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Original Research Article

# Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils

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ABSTRACT

New Zealand cold-pressed hemp, flax and canola seed oils were analyzed for their fatty acid compositions, tocopherols, β-carotene, chlorophyll, total phenolics, flavonoids, color, quality, melting and crystallization characteristics. The dominant fatty acid of canola, hemp and flax seed oils was oleic (57.0 ± 0.0%), linoleic (55.7 ± 0.3%) and linolenic acids (58.7 ± 1.2%) respectively ($p < 0.05$). Hemp seed oil contained the highest tocopherol, flavonoid and phenolic acid contents. There was a significant difference in color for the oils ($p < 0.05$) due to the chlorophyll content in the oil. Melting and crystallization transitions and $\Delta H$ values varied for the three oils in the order canola > flax > hemp. All oils had low moisture and volatiles, unsaponifiable matter and free fatty acids. Peroxide value, *p*-anisidine, conjugated dienoic acid, acid value, specific extinction of cold-pressed oils at 232 and 270 nm were under the limits allowed in general regulations.



## 1. Introduction

Cold-pressed oils refer to oils that are extracted by cold-pressing plant seed with a screw press or hydraulic press. Cold-pressing is used to extract oil from plant seed instead of conventional solvent extraction method because cold-pressing does not require the use of organic solvent or heat (CAC, 2001). Hence, cold-pressing is able to retain bioactive compounds such as essential fatty acids, phenolics, flavonoids and tocopherol in the oils.

Hemp (*Cannabis sativa*), flax (*Linum usitatissimum*) and canola (*Brassica napus*) seed oils are examples of oils that are extracted by cold-pressing. Hemp, flax and canola seeds contain approximately 31, 40 and 42% of oil content respectively (Callaway et al., 2002; Oomah, 2001; Carvalho et al., 2006). Cold-pressed oils are considered as healthy oils that are important to human nutrition due to their favorable polyunsaturated fatty acid content, notably α-linolenic acid (C18:3; *n*-3) and linoleic acid (C18:2; *n*-2) (Simopoulos et al., 2000)

Hemp seed oil possesses a well-balanced proportion of linoleic acid (C18:2; *n*-6) and α-linolenic acid (C18:3; *n*-3) in the ratio of between 2:1 and 3:1 (Simopoulos et al., 2000). The authors documented that *n*-6/*n*-3 ratio of hemp seed oil is similar to the *n*-6/*n*-3 ratio found in Mediterranean and Japanese diets where low numbers of coronary heart disease were found in people who had these diets. The *n*-6/*n*-3 ratio of flax seed oil was approximately 1:3, where the *n*-3 content was about 50% (Daun et al., 2003). Canola seed oil contains about 70% oleic acid and 10.30% linoleic acid (Batista et al., 2011).

Essential fatty acids namely linolenic acid and linoleic acid, cannot be synthesized by the human body. Hence, they can be only obtained from food. Linolenic acid possesses beneficial properties to human health such as anti-inflammatory, antihypertension, anti-vasoconstrictive, anti-cancer, anti-thrombotic, lowering of low-density lipoprotein (LDL), increasing metabolic rates and fat burning (Erasmus, 1999). In addition, essential fatty acids play a vital role in the development of membrane phospholipid bilayers of cellular and organelle membranes (Oliwiecki et al., 1991). Previous clinical studies proved that essential fatty acids and other polyunsaturated fatty acids are able to heal wounds and increase immunity (Harbige et al., 2000).

Cold-pressed seed oils contain other bioactive compounds that exert health benefits. This has drawn interest among researchers to study the bioactive compounds in the oils. For example, tocopherol contents in the oils function as powerful antioxidants, vitamin E analogs, anticancer and anti-cardiovascular disease agents (Wolf, 1997; Léger, 2000). In addition, polyphenols such as phenolics and flavonoids exhibit therapeutic properties such as anti-microbial, anti-inflammatory,

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anti-thrombotic, anti-allergenic, anti-artherogenic, antioxidant, anti-cardiovascular disease and vasodilatory effects (Balasundram et al., 2006).

However, the high amounts of polyunsaturated fatty acids in cold-pressed oils are prone to lipid oxidation. Hence, the quality of cold-pressed oils needs to be monitored for consumer safety purposes. Most of the oil quality tests such as peroxide, non-volatile carbonyl compounds and conjugated diene hydroperoxides are used to determine the oxidation compounds in the oils. The objective of the research was to analyze the fatty acid compositions and bioactive compounds in cold-pressed hemp, flax and canola seed oils and monitor the physicochemical quality characteristics of the oils. As there is no specific standard for the oil quality, Codex Alimentarius Commission (1999) standard for virgin oils and cold-pressed fats and oils, New Zealand Food Regulation (1984) for edible and virgin fats and oils, and Australia New Zealand Food Authority (2000) for edible oils are used as a guideline to monitor the quality of cold-pressed oils.

## 2. Materials and methods

### 2.1. Materials

Cold-pressed hemp and flax seed oils were supplied by Oil Seed Extractions Limited, Ashburton, New Zealand. Cold-pressed rape seed oil was sourced from New Zealand Vegetable Oil Limited, Canterbury, New Zealand. There was only one sample for each type of oil. The oils were stored under $N_2$ gas at 0 °C prior to analysis. All the chemicals used were of analytical grade unless stated otherwise.

### 2.2. Fatty acid composition

Fatty acid methyl esters (FAME) were prepared from the cold-pressed oils according to the method of Choo et al. (2007) which is the partially modified method of Van Wijngaarden (1967). Oil sample (20 mg) was mixed with 2 mL methanolic sodium hydroxide (0.5 N) in a Teflon-lined screw cap glass tube. The mixture was boiled for 20 min and cooled to room temperature. Diethyl ether (2 mL) and 5 mL water were added into the mixture before it was mixed. The ether layer (upper layer) was separated for further analysis while the remaining aqueous layer was acidified with concentrated HCl until acid to Litmus. The fatty acids were recovered into diethyl ether (2 mL) and backwashed with NaCl (5%). Later, $BF_3$-methanol reagent (BDH Laboratory Supplies, Poole, England; 1 mL) was added and the solution refluxed for 20 min, followed by further backwashing of the FAME (upper layer). The fatty acid composition of each oil sample was determined by gas chromatography using an Agilent 6890N with an autosampler injector (Agilent 7683), a flame ionization detector (Agilent Technologies Inc., Wilmington, DE, USA), BPX70 capillary column (50 m × 330 μm, SGE International Pvt. Ltd., Victoria, Australia) and HP Chemstation software (Hewlett Packard, Agilent Technologies Inc., Wilmington, DE, USA). Carrier gas, hydrogen, was set at a flow rate of 2 mL/min while the temperature of the injector and detector was set at 250 °C. Injection sample was 1 μL with a split ratio of 40:1. The temperature of column was set from 35 to 205 °C (2.5 °C/min) and then to 230 °C (10.0 °C/min). The FAME Q005 mixture (Nu-Check Prep Inc., Elysian, MN, USA) was used as the FAME standard for the determination of FAME in oil samples.

### 2.3. Tocopherol and β-carotene composition

The ether layer (upper layer) which contained the unsaponifiable matter during preparation of FAME was evaporated and the extract dissolved in 200 μL of mobile phase, acetonitrile/methanol/chloroform (47/47/6, v/v/v). Extract solution (25 μL) was injected into an HPLC system (Agilent 1100 series) with the flow rate of mobile phase, 1.2 mL/min isocratically, The HPLC system was equipped with a G1311A Quaternary Pump, G1315A Diode Array Detector, G1313A Autosampler, G1322A Vacuum Degasser, guard column (Altech, United States), an analytical C18 column (150 mm × 460 mm, 5 μm, Phenomenex, Torrance, CA, USA) and an Agilent Chemstation for LC3D flev. A. 10.02 (1757) integration software. Beta-carotene (Sigma Chemical Co., St. Louis, MO, USA) and mixed tocopherol standards that consisted of α-, γ-, δ-tocopherols (Sigma Chemical Co., St. Louis, MO, USA) and rac-β-tocopherol (Supelco, Bellefonnte, PA, USA) were prepared with a known concentration in hexane. All the chemicals used were HPLC grade.

### 2.4. Chlorophyll content

Chlorophyll content of oil samples was determined according to A.O.C.S. Method Cc 13i-96.

### 2.5. Total phenolic acids and flavonoids

In order to extract the phenolic acids and flavonoids, 50 mL hexane was added into 10 g of oil followed by triple extraction of 20 mL aqueous methanol (60%, v/v) and the combined extracts were dried in a vacuum rotary evaporator at 40 °C (AOCS, 2003). About 1 mL of methanol was added into the dry residue which was stored at −20 °C prior to analysis. The determination of total phenolic acids in oil was based on the method of Gutfinger (1981). Firstly, 0.1 mL methanolic extract was topped up to 5 mL with distilled water in a 10-mL volumetric flask, followed by 0.5-mL 2 N Folin-Ciocalteu's phenol reagent (Sigma Chemical Co., St. Louis, MO, USA). About 1 mL of saturated (35%, w/v) sodium carbonate solution was added into the mixture after 3 min. The mixture was topped up to 10 mL with water. After 1 h, the mixture was measured spectrophotometrically at 725 nm against a reagent blank. Gallic acid (Sigma Chemical Co., St. Louis, MO, USA) in the concentration range of 0–400 μg/mL assay solution was used to prepare the standard curve for the total phenolic acids content. The determination of flavonoids in oil was based on the method of Oomah et al. (1996). Methanolic extract (1 mL) was diluted three times with distilled water and then 100 μL diphenylboric acid 2-aminoethyl ester solution (1%, v/v; Sigma Chemical Co., St. Louis, MO, USA) was added into the extract before it was measured spectrophotometrically at 404 nm. Luteolin (Sigma Chemical Co., St. Louis, MO, USA) with concentration range of 0–42 μg/3 mL assay solution in 82% methanol was used as the standard for the calibration curve.

### 2.6. Color

Oil color was measured using CIELab color scales with a MiniScan XE spectrocolorimeter (Hunterlab, Reston, VA, USA) that recorded "$L^*$" (lightness), "$a^*$" ($-a$ greenness; $+a$ redness) and "$b^*$" ($-b$ blueness; $+b$ yellowness) with a measuring head hole of 22 mm, illuminance of $D_{65}$ and 10° standard observer, calibrated to a white plate ($X$: 79.61, $Y$: 84.45, $Z$: 90.64). Samples were filled to 3 cm from the top of a white 22 mL container and the lens placed over the container to exclude external light.

### 2.7. Melting and crystallization profiles

Melting and crystallization of cold-pressed oils were determined using a differential scanning calorimeter (DSC; TA Instruments, New Castle, Delaware, United States), equipped with Refrigerated Cooling System RCS90 that reaches the temperature