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Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed cakes

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ABSTRACT

The effectiveness of ultrasonic extraction of phenolics and flavonoids from defatted hemp, flax and canola seed cakes was compared to the conventional extraction method. Ultrasonic treatment at room temperature showed increased polyphenol extraction yield and antioxidant capacity by two-fold over the conventional extraction method. Different combinations of ultrasonic treatment parameters consisting of solvent volume (25, 50, 75 and 100 mL), extraction time (20, 30 and 40 min) and temperature (40, 50, 60 and 70 °C) were selected for polyphenol extractions from the seed cakes. The chosen parameters had a significant effect ($p < 0.05$) on the polyphenol extraction yield and subsequent antioxidant capacity from the seed cakes. Application of heat during ultrasonic extraction yielded higher polyphenol content in extracts compared to the non-heated extraction. From an orthogonal design test, the best combination of parameters was 50 mL of solvent volume, 20 min of extraction time and 70 °C of ultrasonic temperature.

1. Introduction

Hemp (*Cannabis sativa*), Flax (*Linum usitatissimum*) and Canola (*Brassica napus*) seed oils have been favoured for human consumption due to their high amount of essential fatty acids namely α-linolenic acid (C18:3; *n*-3) and linoleic acid (C18:2; *n*-6). The production of plant seed oils has generated tonnes of processing wastes called seed cakes. The seed cakes are then further processed into animal feed due to their high protein and energy contents. Previous study has shown that calves fed with seed cakes had similar live weigh gain with calves fed a mixture of soybean meal and barley [1]. However, the by-products of the oil production also contain secondary metabolites namely phenolic acids and flavonoids that have not been studied extensively. The polyphenols that exhibit functional and nutraceutical properties could be used in functional foods. Previous studies show that the antioxidant compounds improve human health such as healing human chronic ulceration [2], anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antiviral, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [3,4], inhibition of cancer cells, improving the condition of cardiovascular diseases and diabetes [5,6].

Polyphenols of seed cakes are enclosed in hard and insoluble structures such as vacuoles, lipoprotein bilayers, lignin, hull and cell walls [7]. Phenolic acids occur as esters, glycosides and bound complexes in plants. These factors have hindered polyphenol extraction from seed cakes by conventional extraction methods. Ultrasonic treatment is an economic, environmentally friendly, energy saving and sustainable technology used industrially to increase mass transfer phenomena in plant cellular tissues [8]. Ultrasound generates low frequency energy (20 kHz to 1 MHz) for purposes such as cleaning, sterilizing, degassing, precipitation, leaching, digestion, crystallization and extraction [9–13]. The energy generates cavitation force to increase mass transfer rates where resulting bubbles in the liquid/solid extraction explosively collapse. This phenomenon generates localized pressure to disrupt the plant tissue and release the intracellular bioactive compounds [14]. Various studies have incorporated ultrasound into oil extraction of *Jatropha curcas*, Pongamia [15] and hemp seed [16] where ultrasonic treatment successfully increased the oil yield in shorter operating times compared to solvent extraction. Other studies that applied ultrasound include anthocyanin extraction from grape by-products [7] and phenolics extraction from cranberry products [9], coconut shell powder [17] and *Sclerocarya birrea* kernel oil cake [18].

The application of ultrasound for optimization of yield of polyphenols from seed cakes contributes to industrial applications economically and environmentally since it reduces the usage of organic solvent and extraction time. Furthermore, the polyphenols extracted from the seed cakes by ultrasonic treatment would be a good source in product development for nutraceuticals and functional foods that could extend product shelf-life. The objectives

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of this study are: (i) to investigate the differences of polyphenol extraction yield in seed cakes between ultrasonic and conventional methods; (ii) to identify the optimum parameters of ultrasonication for maximum polyphenol yield from seed cakes; (iii) to evaluate the antioxidant capacity of the ultrasonicated seed cake extracts.

2. Materials and methods

2.1. Materials

Methanol and acetone were analytical grade and purchased from Labserv™, Biolab (Aust) Ltd., Victoria, Australia. Defatted hemp (*C. sativa*) and flax seed (*L. usitatissimum*) cakes were obtained from Oil Seed Extractions Limited, Ashburton, New Zealand while canola (*B. napus*) seed cake was supplied by New Zealand Vegetable Oil Limited, Canterbury, New Zealand. The seed cakes were milled into powder using a Cemotec Sample Mill 1090 (FOSS Tecator, Hoganas, Sweden) and the powders were sieved to produce particles to pass a 450 μm sieve. The seed cake powders were vacuum-packed and stored at 4 °C prior to analysis.

2.2. Methods

2.2.1. Ultrasonic extraction

Seed cake powders (5.00 g) were mixed with methanol:acetone:water (MAW, 7:7:6 v/v/v) in a conical flask. The flask was covered with petri film to avoid solvent evaporation before ultrasonication. The experiments were conducted using an ultrasonic bath (Elma®, Germany) with a fixed power (200 W). Various parameters such as solvent volume (25, 50, 75, 100 mL), extraction time (20, 30, 35 min) and temperatures (40, 50, 60, 70 °C) were used in the study. The extracts were filtered through filter paper (0.45 μm, Whatman™) by vacuum. Filtrates were stored in a dark ambient glass bottle at 4 °C prior to analysis.

2.2.2. Control extraction

In order to compare ultrasonic treatment, similar conditions are needed for the setup of the control. Seed cake powders (5.00 g) were mixed with 50 mL of mixed solvent (MAW) in a conical flask. The mixtures were stirred with a magnetic stirrer (4.5 × 0.5 cm) at 1000 rpm without application of heat for 30 min at room temperature (25 ± 1 °C). The extracts were filtered through filter paper (0.45 μm, Whatman™) by vacuum before they were stored at 4 °C prior to analysis.

2.2.3. Determination of total phenolics in seed cake extracts

The total phenolic content in the extracts was determined based on the method of Gutfinger [19]. Extract (0.1 mL) was made up to 5 mL with distilled water in a 10-mL volumetric flask. Folin–Ciocalteau's phenol reagent (0.5-mL; 2 N) was added into the mixture. After 3 min, saturated (35% w/v) sodium carbonate solution (1 mL) was added into the mixture, following by topping up the mixture to 10 mL with water. The mixture was measured at 725 nm using a spectrophotometer against a reagent blank that consisted of all reagents without the extract. The standard curve consisted of gallic acid within the concentration range of 0–400 μg/mL assay solution. Results were expressed as mg gallic acid equivalents (GAE)/100 g of fresh weight.

2.2.4. Determination of total flavonoids in seed cake extracts

The flavonoids were determined following the method of Oomah, Mazza, and Kenaschuk [20]. Distilled water (3 mL) was mixed with 1 mL of extract, followed by 100 μL of diphenylboric acid 2-aminoethyl ester solution (1% v/v) before the mixture was measured spectrophotometrically at 404 nm. Luteolin (0–42 μg/3 mL assay solution in 80% methanol) was used as the standard for the calibration curve. Results were expressed as mg luteolin equivalents (LUE)/100 g of fresh weight.

2.2.5. Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable radical that is widely used in estimating the antioxidant properties where free radical scavengers react quickly with DPPH radicals, causing a decrease in the absorbance measured [20]. Thus, a lower absorbance represents a higher DPPH scavenging activity and vice versa. The DPPH free radical-scavenging assay was based on the method of de Ancos et al. [21] with some modification. Extract (10 μL) was mixed with 3.99 mL of 25 mM DPPH· in MAW solution. The mixture was vortexed and kept in the dark for 30 min. The mixture was measured spectrophotometrically at 515 nm, against a blank solution without the presence of DPPH·. Results were expressed as percentage inhibition of the DPPH· as shown in the following equation:

$$\%\ \text{inhibition of DPPH} = (\text{Absorbance control} - \text{Absorbance sample})/\text{Absorbance control} \times 100$$

where absorbance control is the absorbance of DPPH· solution without extract.

2.2.6. Determination of ferric reducing/antioxidant power (FRAP) assay

Ferric reducing/antioxidant power (FRAP) of bioactive compound extract is the determination of the antioxidant capacity to reduce ferric-tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous (FeII) form, which is a dark blue color at the UV absorption of 593 nm. In this case, the antioxidant compounds represent the role of electron donor in order to reduce the ferric complex to ferrous form. FRAP assay was carried out following the method of Benzie and Strain [22] with modification. FRAP reagent was made by mixing 200 mL of acetate buffer (300 mM, pH 3.6), 20 mL of 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) solution (10 mM), 20 mL of $FeCl_3$ solution (20 mM) and 24 mL distilled water. TPTZ and $FeCl_3$ solutions were prepared freshly every day prior to analysis. The straw colored FRAP reagent was kept in 37 °C water bath prior to

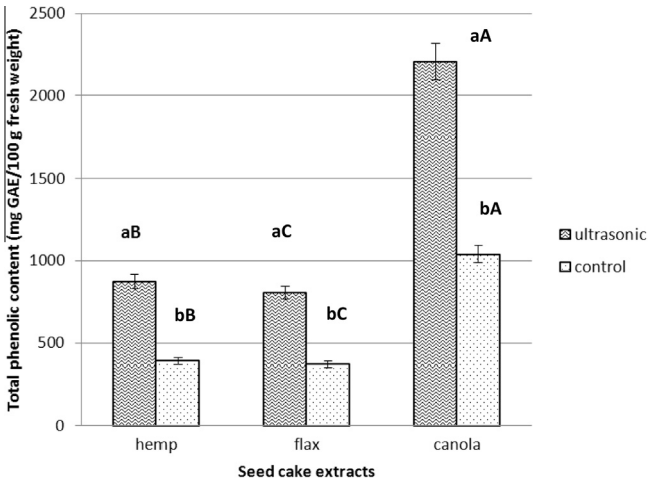

Fig. 1. Total phenolic content of the seed cake extracts by ultrasonic treatment and the control. Values are mean ± standard deviations of three ($n = 3$) measurements. Lower case letters (a and b) within bars of the same sample with different extraction methods and capital alphabet letters (A, B and C) within bars of the different samples are significantly different at $p < 0.05$.