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Fatty acid, carotenoid and tocopherol compositions of 20 Canadian lentil cultivars and synergistic contribution to antioxidant activities

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ABSTRACT

Understanding the profile of lipophilic phytochemicals in lentils is necessary to better understand the health benefits of lentils. The fatty acid, carotenoid and tocopherol compositions and antioxidant activities of the lipophilic extracts of 20 lentil cultivars (10 red and 10 green) were therefore examined. Lentils contained 1.52–2.95% lipids, of which 77.5–81.7% were unsaturated essential fatty acids. Total tocopherols ranged from 37 to 64 μg/g DW, predominantly γ-tocopherol (96–98% of the tocopherol content), followed by δ- and α-tocopherol. *trans*-Lutein was the primary and major carotenoid (64–78%) followed by *trans*-zeaxanthin (5–13%). Carotenoids and tocopherols showed weak correlation with 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity ($r = 0.4893$ and 0.3259, respectively), but good correlation when combined ($r = 0.6688$), suggesting they may act synergistically. Carotenoids were found to contribute the most to the strong antioxidant activity measured by photochemiluminescence (PCL) assay. Results from this study contribute to the development of lentil cultivars and related functional foods with increased health benefits.

1. Introduction

Legumes are the oldest crops cultivated by humans and are one of the most important foodstuffs consumed in Europe, the Middle East, Africa, and South Asia (Gumienna, Lasik, & Czarnecki, 2009). In some developing countries, legumes are consumed as a basic staple food with high quality and low cost protein alternatives to animal proteins (Marathe, Rajalakshmi, Jamdar, & Sharma, 2011).

Among legumes, lentils (*Lens culinaris*) have been gaining increasing attention for their nutritive value. They are considered as a potential whole food source for people affected by micronutrient malnutrition (Thavarajah, Thavarajah, Sarker, & Vandenberg, 2009). Canada is by far the world's largest lentil producer with an annual production of approximately 4 megatons accounting for 25% of the total world lentil output (Thavarajah et al., 2009). On average, while global pulse consumption is declining, the annual consumption of lentils is steadily increasing (Zou, Chang, Gu, & Qian, 2011). Lentils are an excellent source of both macronutrients, micronutrients and phytochemicals (Dueñas, Hernández, & Estrella, 2002). Phytochemicals of lentils may provide potential health benefits for humans. Epidemiological and interventional studies suggest that legume consumption, including lentils, is inversely associated with the incidence of several chronic diseases, for example, coronary heart disease, type II diabetes mellitus, cardiovascular diseases, cancer and aging (Amarowicz & Pegg, 2008; Villegas et al., 2008). However, the use of lentils in food products has been limited in western countries, due to traditional eating customs, lack of consumer understanding, processing techniques and available diversified food products. Incorporation of lentils into western diets has been highly recommended (Aguilera et al., 2010; Han & Baik, 2008).

While many research groups have focused their studies on bioactive components and attributed the potential health benefits to the antioxidant activities of hydrophilic phytochemicals such as phenolics in lentils, available information on the bioactive compositions and antioxidant activities of lipophilic compounds from lentils are lacking (Dueñas, Sun, Hernández, Estrella, & Spranger, 2003; Xu & Chang, 2010). There are a few studies on other legumes and cereals in terms of their fatty acid profile and lipophilic

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phytochemical contents, such as tocopherols and carotenoids (Boschin & Arnoldi, 2011; Konopka, Czaplicki, & Rotkiewicz, 2006). In these legumes and cereals, lutein was found to be the main carotenoid (Abdel-Aal, Young, Rabalski, Hucl, & Fregeau-Reid, 2007). Tocopherols and carotenoids are strong antioxidants. Lutein is a non-provitamin A oxygen-containing carotenoid or xanthophyll that has been associated with reduced incidence of age-related macular degeneration, cataracts, cancer, and cardiovascular disease (Olmedilla, Granado, Blanco, Vaquero, & Cajigal, 2001; Osganian et al., 2003). It has also been found to play significant roles in promoting the health of eyes and skin (Yao et al., 2013). Understanding the profile of lipophilic phytochemicals in pulse foods, particularly lentils is therefore necessary, in order to better understand how these micronutrients in lentils contribute to health benefits and how these bioactive components are retained at maximal levels throughout the food processing chain.

The new trend in pulse consumption and its potential health benefits require a closer look at the possible bioactive components in grain legumes like lentils. There are many commercial lentil cultivars, but the largest collection of such are found in Canada. In the present study, we have selected 20 popular red and green Canadian lentil cultivars, and conducted a comprehensive analysis of the fatty acid profile, the contents of total and individual carotenoids, tocopherols, as well as the antioxidant activities of the lipophilic extracts that contained these compounds using two chemical-based models, i.e. the photochemiluminescence (PCL) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Results from this study will provide support for selecting lentil cultivars with improved nutritional value, and lead to further study on how these bioactive components contribute to human health.

2. Materials and methods

2.1. Plant materials

The 20 lentil cultivars and breeding lines used for this study were received from Saskatchewan Pulse Growers (Canada) on September 26, 2012. The whole lentil samples were ground into fine powder, and stored in sealed plastic bags at −4 °C prior to analysis. The 20 tested lentils are categorised into 2 groups based on their colours: 10 red lentils: Blaze, Redcliff, Maxim, Rouleau, Redbow, Redberry, Impact, Imperial, Rosetown, and Dazil; 10 green lentils: Imvincible, Greenland, Asterix, Imigreen, Impower, Improve, Sovereign, Milestone, Eston and Plato.

2.2. Chemicals and reagents

All-*trans*-lutein and all-*trans*-zeaxanthin standards were purchased from Indofine (Belle Mead, NJ); fluorescein, Trolox and DPPH were obtained from Sigma (St. Louis, MO). All HPLC-grade solvents, including methanol, methyl *tert*-butyl ether (MTBE), tetrahydrofuran, hexane and isopropanol were purchased from Caledon Laboratories (Georgetown, ON, Canada). All other chemical reagents used were of analytical grade.

2.3. Colorimetric study

The colour of ground samples was measured at room temperature using a Minolta Chromometer (Chroma Meter CR-300; Minolta Camera Co. Ltd., Osaka, Japan) according to the method of Li et al. (2011). The chromometer consisted of an 8-mm-diameter measuring area and diffuse illumination/viewing. The tristimulus values of CIE L^*, a^*, b^* readings were calibrated against a standard calibration white plate. CIE 1976 uniform colour space was taken into account for the colorimetric analysis. Within the CIELAB uniform space, psychometric index of brightness L^* measures the whiteness value of a colour and ranges from black at 0 to white at 100, while chromaticity coordinate a^* is measured for redness when positive and greenness when negative, and b^* indicates yellowness when positive and blueness when negative. The values a^* and b^* were used to calculate the hue angle (H = arctan (b^*/a^*)) and metric chroma value ($C = (a^{*2} + b^{*2})^{1/2}$). The data of each measurement are the average of triplicate measures on equidistant points of the sample.

2.4. Extraction of lipophilic fraction

The lipophilic fraction of lentils was extracted from the finely ground samples following a slightly modified version of the method described by Villalobos Solis, Patel, Orsat, Singh, and Lefsrud (2013). Briefly, *ca.* 1 g lentil sample was accurately weighed and placed in a 15-mL Teflon-lined screw-capped glass centrifuge tube and extracted with 10 mL hexane/isopropanol (3:2, v/v) at room temperature with constant rolling on a rotary shaker (Scientific Industries Inc., Bohemia, NY) at 150 rpm for 5 h. The mixture was then vortexed for 30 s followed by centrifugation (Eppendorf centrifuge 5810R, Brinkman Instruments Inc., Westbury, NY) at 3000*g* for 10 min and the supernatant was collected in a glass centrifuge tube. The residue was re-extracted with the same method until it was colourless. The combined supernatant was then evaporated under nitrogen stream at room temperature, and re-dissolved in 1 mL tetrahydrofuran and stored at −20 °C before being used for different analyses. All extraction experiments were conducted under subdued light, and the extraction tubes were wrapped with aluminium foil to avoid sample degradation by photooxidation.

2.5. Fatty acid analysis by gas chromatography

The fatty acid composition of the lipophilic fraction extracted from lentils was analysed according to the method described by Kramer et al. (1997). Fatty acid methyl esters (FAME) were prepared using base-catalysed reactions. Briefly, 10 mg of lipids were placed in a 15-mL glass tube equipped with Teflon-lined screw cap and dissolved in 80 μL of toluene. One millilitre of $NaOCH_3$/methanol (0.5 N) was then added for the methylation and heated for 30 min at 50 °C. After cooling to room temperature, 1 mL of water was added to the solution, and the esters were extracted with 2 mL of hexane. FAME were analysed by GC (Model 6890; Hewlett–Packard, Palo Alto, CA) using a CP-Sil 88 WCOT fused silica column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Chrompack, Middleburg, The Netherlands). Column was operated at 45 °C for 4 min, then temperature-programmed at 13 °C/min to 175 °C, held for 27 min, programmed at 4 °C/min to 215 °C, and finally held for 31 min; total run time was 80 min, the sample injection volume was 1 μl, injection mode was splitless. Flame ionisation detection (FID) was used.

2.6. Determination of tocopherols by HPLC

Tocopherols were analysed following a published procedure with slight modification (Li, Tsao, Yang, Kramer, & Hernandez, 2007). Agilent Technologies 1100 series HPLC system equipped with a quaternary pump, a degasser, a thermostatic autosampler, a diode-array detector (DAD) and a fluorescence detector was used for the analysis of tocopherols in the lipophilic extracts of lentils. The latter detector was used for the quantification of individual tocopherols. The detection was set at 295 nm for DAD, and λ_{exc} = 290 nm, λ_{emis} = 330 nm, for the fluorescence detector. A Phenomenex (Torrance, CA) silica column (250 × 4.6 mm, 5 μm) was used for separation. The mobile phase contained 10% *tert*-butyl methyl ether in hexane (*v/v*), and the flow rate was kept constant