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A new ultrasound-assisted bleaching technique for impacting chlorophyll content of cold-pressed hempseed oil

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ABSTRACT

Ultrasonic treatment was applied to reduce chlorophyll of cold-pressed hempseed oil in conjunction with various clays. Bleaching clays such as bentonite (activated/non-activated), sepiolite and clay from a canola oil refining industry (industrial clay) were used. Ultrasonic bleaching of hempseed oil for 20 min at 20, 40 and 60% power (expressed as ultrasound amplitude) with bleaching clays (40 g/kg) reduced chlorophyll in decreasing order of effectiveness from industrial clay > activated bentonite > sepiolite > non-activated bentonite together with an increase in ultrasound power to 40%. At 20% ultrasound power, chlorophyll reduction (%) was found to be 99.4% (industrial clay) > 97.8% (activated bentonite) > 82.7% (sepiolite) > 47.1% (non-activated bentonite). The reduction in total phenolic content was 27.3%, 33.4%, 27.9% and 34.7% when treated at 20% ultrasound power with 40 g/kg clay from canola industry, activated bentonite, non-activated bentonite and sepiolite, respectively. The corresponding reduction in the peroxide value for the oil samples, due to the adsorptive nature of the clay, followed the trend of industrial clay > sepiolite > non-activated bentonite. This process proved promising for impacting hempseed oil color through reduction of chlorophyll.

1. Introduction

The optimal linoleic acid to alpha-linolenic acid ratio (3:1) and the presence of gamma-linolenic acid, make hemp (*Cannabis sativa* L.) seed oil an important functional and nutritional vegetable oil (Callaway, Tennila, & Pate, 1997; Deferne & Pate, 1996). Cold pressing of hemp seed, the common method to obtain oils, could only recover~65% of the oil with a substantially high content of chlorophyll pigments. Chlorophyll initiates lipid oxidation and quality deterioration of hempseed oil, which necessitates storage in dark or opaque containers. However, to enhance its appeal, hempseed oil is generally marketed in transparent bottles. This can lead to a color change from green to yellow which is accompanied by a corresponding increase in oxidative deterioration and rancidity (Matthäus & Brühl, 2008).

Bleaching is used commercially during edible oil refining to reduce the pigments such as chlorophylls and carotenoids as well as to remove undesired minor components such as metals, phospholipids and oxidation products (Makhoukhi, Didi, Villemin, & Azzouz, 2009). The mechanism of bleaching involves either adsorption, heating or chemical oxidation (Gunstone & Norris, 1983). Bleaching with clays/earths is primarily an adsorption process and is commonly practiced in the oil refining industries. This adsorption process involves complicated interactions between clay particles and undesired molecules. These interactions include physical interactions such as molecular sieves effect wherein undesired compounds are trapped inside the holes of clays, attraction effect of Van der Waals forces, and sometimes chemical reactions occurring via the ionic bonds (Kuuluvainen et al., 2015). Various attributes of bleaching clays such as particle-size distribution, micro-porosity, capillary structure and grain size affect the bleaching efficiency (Galan, 1996). Specific surface area of the bleaching clay also plays critical roles in the bleaching processes because the larger surface area of the clay indicates more

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opportunities of contact between the adsorbents and the undesired compounds. In general, adsorbents from four categories can be utilized as bleaching clays, which are active carbon, naturally active clays, activated clays and other adsorbents such as alumina powder, silica gel, and aluminum and magnesium-silicate (Zschau, 2001). Specific bleaching clays such as bentonite, sepiolite and palygorskite are commonly used in bleaching of vegetable oils. Acid activation using sulfuric acid or hydrochloric acid with elevated temperature has proven to significantly improve the adsorbility of these clays (Didi, Makhoukhi, Azzouz, & Villemin, 2009; Su et al., 2013) possibly due to the increase in the surface area of the clays by replacing the large metal ions with hydrogen ions (Shahidi, 2005). Pigment removal by bleaching can prevent lipid oxidation catalyzed by chlorophyll and its derivatives. However, increasing the treatment time and amount of clay is associated with such as oil retention, filtration and environmental issues (Hussin, Aroua, & Daud, 2011); Lipid oxidation of vegetable oils would be intensified and the loss of oils and disposal costs of the clays increased. The current drawbacks of the bleaching clays necessitate exploring other sustainable, eco-friendly and rapid alternate approaches of pigment removal.

Ultrasound treatment can be an alternative to bleaching clay even though its use in bleaching of vegetable oil is limited to only a few recently reported studies (Abedi, Sahari, Barzegar, & Azizi, 2015; Su et al., 2013). In these studies, ultrasound treatment was applied to oil samples alone or in combination with bleaching clays during the bleaching process and referred to as ultrasonic bleaching. The degradation of carotenes during the ultrasonic bleaching of rapeseed oil was attributed to free radical oxidation which increased the primary oxidation products (Su et al., 2013). Previously, a non-aqueous bleaching process involving ultrasound was effectively used for olive oil (Jahouach-Rabai et al., 2008). Recently, the application of ultrasonic bleaching on soybean oil and corresponding changes in its physico-chemical properties were studied (Abedi et al., 2015). There are no reports available on the bleaching of hempseed oil using either clay or combined application of clay and ultrasound; however, there is a report on the ultrasonic extraction of hempseed oil (Lin et al., 2012).

Ultrasound treatment, a sustainable and eco-friendly technology (Toepfl, Mathys, Heinz, & Knorr, 2006), has already been widely applied in food industries especially for extracting the bioactive compounds such as polyphenols and carotenoids (Maki-Arvela, Hachemi, & Murzin, 2014; Santos-Buelga, Gonzalez-Manzano, Dueñas, & Gonzalez-Paramas, 2012). The low frequency energy of ultrasound (20 kHz–1 MHz) results in a cavitation force and ensuing localized pressure which disrupts plant cell wall, thereby enhancing the release of the bioactive components and facilitating the interaction between bioactive components and solvents in an extraction process. Ultrasound assisted extraction of oils from hempseed, Jatropa, Pongamia and many other oil seeds produced higher oil yields as well as greater amount of anthocyanin from grape byproducts and phenolics from coconut shell powder, cranberry, and marula kernel oil cake (Drosou, Kyriakopoulou, Bimpilas, Tsimogiannis, & Krokida, 2015; Rodrigues & Pinto, 2007; Wang & Zuo, 2011). Ultrasonic extraction of phenolics and flavonoids from defatted hemp, flax and canola seed cakes were carried out and validated against results using conventional extraction methods (Teh & Birch, 2014). Utilizing ultrasound in oil bleaching is a novel application of ultrasound and a potential alternative to commercial bleaching, because it could improve the bleaching efficiency and reduce the amount of clay. However, ultrasound treatment of oil generates heat, the amount of which is directly proportional to the ultrasound power used. A general concern is that the ultrasound wave and the heat generated could not only reduce the pigments, but also have an adverse impact on the bioactive compounds and hence the quality of oil. Peroxide value (PV) is commonly used to estimate the level of oxidative deterioration in heated oils.

Ultrasound treatment is also used in the extraction of chlorophyll. In addition to the extraction techniques, solvents have a more significant impact on the extraction efficiency. Various solvents, namely diethyl ether, methanol, ethanol, acetone and chloroform have been used for the extraction of chlorophyll from different plant samples. Acetone and diethyl ether are effective solvents for extracting chlorophylls but their volatile and flammable properties are a major concern, on the other hand methanol and ethanol are not as efficient (Dere & Güneş, 1998; Ritchie, 2006). Besides, the optimal solvent for chlorophylls extraction varies depending on the plant matrices.

The main objective of this work was to reduce chlorophyll in the cold-pressed hempseed oil by simultaneous application of ultrasound and clay. Another objective was to evaluate the effect of ultrasonic bleaching on the phenolic compounds and oxidative deterioration of hempseed oil. This is the first ever report showing the efficacy of ultrasound treatment minimizing the amount of clay required for effective removal of chlorophyll in hempseed oil. The study extends new applications by producing hempseed oil with varying color attributes for food and non-food applications.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade. Standards of chlorophyll-*a*, chlorophyll-*b* and gallic acid, cumene hydroperoxides, quercetin, diethyl ether, methanol, and chloroform were purchased from Sigma Aldrich, Canada. Cold-pressed hempseed oil, a product from Hemp Oil Canada Inc. (Ste. Agathe, Manitoba, Canada), was obtained and stored in opaque containers and flushed with nitrogen at 4 °C. A sample of clay used for refining canola oil was obtained from a commercial canola oil refining industry in Canada and was referred to as industrial clay in this paper. Sepiolite (original sepiolite, particle size $\leq$75 μm, surface area $\geq$223 m^2/g) and bentonite (particle size $\leq$75 μm, surface area $\geq$130 m^2/g) were purchased from Sigma–Aldrich, Canada. A fine bentonite suspension in water (320 g/L) was treated with concentrated H_2SO_4 (1.83 kg/L) at 90 °C for 6 h, followed by washing the clay to neutral pH using distilled water to produce activated bentonite. The acid treated clay was dried at 100 °C for 6 h and ground and sieved to 75 μm particle size.

2.2. Extraction of chlorophyll of hempseed oil

A modified method was used for the extraction of chlorophyll *a and b* (Dere & Güneş, 1998). About 0.5 ± 0.01 g of the hempseed oil in a 50 mL centrifuge tube was mixed thoroughly with 25 mL of extraction solvent (diethyl ether: absolute or 950 mL/L aqueous solution; methanol: absolute or 960 mL/L aqueous solution). The chlorophyll was extracted by immersing an ultrasound probe (40% power) in the mixture for 1 min ultrasound treatment. The homogenate was centrifuged at 875 g (radius 8.7 cm) for 10 min at 4 °C and the pigment layer was collected for spectrophotometric analysis. All extractions were carried out in duplicate (Fig. 1).

2.3. Quantification of chlorophyll of hempseed oil

Standards of chlorophyll-*a* and chlorophyll-*b* were used in the respective solvents to determine maximum wavelengths of absorption against absolute solvent as blank. Based on the results, chlorophyll-*a* and chlorophyll-*b* of diethyl ether extracts were estimated at 640 nm and 663 nm respectively and that of methanol extracts were estimated at 650 nm and 664 nm respectively.