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CONTENT OF TOCOPHEROL ISOMERS IN OILSEED RADISH CULTIVARS – A SHORT REPORT

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Three oilseed radish (*Raphanus sativus* [cv. Daikon, China Rose, and German]) cultivars from two research farms in Saskatchewan, Canada, were analysed for their tocopherol contents. The findings were compared to tocopherol levels determined in an oriental mustard, a field mustard, a canola, and an arugula cultivar. HPLC data revealed that the oilseed radish cultivars were devoid of both α- and β-tocopherol, but contained γ-tocopherol at levels similarly found in many canola varieties (*i.e.*, 11.4 mg/100 g seed). In addition, they possessed a small content of δ-tocopherol (~1-1.4 g/100 g seed). Application of a YMC™ Carotenoid (C_{30}) column successfully separated and allowed quantification of all tocopherol isomers using reversed--phase HPLC with UV detection in <15 min.

INTRODUCTION

Tocopherols play important roles in the oxidative stability of vegetable oils and in the nutritional quality of crop plants for human and livestock diets [Kamal-Eldin & Appelquist, 1996]. Rapeseed is a rich source of tocopherols, especially of γ-tocopherol [Goglewski *et al.*, 2000; Barrera-Arellano *et al.*, 2002; Matthäus, 2006; Wakamatsu *et al*., 2005]. Antioxidant activity of tocopherols has been confirmed in bulk oil and emulsion model systems [Wagner *et al.*, 2004; Matthäus, 2006] as well as in shelf life and Oxidograph tests [Nogala-Kalucka *et al.*, 2004].

Oilseed radish (*Raphanus sativus* [L.] or *R. sativus* [L.] var. *oleiferus* Metzg [Stokes]) belongs to the *Brassicaceae* family, commonly called mustards. It was originally developed for oil production by crossing radish, fodder rape and rapeseed [Marshall, 2007]. More recently, it has been used as a green manure crop in organic cropping systems, due to its ability to establish easily and produce large amounts of dry matter early in the season. Due to its high lipid content and the fact that oilseed radish cultivars can withstand the stress of cool weather, the plant may also be a rich source of natural tocopherols. Owing to a general lack of information on the profile and content of tocopherols in this cover crop, this study was undertaken. Two mustard samples (*i.e.*, a field and an oriental mustard), a canola cultivar, and an arugula sample were also analysed for comparative purposes.

MATERIALS AND METHODS

Chemicals

A set of high-purity α, β, γ, and δ-tocopherol standards was acquired from EMD Biosciences, Inc. (San Diego, CA).

Plant material

Three oilseed radish varieties (*Raphanus sativus* [cv. Daikon, China Rose, and German]), one oriental mustard (*Brassica juncea* L. [cv. Cutlass]), one field mustard (*Brassica rapa* L. [cv. AC Sunbeam]), one canola (*Brassica napus* L. [cv. 5020]), and one arugula (*Eruca sativa* [cv. Rocket]) were grown at Indian Head (Indian Head Research Farm, Saskatchewan, Canada) and Melfort (Melfort Research Farm, Saskatchewan, Canada) in 2006. All trials had a randomized complete block design with four replications. At harvest time, seeds from these trials were collected and presented for tocopherol analyses.

Preparation of samples for HPLC analysis

All seven seed samples (in triplicate) were extracted 3H for 10 min using a Kinematica® Polytron benchtop homogenizer (Model PT3100; Brinkmann Instruments, Inc., Westbury, NY). Chloroform:methanol (2:1, v/v) at a material-to-solvent ratio of 15 g/100 mL was used. After each extraction, the sample was gravity filtered through Whatman No. 1 filter paper. The solid residue was re-extracted with fresh solvent as described above. During this step, lipids and tocopherols were collected. The solvent was then removed using a Rotavapor® R (BÜCHI Corporation, New Castle, DE) under reduced pressure at ≤45°C. To 4.5 g of the resultant preparation, 50 mL of anhydrous methanol were added. Tocopherols were then extracted into the methanol for 10 min using an Ultrasonik™ Cleaner (Model 104X; Dentsply International/Ceramco Division, York, PA). To remove turbidity, each sample was transferred to a 50-mL polypropylene tube and centrifuged for 5 min. Before chromatographic analysis, the sample was passed through a Whatman GD/X syringe filter (0.45-μm nylon membrane, 25-mm diameter).

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HPLC analysis

The tocopherol contents in the seven varieties of oilseeds were determined by an HPLC protocol. An Agilent 1200 series quaternary pump with degasser, autosampler, diode array detector and ChemStation software were employed. A YMC™ Carotenoid (C_{30}) HPLC column (4.6 H 250 mm, 3 μm – Waters Corporation, Milford, MA), YMC™ Carotenoid S-3 DC guard cartridge (4.0 H 200 mm) and YMC™ direct connect endfitting were used. An isocratic elution was employed at a flow rate of 0.5 mL/min with 100% methanol as the mobile phase. An injection volume of 20 μL was made. UV detection was monitored at 295 nm.

Statistical analysis

Results were reported as mean values ± standard deviation. Regression equations and correlation coefficients between the contents of tocopherols in the samples originating from the two farms (*i.e.*, Indian Head and Melfort, Saskatchewan) were calculated using Excel 2000.

RESULTS AND DISCUSSION

Tables 1 and 2 summarise the mean ± standard deviation for the HPLC tocopherol analyses of the three oilseed radish, two mustard, one canola, and one arugula seed samples supplied from the Indian Head and Melfort farms in Saskatchewan, Canada, respectively. Of the preparations tested, the dominant tocopherol isomer was γ-tocopherol, and it was found in all samples. α-Tocopherol was not detected in any of the oilseed radish cultivars but was present in significant amounts in the field mustard samples, though not statistically ($p>0.05$) different from that of the canola seed sample from the Indian Head farm. Furthermore, δ-tocopherol was only detected in the oilseed radish cultivars and arugula sample. No β-tocopherol or tocotrienols were determined in any of the seven oilseed varieties investigated. As there are no reports to our knowledge on tocopherol contents in oilseed radishes, we have no basis in which to compare these cultivars grown in Saskatchewan with those from other regions.

Even though the field mustard and canola seed samples from the Indian Head farm contained the greatest quantity of tocopherol isomers at 13.9 mg/100 g seed and 13.0 mg/100 g seed, respectively, the oilseed radish samples generally contained between 11 and 12 mg tocopherol/100 g seed. The greatest content of γ-tocopherol (11.4 mg/100 g) was determined in the oilseed radish variety of China Rose, originating from the Indian Head farm.

The content of tocopherols in the same cultivars for each crop originating from the two Saskatchewan farms was well correlated (Figure 1). The best correlation coefficients were found for δ-tocopherol ($r=0.937$) and for α-tocopherol ($r=0.864$). On the other hand, the poorest correlation of $r=0.67$ was determined when the total tocopherol contents from the two farms were compared.

Fully-resolved separations of α-, β-, γ-, and δ-tocopherol (*i.e.*, for both standards and samples) were achieved within a short analysis time (*i.e.*, <15 min) for this work using the YMC™ Carotenoid (C_{30}) column (Figure 2). The YMC™ Carotenoid (C_{30}) column functions as a reversed-phased HPLC column. Normal-phase HPLC is typically employed for tocopherol analyses; however, this new reversed-phase column gave excellent separations with elutions in the order of δ-, γ-, β-, and α-tocopherol. The reversed-phase column allows UV detection to be employed without overlapping of absorbance signals resulting from residual lipid constituents, such as free fatty acids. This simplifies tocopherol analyses when a fluorescent detector is not at hand. Using a silica column, the presence of lipid residues in samples being analysed can, and often do, create problems with the quantitative determination of α-tocopherol. Under these circumstances, a fluorescence detector is necessary to resolve the issue.

TABLE 1. Content of tocopherols in seeds from Indian Head (mg/100 g)[1].

Crop	Cultivar	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
Oilseed radish	Daikon	ND[2]	9.8±0.2	1.4±0.1	11.2±0.3
Oilseed radish	China Rose	ND	11.4±0.3	0.9±0.05	12.3±0.3
Oilseed radish	German	ND	10.0±0.2	1.4±0.1	11.4±0.3
Arugula	Rocket	0.9±0.05	7.9±0.2	0.4±0.05	9.2±0.3
Oriental mustard	Cutlass	2.1±0.1	7.1±0.2	ND	9.2±0.3
Field mustard	AC Sunbeam	2.6±0.1	11.3±0.3	ND	13.9±0.4
Canola	5020	2.8±0.1	10.2±0.3	ND	13.0±0.4

[1]No α, β, γ, or δ-tocotrienols nor β-tocopherol were detected in the seven cultivars investigated. [2]ND – not detected; hence, either absent or below the HPLC's detection limit of 0.1 mg tocopherol/100 g sample.

TABLE 2. Content of tocopherols in seeds from Melfort (mg/100 g)[1].

Crop	Cultivar	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
Oilseed radish	Daikon	ND[2]	9.6±0.2	1.8±0.1	11.4±0.3
Oilseed radish	China Rose	ND	9.7±0.3	1.0±0.05	10.7±0.3
Oilseed radish	German	ND	7.7±0.2	1.3±0.1	9.0±0.3
Arugula	Rocket	0.9±0.05	7.6±0.2	0.4±0.05	8.9±0.2
Oriental mustard	Cutlass	1.9±0.1	6.5±0.2	ND	8.4±0.3
Field mustard	AC Sunbeam	2.4±0.1	9.1±0.3	ND	11.5±0.3
Canola	5020	1.8±0.1	7.2±0.3	ND	9.0±0.3

[1]No α, β, γ, or δ-tocotrienols nor β-tocopherol were detected in the seven cultivars investigated. [2]ND – not detected; hence, either absent or below the HPLC's detection limit of 0.1 mg tocopherol/100 g sample.

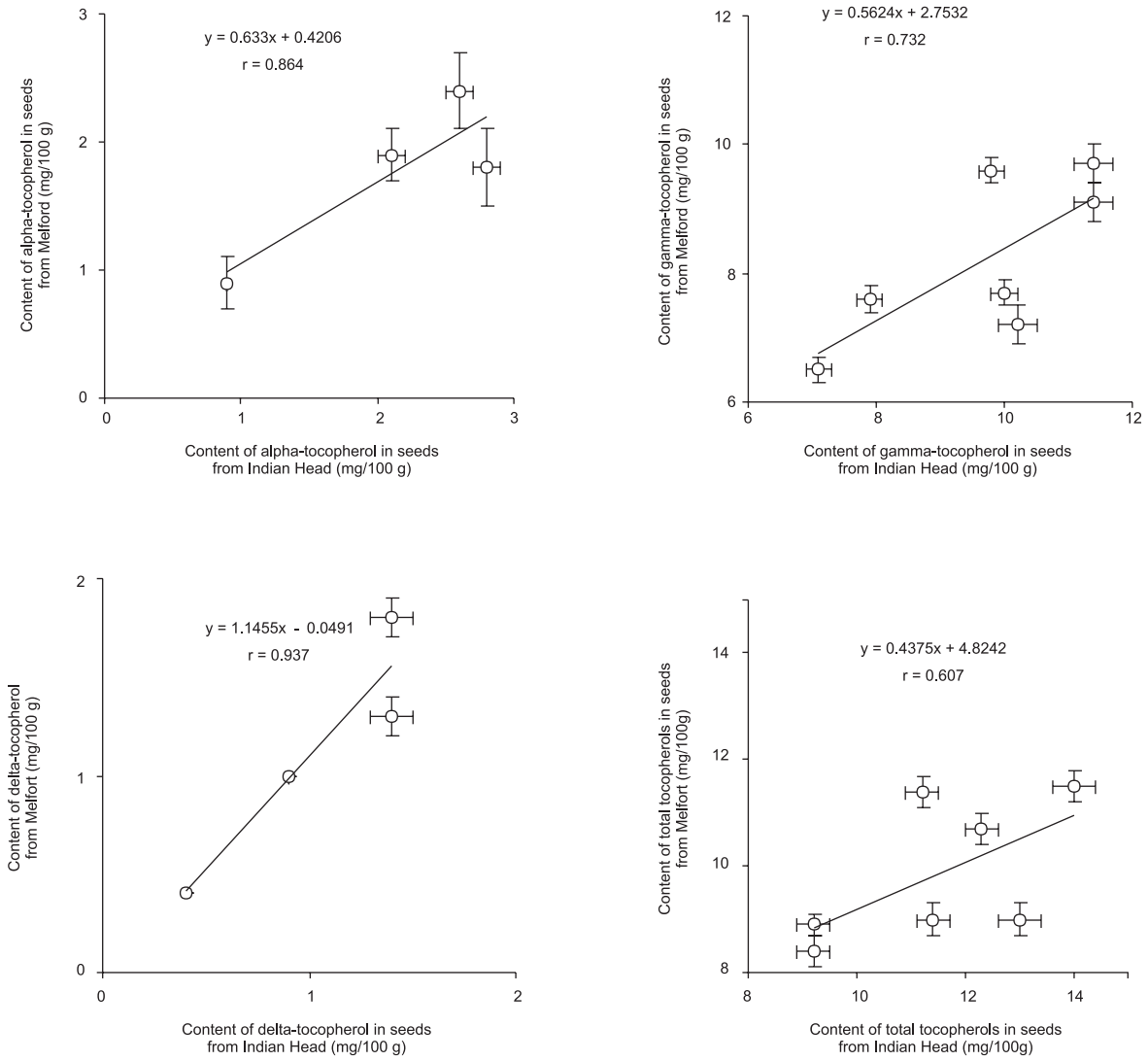

FIGURE 1. Correlation between the contents of tocopherol isomers in the samples originated from the two Saskatchewan farms.

The content of γ-tocopherol in oilseed radish found in this study (*i.e*., 11.4 mg/100 g seed) is typical for rapeseed [Goglewski *et al.*, 2000; Nogala-Kalucka *et al*., 2004; Matthäus, 2006], mustard [Mortuza, 2006], sorghum and mung bean [Choi *et al*., 2007], rice bean [Mukherjee & Darkar, 1991] as well as buckwheat [Zieliński *et al*., 2001]. A high content of α-tocopherol has been reported in rapeseed (*i.e.*, the same value as that of γ-tocopherol) by Wakamatsu *et al*. [2005] and Goffman & Becker [2001]. α-Tocopherol was completely devoid in the three oilseed radish cultivars, which is somewhat surprising considering that they are a product from the crossing of radish, fodder rape and rapeseed. α-Tocopherol is the dominant isomer for wheat, rye, oat, rice, bran, barley, amaranth, olive, sunflower, and palm [Bustamante-Rangel *et al*., 2007; Aguilar-Garcia *et al*., 2007; Petersom, 1995; Holasova 1997; Petersom & Qureshi, 1993; Lehmann *et al.*, 1994; Barrera-Arellano *et al.*, 2002]. Small quantities of δ-tocopherol (~1-1.4 mg/100 g) were detected in all oilseed radish cultivars; a high content of δ-tocopherol is typical for pumpkin seed oil [Stevenson *et al.*, 2007].

The content of lipid in oilseed radish samples was *ca*. 35%. The contents of γ-tocopherol determined in the oil from the oilseed radish cultivars were higher or similar to those reported for rapeseed oil: 32 mg/100 g oil [Barrera-Arellano *et al.*, 2002], 33.4 mg/100 g oil [Matthäus, 2006], 31.6 mg/100 g oil [Goglewski *et al.*, 2000], and 33.0 mg/100 g oil [Wakamatsu *et al*., 2005].

The dominant content of γ-tocopherol in oilseed radish is a very positive observation. According to Wagner *et al.* [2004], γ-tocopherol is more effective than α-tocopherol against autoxidation in emulsions. Sinapic acid derivatives, of which oilseed radishes are a rich source, exhibited a synergetic antioxidant effect in combination with γ-tocopherol in bulk oil systems [Thiyam *et al.*, 2006]. Even though α-tocopherol is regarded as having the greatest bioavailability, emerging research has found that γ-tocopherol may have greater antioxidant activities than α-tocopherol [Wagner *et al.*, 2004; Jiang *et al.,* 2001; Saldeen & Saldeen, 2005] and that the latter may suppress the bioavailability of the former isomer [Wolf, 2006]. In which case, the marked γ-tocopherol content in oil-

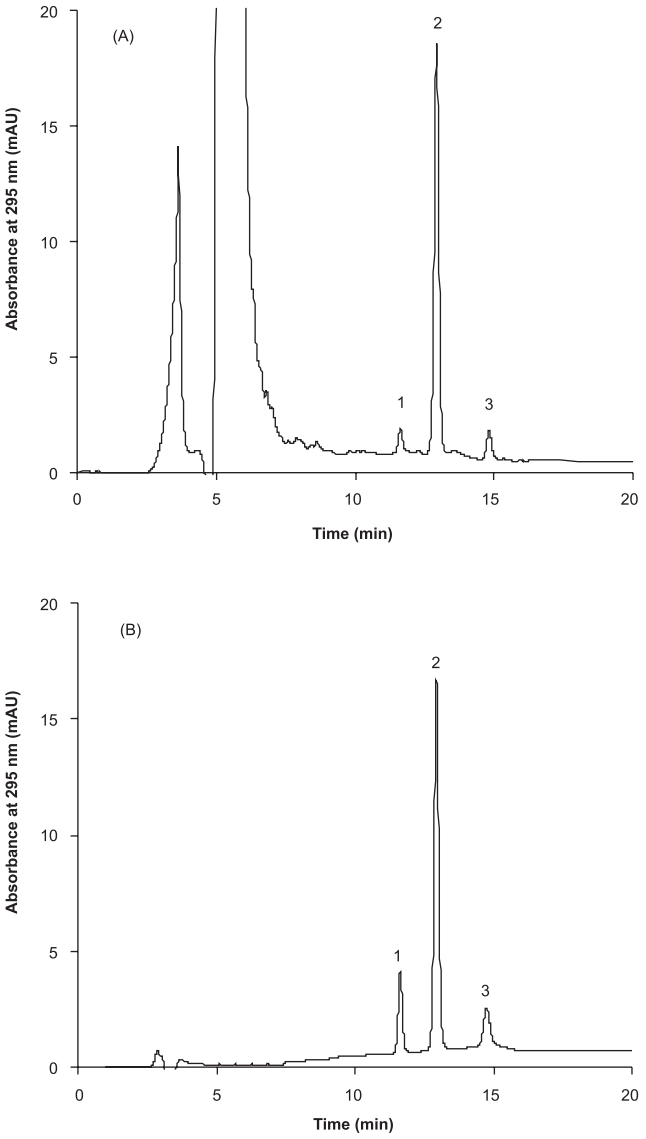

FIGURE 2. HPLC chromatogram for the δ-, γ-, and α-tocopherol standards (*i.e.*, peaks 1, 2, and 3, respectively) (A) and the respective tocopherols extracted from the China Rose cultivar of oilseed radish (B).

seed radish cultivars could make the oil attractive from a nutritional perspective.

CONCLUSIONS

This study is the first which reports on the tocopherol content in oilseed radish (*Raphanus sativus* [L.] var. *oleiferus* Metzg [Stokes]). The investigated oilseed radish cultivars (Daikon, China Rose, and German) contained neither α- or β-tocopherol, but had a prominent content of γ-tocopherol at levels commonly determined in many canola varieties (*i.e.*, 11.4 mg/100 g seed), as well as containing a small amount of δ-tocopherol (~1-1.4 g/100g seed). The dominant content of γ-tocopherol in oilseed radish could be very important for future applications of this cover crop. Recent research has suggested that γ-tocopherol may have greater significance biologically than previously thought. Hence, the marked γ-tocopherol content in oilseed radish cultivars could make the oil attractive in functional food and nutraceutical formulations.

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