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Short Communication

Seed composition of ten industrial hemp cultivars approved for production in Canada

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ABSTRACT

The objective of this study was to determine the seed chemical composition of ten industrial hemp cultivars grown in Québec. The fatty acid and tocopherol composition, as well as the concentrations of crude protein, oil, ash, cellulose, hemicellulose and lignin were quantified. The seed oil concentration varied between 269 and 306 g/kg, while the crude protein concentration ranged between 238 and 280 g/kg. The hemp seed oil is mainly composed of unsaturated fatty acids, and the dominant fatty acids are linoleic acid (597 g/kg) and α-linolenic acid (170 g/kg). For all ten cultivars, γ-tocopherol was present at a much higher concentration than δ-tocopherol (2481 vs. 774 μg/g). Out of the ten cultivars analyzed, Anka was the richest in phenolics (5.16 g/100 g), whereas CRS-1 had the lowest phenolic content (1.37 g/100 g). Seed ash concentration ranged between 51 and 58 g/kg, while neutral detergent fibre and acid detergent fibre concentrations ranged between 327 and 388, and 259 and 298 g/kg, respectively. In conclusion, our results reveal noticeable differences among cultivars in terms of the essential fatty acid, oil, protein, and antioxidant content of industrial hemp seed. Collectively, this study suggests that the seed of Canada-grown hemp is a balanced health product.

1. Introduction

Hemp (*Cannabis sativa* L., Cannabaceae) is an herbaceous, wind-pollinated annual plant that is believed to have originated in central Asia (Li, 1973, 1974; Zlas et al., 1993; Oliver and Joynt, 1999; Oomah et al., 2002; Small and Antle, 2003; van Bakel et al., 2011). Traditionally considered a multiuse crop, industrial hemp has been widely cultivated and used throughout history for its fibre, nutritional and medicinal properties (Kriese et al., 2004; Vera and Hanks, 2004; Tang et al., 2006; House et al., 2010). The bast fibre isolated from the stalk is still used today for the production of rope and paper (Ranalli and Venturi, 2004). In addition, fast-growing market segments for hemp products include hemp food and body care products. Hemp seed oil can be used in the manufacturing of cosmetics and soaps (Ranalli and Venturi, 2004). Markedly, hemp grain and hemp seed oil are ingredients in many certified organic foods, which have been gaining greater popularity in recent years.

Despite its versatility, the cultivation of hemp in Canada was prohibited beginning in the 1930s due to the presence of the secondary metabolite delta-9-tetrahydrocannabinol (THC) in parts of the plant (Oomah et al., 2002; Tang et al., 2006). This ban was partly lifted in 1998 and the cultivation of hemp varieties containing less than 0.3% THC is currently permitted in Canada, provided that a license from Health Canada has been acquired (Oliver and Joynt, 1999; Vera and Hanks, 2004; Tang et al., 2006).

Industrial hemp plants can potentially reach heights of up to 5 m at maturity, although some oil cultivars (such as Finola) can be quite short in comparison to those bred primarily for fibre production (Oliver and Joynt, 1999; Callaway, 2004). In the cultivars bred mainly for the production of fibre, the seed component is often overlooked simply as a by-product (Oomah et al., 2002; Kriese et al., 2004). Nonetheless, hemp seeds have proven to be of great nutritional value, generally composed of

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250–350 g/kg lipids, 20–25 g/kg protein, and 20–30 g/kg carbohydrates (Oliver and Joynt, 1999; Leizer et al., 2000; House et al., 2010). In particular, the lipid portion of hemp seeds is very rich in essential fatty acids, consisting of large amounts of linoleic acid (omega-3) and α-linolenic acid (omega-6), often in a favourable 3:1 ratio (Deferne and Pate, 1996; Oomah et al., 2002; Carvalho et al., 2006; Matthäus and Brühl, 2008; Chen et al., 2010; Da Porto et al., 2012; Teh and Birch, 2013). Notably, hemp seeds are also a good source of highly digestible protein, well-suited for human and animal consumption (Deferne and Pate, 1996; Mustafa et al., 1999; Callaway, 2004; Tang et al., 2006).

The objective of this study was to determine the seed chemical composition of ten industrial hemp cultivars grown in southern Québec, which are also currently widely cultivated and transformed throughout Canada. More specifically, the seed fatty acid and tocopherol composition, crude protein, oil, ash, and cellulose, hemicellulose, and lignin concentrations were determined and quantified for high-yielding hemp grain varieties (CanMa, CFX-2, CFX-1, CRS-1 and Finola) and dual-purpose varieties suitable for both fibre and seed uses (Alyssa, Anka, Delores, Jutta and Yvonne).

By comparing several varieties that display differing characteristics, this integrative study provides more comprehensive information than studies focusing on the nutritional value of a single variety/product. Importantly, this work characterizes multiple common Canadian industrial hemp cultivars that have yet to be studied in depth. Overall, this study better defines hemp as a natural source of functional ingredients and will serve as a stepping stone for further research initiatives fostered by the recent ratification of the 2014 Agricultural Act of the United States of America, which authorizes institutions of higher education or state departments of agriculture to regulate agricultural pilot programs for hemp (U.S. Government Printing Office, House of Representatives, 2014).

2. Materials and methods

2.1. Materials

The seeds of ten industrial hemp cultivars were obtained from approved suppliers: CanMa (Ferme Éliro/Moulin A. Coutu Inc., Canada); Anka, Jutta and Yvonne (Stone Farms, Canada); Delores (Parkland Industrial hemp Growers, Canada); CFX-1, CFX-2 and CRS-1 (Hemp Genetics International Inc., Canada); Finola (Hemp Oil Canada Inc, Canada).

The ten cultivars of industrial hemp were grown in 2012 in Sainte-Anne-de-Bellevue, QC, Canada (45°25′ N, 73°56′ W) in a randomized complete block design with three replications according to the term specified in the licenses #12-C0142-R-01, 13-C0142-R-01, 14-C0142-R-01 delivered to Dr. Jean-Benoit Charron by Health Canada (Minister of Justice, 2014). Planting was done in a sandy soil at a rate of 43 kg/ha in late May using a disk-drill in a prepared seedbed. Plots were 1.3 by 5 m and row spacing was 18-cm. Plants were harvested at maturity in late August and September, date varying depending on the cultivar. Plants were harvested by hand and threshed using a grain combine (Wintersteiger, Austria). The THC concentration of plants during the growing season was determined by gas chromatography using a governmental accredited laboratory (RPC, NB, Canada). The concentrations observed ranged between 0.04 and 0.23%, which are below the authorized limit of 0.3% set by Health Canada.

2.2. Proximate analysis

Dry matter, ash, crude protein ($N \times 6.25$), and ether extract concentrations were determined according to the standard procedures of the Association of Official Analytical Chemists (AOCS, 2003). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed using an Ankom fibre Analyzer (Ankom Technology, Macedon, NY, USA). The analysis for NDF was performed without the inclusion of sodium sulfite and with the inclusion of heat-stable α-amylase (Van Soest et al., 1991). Acid detergent lignin analysis of feed samples was conducted following AOACS procedures (AOCS, 2003).

2.3. Tocopherol composition

Tocopherol concentrations were determined using a modified version of the protocol of Kitamura et al. (2007) as presented in Chennupati et al. (2011).

2.4. Fatty acid composition

Lipid extraction and methyl ester synthesis were conducted according to O'Fallon et al. (2007). Fatty acid composition of the fatty acid methyl esters was determined by capillary gas chromatography (Varian model 3900 equipped with flame ionization detector at 260 °C and model 1177 auto injector) fitted with a fused silica capillary column (CP7489, 100 m × 0.25 mm; Varian, CA, USA). The carrier gas was H_2, and the flow rate was 0.8 mL/min. Injector and detector temperatures were 260 °C, and the split ratio was 50:1. Column temperature was set at 70 °C for 4 min, and then increased to 130 °C at a rate of 12.0 °C/min and was maintained for 3 min. It was then increased to 175 °C at a rate of 4 °C/min and was maintained for 27 min. Finally, the temperature was increased to 214 °C at a rate of 4 °C/min and maintained for 11 min and increased to 225 °C at a rate of 4 °C/min and held for 5.5 min; therefore, total run time was 79.25 min. Fatty acids were identified by comparing their retentions times with fatty acid methyl standards (NuCheck Prep Inc., Elysian, MN, USA).

2.5. Determination of total phenolic compounds

Finely powdered, dried samples of 0.1 g were placed into 1.5 mL Eppendorf tubes and 900 μL of 90% methanol was added and vortexed. The samples were sonicated in a darkened cold room for 30 min and centrifuged for 10 min at 3000 rpm at 4 °C. The supernatant was collected, 600 μL of 90% methanol was added to the pellet, the extraction and centrifugation process repeated, and supernatants combined for antioxidant assessment. Total phenolics were measured using the Folin-Ciocalteu method (Singleton and Rossi, 1965). A 100 μL volume of methanolic sample extract or standard was added to 2 mL of distilled water. Then, 200 μL of Folin-Ciocalteu reagent (2 N) was added to the sample, vortexed, and incubated at room temperature for 30 min. Following incubation, 1.0 mL of aqueous sodium carbonate solution (20%) was added, the solution vortexed again, and then kept at room temperature for 1 h before sample absorbance was read at 765 nm using a Beckman Spectrophotometer (Beckman Coulter, CA, USA). The total phenolic concentration of the sample was expressed as gallic acid equivalents (GAE) in mg per 100 g dry weight (DW).

2.6. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in SAS to identify significant model and treatment effects (SAS Institute, 2003).

3. Results and discussion

3.1. Proximate composition

Typically, whole hemp seed contains approximately 250–350 g/kg oil and 200–250 g/kg crude protein (House et al., 2010;