Portulaca oleracea* cv. *sativa*, (Green Leaf French Purslane, Richters Herbs, Goodwood, ON)] was sown in 12.7 cm pots and thinned to three plants per pot following germination, about 1 wk after sowing. Leaf tissue was harvested at approximately 6 wk after thinning and was processed for fatty acid analysis as was the ostrich fern tissue (see above).

Preparation of Fatty Acid Methyl Esters

The fatty acid methyl esters (FAME) were prepared by direct sulphuric acid-catalysed transesterification (Dobson et al. 2004) of freeze-dried ostrich fern and purslane tissue. To 100 mg of dry tissue, toluene (0.75 mL), nonadecanoic acid methyl ester [internal standard (312 mg in 100 mL methanol; 0.5 mL)], and 1% (vol/vol) methanolic sulphuric acid (3 mL) were combined in a screw-cap glass culture tube, and then heated at 50°C overnight (approx. 18 h). After cooling, 5% (wt/vol) sodium chloride (5 mL) and hexane (3 mL) were added followed by shaking and centrifugation at 2200 rpm. The upper organic layer was removed while the aqueous layer was re-extracted with hexane (3 mL). The combined organic layers were washed with 2% (wt/vol) potassium hydrogen carbonate (3 mL) and then passed through a short (3 cm) column of anhydrous sodium sulphate prepared in a Pasteur pipet. The column was washed with hexane, and the combined eluents, containing the FAME, were evaporated to dryness under nitrogen at 30°C in a water bath. The FAME were dissolved in hexane and kept at -20°C until they were analyzed. All chemicals used in the FAME preparation were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON), while the fatty acid standards were obtained from Cedarlane (Burlington, ON).

GC-MS Analysis of the FAME

Samples were analyzed with a Varian 4000 gas chromatograph mass spectrometer (Varian Inc., Walnut Creek, CA) equipped with a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland). A 1.0 μL sample was injected (injection temperature 250°C) on to a Factor Four VF-WAXms 30 m $\times$ 0.25 mm $\times$ 1.00 μm column (Varian Inc., Lake Forest, CA) used in constant flow mode of 1.0 mL min^{-1} of 99.99% helium (Praxair Canada, Mississauga, ON) throughout the temperature gradient where the column was held at 170°C for 2.0 min, increased to 250°C at a rate of $3^{\circ}C \cdot min^{-1}$, and held for 11.33 min (total of 40 min). The analyses were performed with the mass spectrometer in external mode with the damping gas flow set at 1.0 mL min^{-1}. The separated compounds were analyzed in electron ionization mode with the following settings: (i) mass range for analysis was m/z 50 to 500 amu, (ii) scan time

set to 0.85 s scan^{-1}, (iii) target total ion chromatogram at 20 000, and (iv) emission current at 25 μamps. Peak areas were calculated from selected "quan" ions based on relative abundance, stability and resolution from co-elutors. Individual fatty acid species were identified through comparison with reference spectrum and retention times of available standards (Cedarlane, Burlington, ON) and with the National Institutes of Standards and Technology 2008 Mass Spectral Library (ChemSW, Fairfield, CA).

Analysis of Ascorbate

Tissue ascorbate concentration was determined according to Bartoli et al. (2006) with some modification. In a cold mortar and pestle, approximately 5 g of fresh fiddlehead tissue were ground in 15 mL of 5% metaphosphoric acid. The slurry was decanted in to a 50-mL plastic centrifuge tube and centrifuged at $10\,000 \times g$ at 4°C for 15 min. The supernatant was then removed to a cooled clean test tube. For reduced ascorbate determination, a 200 μL supernatant aliquot was combined with 1000 μL of 150 mM phosphate buffer (pH 7.4; 5 mM ethylenediaminetetraacetic acid) and 200 μL deionized water. For total ascorbate, the same procedure was followed except that 200 μL of 5 mM dithiothreitol was substituted for 200 μL deionized water. The standards were prepared similarly as the reduced ascorbate determination, except that 200 μL of each standard was substituted for the sample aliquot. Samples and standards were incubated at room temperature for 15 min in the dark. O-phosphoric acid (100 μL) was added to each sample and standard to neutralize dithiothreitol and to acidify the solution for high performance liquid chromatography (HPLC) analysis. Samples were then filtered with 0.2 μm polyvinylidene fluoride filters fitted to a glass syringe, which was rinsed three times with methanol between samples. All chemicals used in the ascorbate analysis were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON).

Reduced and total ascorbate were quantified with an isocratic HPLC procedure on a photodiode array-equipped HPLC (Waters Corp., Milford, MA) with a C18 guard and analytical column (Luna 5 μm 150×4.6 mm i.d., Phenonomex, Torrance, CA). The samples were kept at 4°C in the HPLC autosampler, while the analytical column was at 25°C. The mobile phase consisted of 100 mM KH_2PO_4 at a flow rate of 0.6 mL min^{-1} for 15 min with a detection wavelengths set at 243 nm and a scan interval between 190 and 400 nm. Recoveries for ascorbate were >95%.

Determination of Carotenoids and Tocopherols

Ostrich fern carotenoid and tocopherol content was determined according to Lester et al. (2010) with some modification. Fiddlehead fern samples (0.10 g of freeze dried tissue) were weighed into a 15 mL screw-cap glass culture tube containing 7.5 mL 1% butylated hydroxytoluene in ethanol and 500 μL of the two internal standards (120 μM β-Apo-8; 211.5 μM α-tocopherol acetate) in acetone. The tube was capped under a stream of N_2 and sonicated, placed on a benchtop at room temperature for 10 min, mixed and left for another 10 min at room temperature. Three millilitres of deionized water and 3 mL of hexane:toluene (10:8 vol/vol) were then added to each tube, followed by mixing, dislodging any pellet formation, mixing and centrifugation at 4°C for 5 min at $1200 \times g$. The organic layer was removed to an 8-mL glass culture tube, which was immediately placed under a stream of N_2 in a 30°C water bath until dry. Extractions with 3 mL of added hexane:toluene mix (10:8 vol/vol) were repeated three additional times for a total of four extractions per sample. The organic solution was dried completely with N_2, after which, the sample was dissolved in 500 μL of 100% acetone and filtered into HPLC vials using 0.2 μm nylon filters attached to a glass syringe.

The constituent carotenoids (lutein, α and β carotenes) and tocopherols (α, γ) were separated isocratically on a photodiode array-equipped HPLC (Waters Corp., Milford, MA) with a C18 guard and analytical column (Luna 5 μm 150×4.6 mm i.d., Phenonomex, Torrance, CA). The samples were kept at 4°C in the HPLC autosampler, while the analytical column was at room temperature. The mobile phase consisted of acetonitrile: (95% ethanol; 5% deionized water) (50:50) at a flow rate of 1.2 mL min^{-1} for 20 min with detection wavelengths set at 290 and 450 nm and a scan interval between 200 and 500 nm. The carotenoids were quantified at 450 nm and tocopherols at 290 nm based on standard curves developed for each compound. Recoveries for the carotenoid and tocopherol compounds were >95%. α-Carotene was purchased from Chromadex Inc., (Irvine, CA) while all other chemicals used in the carotenoid and tocopherol analysis were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON).

Determination of Xanthophylls

The HPLC protocol was adapted from Thayer and Björkman (1990) for the determination of violaxanthin, antheraxanthin and zeaxanthin. Under dimmed room lighting (to avoid chlorophyll degradation), dried ostrich fern samples (0.2 g) were placed in centrifuge tubes on ice with each containing 4.5 mL of 85% acetone; 500 μL of 100 μM β-Apo-8'-carotenal (trans) (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) was added as an internal standard. Each extract was thoroughly mixed for 30 s, had N_2 gas blown over it for 2 min and was then capped before being mixed for an additional 1 min. The extracts were then placed on ice for 15 min before centrifugation (IEC MultiRF, Thermo IEC, Needham Heights, MA) for 4 min at $12\,000 \times g$ at 4°C. The supernatant was transferred to a 10 mL volumetric flask, while the pellet was re-extracted twice in 3 mL of 85% acetone:15% deionized water. Each supernatant was then added to the 10 mL flask which was kept on ice with a steady stream of N_2 gas blowing over it. After the

third extraction, samples were made up to a final volume of 10 mL with 85% acetone:15% deionized water and were then filtered through a 0.2 μm nylon syringe filter into HPLC vials.

The HPLC system consisted of a K1001 pump, a K1500 solvent organizer, dynamic mixer, and a K2800 diode array detector (Knauer, Berlin, Germany), an autosampler (Basic Marathon, Holland Spark, Emmen, the Netherlands) and a column heater (Alltech, Model 330, Deerfield, IL). The xanthophylls were eluted using a gradient system of solvent A [acetonitrile: methanol (85:15)] for the first 15 min, followed by a 2 min linear transition to solvent B [methanol:ethyl acetate (68:32)]. Solvent B then ran isocratically for 13 min followed by a 2-min linear transition to solvent A, which ran for 10 min to equilibrate the column prior to the next injection. The xanthophylls were separated using a Zorbax non-encapped ODS (4.6×250 mm, C18, 5 μm particle size) analytical column preceded by a C18 Zorbax ODS guard column (4.5×12.5 mm, 5 μm) (Agilent Technologies, IL). Data analysis was done with ChromGate Version 3.1.6 (Knauer, Berlin, Germany). Recoveries (>98%) and standard curves were generated for violaxanthin, antheraxanthin and zeaxanthin using standards obtained from Carotenature (Lupsingen, Switzerland). HPLC-grade acetonitrile, methanol, ethyl acetate and acetone were purchased from the Fisher Scientific Company (Ottawa, ON).

Oxygen Radical Absorbing Capacity (ORAC)

The hydrophilic antioxidant capacity (ORAC) in approximately 50 mg of freeze-dried tissue was analyzed on a Fluoroskan Ascent FL microplate reader (Thermo Electron Corp., Vantaa, Finland) using 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), a peroxyl generator and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich Canada Ltd, Oakville, ON) as a standard according to the method of Prior et al. (2003). The lipophilic antioxidant capacity in ostrich fern tissue was negligible and therefore was not reported.

Determination of Phenolic Compounds

Total phenolic compounds were determined spectrophotometrically by the Folin-Ciocalteau method (Singleton and Rossi 1965). Ten to twenty milligrams of freeze-dried fiddlehead tissue was mixed with 15 mL of extraction reagent (70% methanol, 29% deionized water, 1% HCl) and ground in a ball mill for 5 min at 450 rpm. The wet slurry was transferred to 15 mL centrifuge tubes and centrifuged for 15 min at 4600 rpm. A 200 μL supernatant aliquot was pipetted from each sample and then mixed with 2 mL of deionized water and 0.4 mL of Folin-Ciocalteau reagent (1:2 vol/vol Folin:water; prepared fresh daily) and incubated at room temperature for 5 min. Following mixing, 0.1 mL of saturated Na-carbonate (20 g anhydrous NaCl in 100 mL boiling deionized water, then cooled to room temperature) was added and kept for 60 min at room temperature in the dark. Sample absorbance was then read at 640 nm. Phenolic compound concentrations were determined from a standard curve prepared similarly and reported in milligrams of gallic equivalents per gram of dry tissue. Methanol was purchased from the Fisher Scientific Company (Ottawa, ON), while HCl was obtained from BDH® (VWR International, Mississauga, ON). All other chemicals used in the phenolic compound analysis were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON).

Data Analysis

Data for fatty acids (Table 1) and antioxidants (Table 3) were averaged over 1 or 2 yr, with site being designated an experimental replicate. The variation among sites was expressed as the standard error of the mean (SEM) for all sites for a specific measurement. Differences among sites, equivalent to the α probability level of 5%, can be estimated by 3×the SEM. For the ostrich fern and purslane fatty acid comparison (Table 2), site or sample replicates, respectively, from a combined tissue mass from several plants were designated as experimental replicates. The data were separated by the LSD procedure with statistical differences being declared at the α probability level of 5% (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Fatty Acids

Although fatty acid content varied among sites, no location had consistently lower or higher content for a specific fatty acid species (Table 1). The total lipid content of ostrich fern tissue ranged from 0.038 to 0.056 (3.8 to 5.6%) grams per gram of dry mass, which is a high value for green plant tissue. By contrast, total fat content of spinach, buttercrunch lettuce, red leaf lettuce and mustard is 1.7, 0.60, 0.70 and 1.1%, respectively (Simopoulos 2004).

The type and concentration of omega-3 fatty acids in ostrich fern crosiers are unusual for vegetative tissue. For example, gymnosperms and angiosperms do not usually contain arachidonic and eicosapentaenoic acids, although exceptions have been noted among some algae, ferns and mosses (Gellerman et al. 1972; Grimsley et al. 1981; Hansen and Rossi 1990; Horrobin 1992; Guedes et al. 2010). The additional presence of the omega-6 γ-linolenic and dihomo-γ-linolenic acids, [the former being present in evening primrose (~10%) borage (~20%), black currant (~15%) and echium (~25%) seed oils (Velasco and Goffman 1999; Guil-Guerrero et al. 2000; Scrimgeour and Harwood 2007; Mir 2008)], further demonstrates the unique combination of indigenous fatty acids in this fern. While other plants may have a higher concentration of a single species of fatty acid (e.g., α-linolenic acid in purslane; Table 2), ostrich fern tissue, to our knowledge, has the most complete fatty acid spectrum of any edible green plant. As these

Table 1. Fatty acid composition of dried ostrich fern fiddlehead tissue collected from nine eastern Canadian sites

	Fatty acid											
Site	Myristate C14:0	Palmitate C16:0	Palmitoleate C16:1n7	Stearate C18:0	Oleate C18:1n9	α-Linoleate C18:2n6	α-Linolenate C18:3n3	γ-Linolenate C18:3n6	Dihomo-γ-linolenate C20:3n6	Arachido-nate C20:4n6	Eicosapentaenoate C20:5n3	n6/n3 ratio
	($\mu g\ g^{-1}$ dry wt)											
NB1[z]	99.3	11496	389	351	2296	11410	8239	1244	1524	10188	711	2.7
	0.2%	24.0%	0.8%	0.7%	4.8%	23.8%	17.2%	2.6%	3.2%	21.2%	1.5%	
NB2	119.6	11356	277	436	3306	8300	7878	1085	1513	6890	604	2.1
	0.3%	27.2%	0.7%	1.0%	7.9%	19.9%	18.9%	2.6%	3.6%	16.5%	1.4%	
NB3	151.7	15354	362	504	4062	13703	9263	1474	1654	8220	760	2.5
	0.3%	27.7%	0.7%	0.9%	7.3%	24.7%	16.7%	2.7%	3.0%	14.8%	1.4%	
NS1	102.6	9623	280	338	2726	10550	5493	1096	1055	6453	338	3.3
	0.3%	25.3%	0.7%	0.9%	7.2%	27.7%	14.4%	2.9%	2.8%	17.0%	0.9%	
NS2	99.2	10850	346	377	3388	11520	7882	1400	1448	8516	496	2.7
	0.2%	23.4%	0.7%	0.8%	7.3%	24.9%	17.0%	3.0%	3.1%	18.4%	1.1%	
NS3	89.9	10014	267	440	3775	12436	8007	1139	1333	7171	531	2.6
	0.2%	22.2%	0.6%	1.0%	8.4%	27.5%	17.7%	2.5%	2.9%	15.9%	1.2%	
NS4	99.3	10816	366	356	2551	10670	7792	1380	1386	8575	680	2.6
	0.2%	24.2%	0.8%	0.8%	5.7%	23.9%	17.4%	3.1%	3.1%	19.2%	1.5%	
NS5	95.2	10452	346	294	1722	9730	6572	969	1412	8604	616	2.9
	0.2%	25.6%	0.8%	0.7%	4.2%	23.8%	16.1%	2.4%	3.5%	21.1%	1.5%	
PQ	103	10788	286	357	2445	12149	6776	940	1521	7996	515	3.1
	0.2%	24.6%	0.7%	0.8%	5.6%	27.7%	15.4%	2.1%	3.5%	18.2%	1.2%	
Avg.	107	11194	324	384	2919	11163	7545	1192	1427	8068	584	2.7
Avg.%	0.2%	24.9%	0.7%	0.9%	6.5%	24.9%	16.8%	2.7%	3.2%	18.0%	1.3%	
SEM[y]	6.1	556	15.5	21.4	254	528	367	64.3	56.2	374	43.0	

[z]Means for NS1 and PQ comprised six observations, while all other means had $n = 3$.
[y]SEM, standard error of the site means.

Table 2. Fatty acid composition of dried ostrich fern fiddlehead and purslane tissue

Crop	Fatty acid											
	Myristate C14:0	Palmitate C16:0	Palmitoleate C16:1n7	Stearate C18:0	Oleate C18:1n9	α-Linoleate C18:2n6	α-Linolenate C18:3n3	γ-Linolenate C18:3n6	Dihomo-γ-linolenate C20:3n6	Arachidonate C20:4n6	Eicosapentaenoate C20:5n3	n6/n3 ratio
	(μg/g dry wt)											
Ostrich fern	103*b*[z]	11559*a*	235*a*	341*b*	2165*b*	10713*a*	7011*b*	791*a*	1331*a*	7177*a*	1384*a*	2.4*a*
(%)	0.2	26.9	0.6	0.8	5.1	25.0	16.4	1.8	3.1	16.8	3.2	
Purslane	114*a*	874*b*	0*b*	584*a*	2804*a*	3807*b*	17983*a*	0*b*	0*b*	0*b*	0*b*	0.21*b*
(%)	0.4	3.3		2.3	10.8	14.7	69.2					

[z]For each fatty acid, comparison of crop means was performed by the LSD test with differences being declared at the 5% α probability level. Crop means for each fatty acid with different letters are significantly different.

fatty acids have been linked to health promotion (Fan and Chapkin 1998; Wall et al. 2010), the ostrich fern should be considered an important addition to those diets where consumption of nutrient-dense vegetables is desirable.

The n6/n3 fatty acid ratio varied from 3.3 (NS1) to 2.1 (NB2). Recent dietary recommendations for consumption of lipids suggest an n6/n3 ratio of 4:1 or less is ideal in order to ensure adequate synthesis of the longer-chain omega-3 family (e.g., eicosapentaenoic (20:5) and docosahexaenoic (22:6) fatty acids) (Health Canada 2005; Harnack et al. 2009; Wall et al. 2010). At higher n6/n3 ratios, the omega-6 pathway is favoured as the n6 fatty acid substrates out-compete the 18-carbon omega-3s for the desaturase and elongase enzymes necessary for synthesis of the longer chain omega-3 species. The result is less synthesis of eicosapentaenoic acid and more synthesis of arachidonic acid (Liou et al. 2007; Harnack et al. 2009).

Purslane has been dubbed the ''gold standard'' for omega-3 content in vegetative tissue (Simopoulos et al. 1992). Our analysis confirmed that purslane is very high in α-linolenic acid in particular, having about 2.5 times the amount found in fiddlehead tissue. However, arachidonic, γ-linolenic, dihomo-γ-linolenic and eicosapentaenoic acids were not detected in purslane, but in ostrich fern tissue were present at 17, 1.8, 3.1 and 3.2% of the total fatty acid titre on a dry weight basis (Table 2). Purslane also showed a remarkably low n6/n3 ratio of 0.21, while in ostrich fern the ratio was 2.4 (Table 2). As previously mentioned, consumption of foods having an n6/n3 fatty acid of ideally $\leq$4:1 is the recommendation coming from current nutritional studies and health authorities worldwide. In light of this recommendation, the ostrich fern fiddlehead provides an alternate dietary source for those wanting to bolster their intake of omega-3 fatty acids without eating fish or nut or seed crops (or in addition to them). Based on Health Canada's RDA of 1.6 g of omega-3s daily for a healthy adult male (Health Canada 2008), eating three to four cups of cooked ostrich ferns (assuming minimal fatty acid degradation during cooking) would meet this requirement. Thus, the consumption of ostrich fern fiddleheads can help lower the n6/n3 ratio in the diet to the desirable 4:1 (or lower) level.

Although several mosses, fungi, algae and other ferns have the capacity to synthesize long-chain (>18 carbons) polyunsaturated fatty acids (Hansen and Rossi 1990; Girke et al. 1998; Hong et al. 2002; Bhosale et al. 2009), the pathway details in ostrich fern tissue are not presently known. It appears, however, that the Δ^5- and Δ^6- (and possibly the Δ^{17}-) desaturases and the elongases which produce both arachidonic and eicosapentaenoic acids are functional in ostrich fern cells. The presence of γ-linolenate and dihomo-γ-linolenate (Table 1) and the absence of octadecatetraenoic and eicosatetraenoic acids (both omega-3s) indicate that the synthesis of eicosapentaenoic acid may occur through

conversion of arachidonic acid (i.e., the omega-6 synthesis pathway which implies the presence of Δ^{17}-desaturase).

Matteuccia struthiopteris could provide a model for genetic transfer studies, as other work has demonstrated that the genes controlling fatty acid metabolism can be transferred into higher plants (Qi et al. 2004; Ruiz-López et al. 2009; Cheng et al. 2010). Also, in those situations where the growth environment can be controlled (e.g., large greenhouses or horticultural plantations), the inherent fatty acid content may be elevated through: manipulation of the growing temperature (Yaniv et al. 1989; Renaud et al. 1995), nutrient or water stress (Martin et al. 1986; Carvajal et al. 1996) or CO_2 enrichment (Muradyan et al. 2004).

Antioxidant Compounds and Activity

Site differences occurred among the antioxidant compounds and the total antioxidant activity measured (Table 3). Compared with spinach, muskmelon and purslane, fiddlehead tissue had similar levels of cellular ascorbate (Hodges and Forney 2003; Simopoulos 2004; Hodges and Lester 2006). Fiddleheads also had more α-carotene and α- and γ-tocopherol than many other green, leafy and root vegetables (Raju et al. 2007; Rodriquez-Amaya et al. 2008; Isabelle et al. 2010), but four- to eightfold lower β-carotene than did carrot, kale and spinach (Lefsrud et al. 2005, 2006, 2007; Health Canada 2008). Compared with broccoli, ostrich fern tissue had two- to fourfold more β-carotene content (Farnham and Kopsell 2009; Perry et al. 2009).

One of the main functions of the lipophilic tocopherols in plant cells is membrane stabilization and protection of the lipid domains from oxidation due to the generation of peroxyl radicals (Falk and Munné-Bosch 2010). The carotenoids (i.e., α- and β-carotene) also play a significant antioxidant role, particularly as physical quenchers of the singlet oxygen species generated in the PSII reaction centre regions. They also act as accessory pigments for light absorption in the light harvesting complexes (Young 1991; Pallet and Young 1993). The relatively large quantities of polyunsaturated fatty acids in fiddlehead tissue are susceptible to degradation via oxidative stresses and ideally require relatively high concentrations of protective antioxidants for maintenance of structural and functional integrity, roles that the tocopherols and carotenoids are known to play (Pallet and Young 1993; Munné-Bosch and Alegre 2002).

Fiddlehead tissue has a remarkably high degree of antioxidant activity in the hydrophilic soluble cellular fractions (lipophilic ORAC values were negligible) (Table 3). The ORAC activity values ranging from 1097 to 1849 μmol trolox equivalents g^{-1} dry weight were higher than those reported for fruits known to have high antioxidant titre and activity, such as blueberry, cranberry, strawberry, blackberry, cherry, plum and raspberry (Wu et al. 2004; Wolfe et al. 2008); only pecans measured equivalently in a recent study (Wu et al. 2004). Sun and Powers (2007) developed a relative antioxidant activity index (based on several activity assays) for 20 common vegetables and showed spinach having the 3rd highest activity rating (behind garlic and asparagus). Although ostrich ferns were not evaluated, they would likely place highly in that ranking based on the findings of this study.

The ORAC activity of fiddleheads was higher than any commonly grown vegetable, in some cases by an order of magnitude (Wu et al. 2004; Huang et al. 2009; Isabelle et al. 2010; Song et al. 2010). This extraordinarily high antioxidant activity is likely attributable to the high concentration of phenolic compounds (Table 3), which was roughly comparable with blueberries on a dry weight basis (Wolfe et al. 2008; Poiana et al. 2010). In a recent survey of 31 fern species, Ding et al. (2008) found that the higher the cellular phenolic content, the higher the radical scavenging capacity, with ostrich fern having some of the highest values in both categories. The data of this present study support their findings (Table 3). Interestingly, other vegetable and fruit crops showing comparable phenolic content do not have equivalent antioxidant activity (i.e., ORAC) values, perhaps due to differences in the type(s) of phenolic species (Wolfe et al. 2008; Huang et al. 2009; Corral-Aguayo et al. 2008). Thus, it is plausible that ostrich fern tissue has a number of unique phenolic compounds that confer high ORAC activity; it is currently known that this tissue does have unusual phenolic chemistry (Kimura et al. 2004).

The carotenoid xanthophylls pigments – violaxanthan, antheraxanthin and zeaxanthin – form an integral interdependent cycle for the dissipation of excessive photonic energy. Under high light, violaxanthin is converted by de-epoxidation, into the intermediate antheraxanthin and ultimately zeaxanthin (Demmig-Adams and Adams 1996, 2006). In the violaxanthin state, it functions as antenna pigments and transfers energy to chlorophyll *a* molecules and reaction centres, while in the zeaxanthin state, it traps energy which is dissipated as heat (Frank et al. 1994; Demmig-Adams 1996). The de-epoxidation of violaxanthin requires a pH gradient across the thylakoid membrane (Pfundel and Dilley 1993; Munekage et al. 2002), which is generally associated with high light intensity.

In the macular region of the human eye, the pigments zeaxanthin and lutein represent over 70% of the total carotenoid content of the eye (Landrum and Bone 2001) and are found in concentrations approaching 1 mmol, about three orders of magnitude above the range in normal serum (Thurnham 2007). Interestingly, the concentration of zeaxanthin in the fovea (central retina) is approximately twice that of lutein, while lutein is higher in the perifovea (peripheral retina) region. The direct relationship between carotenoid consumption and the development of age-related macular degeneration and cataracts is controversial (Trumbo and Ellwood

Table 3. Antioxidant molecule content and antioxidant activity of dried ostrich fern fiddlehead tissue from eight eastern Canadian sites

Site	Ascorbate[z]	α-Tocopherol[y]	γ-Tocopherol[y]	α-Carotene[y]	β-Carotene[y]	Violaxanthan[y]	Antheraxanthin[y]	Lutein[y]	Zeaxanthin[y]	Phenolics[x]	TAA (ORAC)[w]
NB1[v]	2.7	334	68.8	48.6	119	287	13.9	256	53.3	49.6	1553
NB2	3.0	331	75.4	50.7	143	238	29.1	265	197	47.8	1420
NB3	3.0	275	83.8	50.1	151	214	34.5	270	254	41.5	1097
NS1	3.2	300	71.2	27.5	96.0	160	18.8	190	130	54.8	1574
NS2	3.9	335	72.9	58.6	131	242	19.6	284	104	62.1	1538
NS3	2.9	279	90.3	40.0	127	180	20.3	245	156	52.3	1640
NS4	3.1	306	93.8	36.7	113	254	17.1	228	64.2	50.0	1563
PQ	2.2	350	90.3	38.1	92.6	224	13.9	166	60.4	54.9	1849
Average	3.0	314	80.8	43.8	122	225	20.9	238	127.4	51.6	1529
SEM[u]	0.2	9.9	3.5	3.5	7.3	14.2	2.6	14.6	25.5	2.1	75.3

[z]μmol g^{-1} fresh weight.
[y]μg g^{-1} dry weight.
[x]mg gallic acid equivalents g^{-1} dry weight.
[w]TAA [total antioxidant activity (ORAC assay)] units: μmol trolox equivalents g^{-1} dry weight.
[v]Means for PQ and NS1 comprised six observations, while all other means had $n = 3$.
[u]SEM, standard error of the site means.

2006); however, intake of foods or supplements high in these pigments appears to be beneficial to retinal tissues (Burke et al. 2005; Carpentier et al. 2009; Barker 2010).

Ostrich fern fiddlehead tissue has a relatively high content of the xanthophyll pigment lutein, being comparable with romaine lettuce, but having one-half to one-third the lutein than the highest yielding species, kale or spinach (Perry et al. 2009; Dias et al. 2010). However, it has one of the highest concentrations of violaxanthin and zeaxanthin in any green vegetable tissue and higher content than most fruit. Recently, Murillo et al. (2010) reported on the lutein and zeaxanthin content of 59 cultivated and wild fruits and vegetables: only corozo, South American sapote, membrillo, orange pepper and sastra (fruit) had higher, while corn had similar zeaxanthin content, compared with the results of this study (Table 3). In addition, only eight species (squash, sastra, Indian mustard, beet, spinach, watercress, endive and romaine lettuce) possess equivalent or higher lutein concentrations. In another recent report, the zeaxanthin content in orange peppers (Perry et al. 2009) is similar to that in ostrich fern (Table 3). Also, antheraxanthin levels in several broccoli cultivars were on average 35–40% less compared with ostrich fern, while lutein and violaxanthin content was one-half to one-tenth, respectively (Farnham and Kopsell 2009) (Table 3).

In the present study, the zeaxanthin content of ostrich ferns varied four- to fivefold among the eight sites. Interestingly, the ranking of zeaxanthin levels (i.e., low to high) appeared to be the inverse of violaxanthin (Table 3). Past research has demonstrated that violaxanthin and zeaxanthin are inter-convertible via the activity of epoxidase and de-epoxidase enzymes, which are governed by thylakoid lumen pH levels and external environmental conditions like high irradiance levels (Demmig-Adams and Demmig 2006). The high concentrations of violaxanthin in fiddlehead tissue thus represent a large potential pool of zeaxanthin when the former is converted to the latter. A future research goal would be to fully convert the xanthophyll cycle pigments to zeaxanthin to determine the upper possible concentration limit of this carotenoid in ostrich fiddlehead fern tissue. As rich, natural sources of zeaxanthin are uncommon, fiddlehead consumption may be one of the simplest ways to maintain or augment carotenoid concentrations in human blood sera and in retinal tissue.

CONCLUSIONS

This study demonstrates that ostrich fern fiddlehead tissue is a rich source of ascorbate, α- and β-carotene, α- and γ-tocopherol, lutein, violaxanthin, zeaxanthin and phenolic compounds. The high ORAC values indicate high biological (i.e., antioxidant) activity. For green vegetable tissue, it also has a high and unusual

fatty acid content, which includes the omega-3 eicosapentaenoic acid, and the omega-6 arachidonic, γ-linoleic and dihomo-γ-linolenic acids. Thus, the ostrich fern fiddlehead can be recommended as a healthful vegetable in the human diet and should be consumed where it is seasonally available.

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Barker, F. M. 2010. Dietary supplementation: Effects on visual performance and occurrence of AMD and cataracts. Curr. Med. Res. Opin. **26**: 2011–2023.

Bartoli, C. G., Yu, J., Gómez, F., Fernández, L., McIntosh, L. and Foyer, C. H. 2006. Inter-relationships between light and respiration in the control of ascorbic acid synthesis and accumulation in *Arabidopsis thaliana* leaves. J. Exp. Bot. **57**: 1621–1631.

Bhosale, R. A., Velankar, D. A. and Chaugule, B. B. 2009. Fatty acid composition of the cold-water-inhabiting freshwater red algae *Sirodotia* Kylin. J. Appl. Phycol. **21**: 99–102.

Biltagi, M. A., Baset, A. A., Bassiouny, M., Kasrawi, M. A. and Attia, M. 2009. Omega-3 fatty acids, vitamin C and Zn supplementation in asthmatic children: a randomized self-controlled study. Acta Paediatr. **98**: 737–742.

Birch, E. E., Khoury, J. C., Berseth, C. L., Castaneda, Y. S., Couch, J. M., Bean, J., Tamer, R., Harris, C. L., Mitmesser S. H. and Scalabrin, D. M. 2010. The impact of early nutrition on incidence of allergic manifestations and common respiratory illnesses in children. J. Pediatr. **156**: 869–871.

Bourdy, G. and Walter, A. 1992. Maternity and medicinal plants in Vanuatu I. The cycle of reproduction. J. Ethnopharmacol. **37**: 179–196.

Burke, J. D., Curran-Celentano, J. and Wenzel, A. J. 2005. Diet and serum carotenoid concentrations affect macular pigment optical density in adults 45 years and older. Am. Soc. Nutr. Sci. **135**: 1208–1214.

Carpentier, S., Knaus, M. and Suh, M. 2009. Associations between lutein, zeaxanthin, and age-related macular degeneration: an overview. Crit. Rev. Food Sci. Nutr. **49**: 313–326.

Carvajal, M., Cooke, D. T. and Clarkson, D. T. 1996. Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function. Planta **199**: 372–381.

Cheng, B., Wu, G., Vrinten, P., Falk, K., Bauer, J. and Qiu, X. 2010. Towards production of high levels of eicosapentaenoic acid in transgenic plants: the effects of different host species, genes and promoters. Transgenic Res. **19**: 221–229.

Chhabra, S. C., Mahunnah, R. L. A. and Mshiu, E. N. 1987. Plants used in traditional medicine in eastern Tanzania. I. Pteridophytes and angiosperms (Acanthaceae to Canellaceae). J. Ethnopharmacol. **21**: 253–277.

Corral-Aguayo, R. D., Yahia, E. M., Carrillo-Lopez, A. and González-Aguilar, G. 2008. Correlation between some nutritional components and the total antioxidant capacity measured with six different assays in eight horticultural crops. J. Agric. Food Chem. **56**: 10498–10504.

DeLong, J. M. and Prange, R. K. 2008. Fiddlehead fronds: nutrient rich delicacy. Chron. Hortic. **48**: 12–15.

Demmig-Adams, B. and Adams, W. W. I I I. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. **1**: 21–26.

Demmig-Adams, B. and Adams, W. W. I I I. 2006. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol. **172**: 11–21.

Devore, E. E., Grodstein, F., van Rooij, F. J. A., Hofman, A., Stampfer, M. J., Witteman, J. C. M. and Breteler, M. M. B. 2010. Dietary antioxidants and long-term risk of dementia. Arch. Neur. **67**: 819–825.

Dias, M. G., Oliveira, L., Camões, M. F. G. F. C., Nunes, B., Versloot, P. and Hulshof, P. J. M. 2010. Critical assessment of three high performance liquid chromatography analytical methods for food carotenoid quantification. J. Chromatogr. A. **1217**: 3494–3502.

Ding, Z. T., Fang, Y. S., Gang, Z., Yang, M. H., Xu, Y. Q., Li, F. and Cao, Q. E. 2008. Phenolic content and radical scavenging capacity of 31 species of ferns. Fitoterapia **79**: 581–583.

Dobson, G., Griffiths, D. W., Davies, H. V. and McNicols J. W. 2004. Comparison of fatty acid and polar lipid contents of tubers from two potato species, *Solanum tuberosum and Solanum phureja*. J. Agric. Food Chem. **52**: 6306–6314.

Falk, J. and Munné-Bosch, S. 2010. Tocochromanol functions in plants: antioxidation and beyond. J. Exp. Bot. **61**: 1549–1566.

Fan, Y. Y. and Chapkin, R. S. 1998. Importance of dietary γ-linolenic acid in human health and nutrition. J. Nutr. **128**: 1411–1414.

Farnham, M. W. and Kopsell, D. A. 2009. Importance of genotype on carotenoid and chlorophyll levels in broccoli heads. Hortscience **44**: 1248–1253.

Fetterman, J. W. and Zdanowicz, M. M. 2009. Therapeutic potential of n-3 polyunsaturated fatty acids in disease. Am. J. Health Sys. Pharm. **66**: 1169–1179.

Frank, H. A., Cua, A., Chynwat, V., Young, A., Gosztola, D. and Wasielewski, M. R. 1994. Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. Photosynth. Res. **41**: 389–395.

Freeman, M. P., Fava, M., Lake, J., Trivedi, M. H., Wisner K. L. and Mischoulon., D. 2010. Complementary and alternative medicine in major depressive disorder: The American Psychiatric Association Task Force Report. J. Clin. Psychiatry **71**: 669–681.

Gellerman, J. L., Anderson, W. H. and Schlenk, H. 1972. Highly unsaturated lipids of *Mnium, Polytrichum, Marchantia and Matteuccia*. Bryologist **75**: 550–565.

Girke, T., Schmidt, H., Zähringer, R. R. and Heinz, E. 1998. Identification of a noval 6-acyl-group desaturase by targeted gene disruption in *Physcomitrella patens*. Plant J. **15**: 39–48.

Glew, R. S., Vanderjagt, D. J., Chuang, L. T., Huang, Y. S., Millson, M. and Glew, R. H. 2005. Nutrient content of four edible wild plants from West Africa. Plant Foods Hum. Nutr. **60**: 187–193.

Grimsley, N. H., Grimsley, J. M. and Hartmann, E. 1981. Fatty acid composition of mutants of the moss *Physcomitrella patens*. Phytochemistry **20**: 1519–1524.

Guedes, C. A., Meireles, L. A., Amaro, H. M. and Malcata F. X. 2010. Changes in lipid class and fatty acid composition of cultures of *Pavolva lutheri* in response to light intensity. J. Am. Oil Chem. Soc. **87**: 791–801.

Guil-Guerrero, J. L., Campra-Madrid, P. and Belarbi, E. H. 2000. Linolenic acid purification from seed oil sources by argenated silica gel chromatography column. Proc. Biochem. **36**: 341–354.

Hansen, C. E. and Rossi, P. 1990. Arachidonic acid and eicosapentaenoic acids in *Brachytheciaceae and Hypnaceae* moss species. Phytochemistry **29**: 3749–3754.

Harnack, K., Andersen, G. and Somoza, V.2009. Quantitation of alpha-linolenic acid elongation to eicosapentaenoic and docosahexaenoic acid as affected by the ratio of n6/n3 fatty acids. Nutr. Metab. **6**: doi:10.1186/1743–7075.

Health Canada. 2005. Essential fatty acids. Discussion paper. Report submitted to the Natural Health Products Directorate, Health Canada March Nutritech Consulting Winnipeg, Manitoba. [Online] Available: http://www.hc-sc.gc.ca/dhp-mps/pubs/natur/efa_age-eng.php. [2010 Dec. 22].

Health Canada 2008. Food and nutrition. Nutrient value of some common foods. Vegetables and vegetable products. [Online] Available: http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/nutrient_value-valeurs_nutritives-table4-eng.php [2010 Dec. 22].

Heinrich, M. and Leimkugel, J. 1999. Medicinal plants in the German and European pharmacopoeia. Z. Phytother. **20**: 264–267.

Hodges, D. M. and Forney, C. F. 2003. Postharvest ascorbate metabolism in two cultivars of spinach differing in their senescence rate. J. Am. Soc. Hortic. Sci. **128**: 930–935.

Hodges, D. M. and Lester, G. E. 2006. Comparisons between orange- and greenfleshed non-netted and orange-fleshed netted muskmelons: antioxidant capacity changes following different harvest and storage periods. J. Am. Soc. Hortic. Sci. **131**: 110–117.

Hong, H., Datla, N., MacKenzie, S. L. and Qiu, X. 2002. Isolation and characterization of a 5 desaturase from *Pythium irregulare* by heterologous expression in *Saccharomyces cerevisiae* and oilseed crops. Lipids **37**: 863–868.

Horrobin, D. F. 1992. Nutritional and medical importance of gamma-linolenic acid. Prog. Lipid Res. **31**: 163–194.

Huang, Z., Wang, B., Eaves, D. H., Shikany, J. M. and Pace, R. D. 2009. Total phenolics and antioxidant capacity of indigenous vegetables in the southeast United States: Alabama collaboration for Cardiovascular Equality Project. Int. J. Food Sci. Nutr. **60**: 100–108.

Isabelle, M., Lee, B. L., Meng, T. L., Koh, W. P., Huang, D. and Ong, C. N. 2010. Antioxidant activity and profiles of common vegetables in Singapore. Food Chem. **120**: 993–1003.

Kelley, D. S. 2001. Modulation of human immune and inflammatory responses by dietary fatty acids. Nutrition **17**: 669–673.

Kimura, T., Suzukia, M., Takenakab, M., Yamagishia, K. and Shinmotob, H. 2004. L-O-Caffeoylhomoserine from *Matteuccia struthiopteris*. Phytochemistry **65**: 423–426.

Landrum, J. T. and Bone, R. A. 2001. Lutein, zeaxanthin and the macular pigment. Arch. Biochem. Biophys. **385**: 28–40.

Lefsrud, M. G., Kopsell, D. A., Kopsell, D. E. and Curran-Celentano, J. 2005. Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. HortScience **40**: 2026–2030.

Lefsrud, M. G., Kopsell, D. A., Kopsell, D. E. and Curran-Celentano, J. 2006. Irradiance levels affects growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment. Physiol. Plant **127**: 624–631.

Lefsrud, M. G., Kopsell, D. A. and Kopsell, D. E. 2007. Nitrogen levels influence biomass, elemental accumulations, and pigment concentrations in spinach. J. Plant Nutr. **30**: 171–185.

Lester, G. E., Makus, D. J. and Hodges, D. M. 2010. Relationship between fresh-packaged spinach leaves exposed to continuous light or dark and bioactive contents: effects of cultivar, leaf size, and storage duration. J. Agric. Food Chem. **58**: 2980–2987.

Liou, Y. A., King, J., Zibrik, D. and Innis, S. M. 2007. Decreasing linoleic acid with constant (-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. J. Nutr. **137**: 945–952.

Marc, E. B., Nelly, A., Annick, D. D. and Frederic, D. 2008. Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. J. Ethnopharmacol. **120**: 315–334.

Marik, P. E. and Varon, J. 2009. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. Clin. Cardiol. **32**: 365–372.

Martin, B. A., Schoper, J. B. and Rinne, R. W. 1986. Changes in soybean (*Glycine max* [L.] Merr.) glycerolipids in response to water stress. Plant Physiol. **81**: 798–801.

McClusker, M. M. and Grant-Kels, J. M. 2010. Healing fats of the skin: the structural and immunologic roles of the omega-6 and omega-3 fatty acids. Clin. Dermatol. **28**: 440–451.

Mir, M. 2008. Echium oil: A valuable source of n-3 and n-6 fatty acids. OLC-Oleagineux Corps Gras Lipides. **15**: 252–256.

Munekage, Y., Hojo, M., Meurer, J., Endo, T., Tasaka, M. and Shikanai, T. 2002. PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. Cell **110**: 361–371.

Munné-Bosch, S. and Alegre, L. 2002. The function of tocopherols and tocotrienols in plants. Crit. Rev. Plant Sci. **21**: 31–57.

Muradyan, E. A., Klyachko-Gurvich, G. L., Tsoglin, L. N., Sergeyenko, T. V. and Pronina, N. A. 2004. Changes in lipid metabolism during adaptation of the *Dunaliella salina* photosynthetic apparatus to high CO_2 concentration. Russ. J. Plant Physiol. **51**: 53–62.

Murillo, E., Meléndez-Martínez, A. J. and Portugal, F. 2010. Screening of vegetables and fruits from Panama for rich sources of lutein and zeaxanthin. Food Chem. **122**: 167–172.

Nonato, F. R., Barros, T. A. A., Lucchese, A. M., Oliveira, C. E. C., dos Santos, R. R., Soares, M. B. P. and Villarreal, C. F. 2009. Antiinflammatory and antinociceptive activities of *Blechnum occidentale* L. extract. J. Ethnopharmacol. **125**: 102–107.

Nwosu, M. O. 2002. Ethnobotanical studies on some Pteridophytes of Southern Nigeria. Econ. Bot. **56**: 255–259.

Palacios-Pelaez, R., Lukiw, W. J. and Bazan, N. G. 2010. Omega-3 essential fatty acids modulate initiation and progression of neurodegenerative disease. Mol. Neurobiol. **41**: 367–374.

Pallet, K. E. and Young, A. J. 1993. Carotenoids. Pages 59–89 *in* R. G. Alscher and J. L. Hess, eds. Antioxidants in higher plants. CRC Press, Inc, Boca Raton, FL.

Perry, A., Rasmussen, H. and Johnson, E. J. 2009. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. J. Food Compos. Anal. **22**: 9–15.

Pfundel, E. E. and Dilley, R. A. 1993. The pH dependence of violaxanthin deepoxidaiton in isolated pea choroplasts. Plant Physiol. **101**: 65–71.

Poiana, M. A., Moigradeau, D., Raba, D., Alda, L. M. and Popa, M. 2010. The effect of long-term frozen storage on the nutraceutical compounds, antioxidant properties and color indices of different kinds of berries. J. Food Agric. Environ. **8**: 54–48.

Prior, R. L., Hoang, H., Gu, L., Bacchiocca, M., Howard, L., Hampsch-Woodhill, M., Haung, D., Ou, B. and Jacob, R. 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. J. Agric. Food Chem. **51**: 3273–3279.

Qi, B., Fraser, T., Mugford, S., Dobson, G., Sayanova, O., Butler, J., Napier, J. A., Stobart, A. K. and Lazarus, C. M. 2004. Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nature Biotechnol. **22**: 739–745.

Raju, M., Varakumar, S., Lakshminarayana, R., Krishnakanatha, P. T. and Bashkaran, V. 2007. Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. Food Chem. **101**: 1598–1605.

Renaud, S. M., Zhou, H. C., Parry, D. L., Thinh, L. V. and Woo, K. C. 1995. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzchia paleacea*, and commercial species *Isochrysis* sp. (clone T.ISO). J. Appl. Phycol. **7**: 595–602.

Rodriquez-Amaya, D. B., Kimura, M., Godoy, H. T. and Amaya-Farfan, J. 2008. Updated Brazilian database on food carotenoids: factors affecting carotenoid composition. J. Food Compos. Anal. **21**: 445–463.

Rondanelli, M., Giacosa, A., Opizzi, A., Pelucchi, C., La Vecchia, C., Montorfano, G., Negroni, M., Berra, B., Politi, P. and Rizzo, A. M. 2010. Effect of omega-3 fatty acids supplementation on depressive symptoms and on health-related quality of life in the treatment of elderly woman with depression: a double-blind, placebo-controlled, randomized clinical trial. J. Am. Coll. Nutr. **29**: 55–64.

Ruiz-López, N., Haslam, R. P., Venegas-Calerón, M., Larson, T. R., Graham, I. A., Napier, J. A. and Sayanova, O. 2009. The synthesis and accumulation of stearidonic acid in transgenic plants: a novel source of 'heart-healthy' omega-3 fatty acids. Plant Biotechnol. J. **7**: 704–716.

Sartorelli, D. S., Damião, R., Chaim, R., Hirai, A., Gimeno S. G. A. and Ferreria, R. G. 2010. Dietary ω-3: ω-6 fatty acid ratio predict improvement in glucose disturbances in Japanese Brazilians. Nutrition **26**: 184–191.

Scrimgeour, C. M. and Harwood, J. L. 2007. Fatty acid and lipid structure. The lipid handbook. 3rd ed. CRC Press, New York, NY.

Shargorodsky, M., Debby, O., Matas, Z. and Zimlichman, R. 2010. Effect of long-term treatment with antioxidants (vitamin C, vitamin E, coenzyme Q and selenium) on arterial compliance, humoral factors and inflammatory markers in patients with multiple cardiovascular risks. Nutr. Metab. **7**: 1–8.

Simopoulos, A. P., Norman, H. A., Gillaspy, J. E. and Duke J. A. 1992. Common purslane: A source of omega-3 fatty acids and antioxidants. J. Am. Coll. Nutr. **11**: 374–382.

Simopoulos, A. P. 2004. Omega-3 fatty acids and antioxidants in edible wild plants. Biol. Res. **37**: 263–277.

Simopoulos, A. P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. **233**: 674–688.

Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosophomolybic-phosphotungstic acid reagents. Am. J. Enol. Vitic. **16**: 144–158.

Soejarto, D. D., Fong, H. H. S., Tan, G. T., Zhang, H. J., Ma, C. Y., Franzblau, S. G., Gyllenhaal, C., Riley, M. C., Kadushin, M. R., Pezzuto, J. M., Xuan, L. T., Hiep, N. T., Hung, N. V., Vu, B. M., Loc, P. K., Dac, L. X., Binh, L. T., Chien, N. Q., Hai, N. V., Bich, T. Q., Cuong, N. M., Southavong, B., Sydara, K., Bouamanivong, S., Ly, H. M., Thuy, T. V., Rose, W. C. and Dietzman, G. R. 2005. Ethnobotany/ ethnopharmacology and mass bioprospecting: Issues on intellectual property and benefit-sharing. J. Ethnopharmacol. **100**: 15–22.

Song, W., Derito, C. M., Liu, M. K., He, X., Dong, M. and Liu, R. H. 2010. Cellular antioxidant activity of common vegetables. J. Agric. Food Chem. **58**: 6621–6629.

Spoor, D. C. A., Martineau, L. C., Leduc, C., Benhaddou-Andaloussi, A., Meddah, B., Harris, C., Burt, A., Fraser, M., Coonishish, J., Joly, E., Cuerrier, A., Bennett, S. A. L., Johns, T., Prentki, M., Arnason, J. T. and Haddad, P. S. 2006. Selected plant species from the Cree pharmacopoeia of northern Quebec possess anti-diabetic potential. Can. J. Physiol. Pharmacol. **84**: 847–858.

Sun, T. and Powers, J. R. 2007. Antioxidants and antioxidant activities of vegetables. Pages 160–183 *in* S. Fereidoon and C. T. Ho, eds. Antioxidant measurement and applications. American Chemical Society, ACS Symposium Series 956.

Taha, A. Y., Burnham, W. M. and Auvin, S. 2010. Polyunsaturated fatty acids in epilepsy. Epilepsia **51**: 1348–1358.

Thayer, S. S. and Björkman, O. 1990. Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynth. Res. **23**: 331–343.

Thurnham, D. I. 2007. Macular zeaxanthins and lutein – a review of dietary sources and bioavailability and some relationships with macular pigment optical density and age-related macular disease. Nutr. Res. Rev. **20**: 163–179.

Trumbo, P. R. and Ellwood, K. C. 2006. Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: an evaluation using the Food and Drug Adminstration's evidence-based review system for health claims. Am. J. Clin. Nutr. **84**: 971–974.

Velasco, L. and Goffman, F. D. 1999. Chemotaxonomic significance of fatty acids and tocopherols in Boraginaceae. Phytochemistry **52**: 423–426.

von Aderkas, P. 1984. Economic history of ostrich fern, *Matteuccia struthiopteris*, the edible fiddlehead. Econ. Bot. **38**: 14–23.

Wall, R., Ross, R. P., Fitzgerald, G. F. and Stanton, C. 2010. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. Nutr. Rev. **68**: 280–289.

Wolfe, K. L., Kang, X., He, X., Dong, M., Zhang, Q. and Liu, R. H. 2008. Cellular antioxidant activity of common fruits. J. Agric. Food Chem. **56**: 8418–8426.

Wu, X., Gu, L., Holden, J., Haytowitz, D. B., Gebhardt, S. E., Beecher, G. and Prior, R. L. 2004. Development of a database for total antioxidant capacity in foods: a preliminary study. J. Food Compos. Anal. **17**: 407–422.

Yaniv, Z., Ranen, C., Levy, A. and Palevitch, D. 1989. Effect of temperature on the fatty acid composition and yield of evening primrose (*Oenothera lamarckiana*) seeds. J. Exp. Bot. **40**: 609–613.

Young, A. J. 1991. The photoprotective role of carotenoids in higher plants. Physiol. Plant **83**: 702–708.

Zhang, C. and Bazan, N. G. 2010. Lipid-mediated cell signalling protects against injury and neurodegeneration. J. Nutr. **140**: 858–863.