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Bleaching techniques impact on some guality parameters in two different cold-pressed oils obtained at farm scale

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Abstract

The consumption of cold-pressed oil, such as linseed (Linum usitatissimum L.) and hemp (Cannabis sativa L.) oil has increased recently. In these oils obtained on the farm, a large number of components able to affect oil quality (mainly chlorophylls) are present. Given this, an experiment was conducted with the following aims: (1) to compare different bleaching methods by applying ultrasound waves and earths in cold-pressed hempseed and linseed oils and (2) to evaluate the effect of different types (acid-activated and natural) and amount (2% and 4%) of earths with different processing temperatures (60 and 80°C) in hempseed oil, utilizing a bleaching method with earths alone. Regarding the linseed oil, any bleaching methodologies adopted must not include ultrasound treatment, as the high temperatures reached determine high levels of oxidation to the detriment of the oil. Concerning the hemp oil, the natural earths, especially at farm level, should be preferred, because in addition to removing chlorophyll satisfactorily, when used at 4% even at the lowest temperature (60°C), an improvement of the stability and nutritional value (Vit. E) of the oil was observed.

Practical applications

The use of ultrasound, applied together with bleaching earths, is considered an interesting and efficient bleaching method, in order to remove several pigments able to affect quality of many vegetable oils, with the exception of linseed oil. In fact, the use of ultrasound during the bleaching process determines a high oil temperature (120°C) and this, in an oil such as that of linseed characterized by a very high percentage (> 55%) of α -linolenic acid, negatively affects the oil stability by the formation of high quantity of primary oxidation products, evidenced by very high peroxide values. In the bleaching activity carried out at farm level in particular, natural earths should be preferred to the acid-activated ones. The latter, despite demonstrating an effective bleaching efficiency for few specific pigments, could increase the level of free fatty acids, reduce the antioxidant compounds (tocopherols) naturally present, and release undesirable contaminants in the oil.

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KEYWORDS

hempseed oil, linseed oil, oil pigments, peroxide value, tocopherols

1 | INTRODUCTION

In recent years, consumers have started to pay more attention to nutrition and the quality of food products and a product considered as natural and unprocessed, with a beneficial effect on the human body, is cold-pressed oil. The consumption of cold-pressed oils has increased recently, together with the increased availability on the market of oils from different plant species. Among these, oil obtained from linseed (Linum usitatissimum L.) is one of the most popular cold-pressed oils (Jhala & Hall, 2010; Tańska et al., 2016). Similarly, Giupponi et al. (2020) in a study with the aim of investigating the characteristics of Italian hemp farms, found that almost all of them use this crop for the production of more than one end-product and in 20% of the cases the cultivation leads to the production of hemp (Cannabis sativa L.) food oil on the farm. Since seed oil on the farm is essentially produced by coldpressing, in which neither high temperatures nor chemical treatments are used, a large amount of minor components is extracted and present in the oil. Some of these, mainly chlorophylls in hemp oil, are undesirable pigments as they lower the oil quality by affecting color, flavor, clarity, nutritional value and oxidative stability. Indeed, the stability of vegetable oils depends on various influences, but mainly on their fatty acid composition, the amount of natural antioxidants, the presence of oxygen and different storage conditions (Liang et al., 2015, 2018; Mahoney et al., 2018; Tura et al., 2022). Chlorophylls, fat-soluble pigments present in many raw vegetable oils, especially in hempseed oils, are undesirable compounds, because they are retained in the oil during cold pressing, making it dark and giving it an unpleasant taste, and acting as a pro-oxidant in oils exposed to light. Oxidation of oils and fats causes a rapid deterioration of their sensory and functional properties and leads to a reduction in their nutritional value; this also hastens rancidity and may result in a rancid aroma and off flavors (Aladić, 2015; Izzo et al., 2020; Tarchoune et al., 2019). In general, the chlorophyll content in linseed oil is low if compared with other seed oils, although a certain variability linked to specific varieties was found, highlighted by a different oil color (Mahoney et al., 2018; Suri et al., 2020). On the other hand, several studies have shown that linseed oil is rich in some compounds, such as tocopherol and carotenoids which exhibit strong antioxidant properties, protecting the oil from oxidation as well as providing health benefits to humans (Liang et al., 2015; Tura et al., 2022). At farm level, in order to ensure a guality product from a short supply chain, the main aim is to maintain desirable antioxidant components, while removing unwanted impurities as much as possible, minimizing the oil loss (Chew et al., 2017, 2019; Tura et al., 2022). Tocopherols, tocotrienols (the lasts absent in flax and hemp oils), and other tocochromanol-related compounds are lipophilic antioxidants of great importance for health (Siger & Gornas, 2023). Among homologues tocopherols, α -tocopherol is the only tocochromanol with vitamin E activity that meets the criteria of

preventing vitamin E ataxia, a disease caused by a vitamin E deficiency in humans (Azzi, 2019; Górnas et al., 2022). Hempseed oil is known to be a good source of vitamin E with high antioxidant activity due to its high levels of tocopherols, which could play an important role in the protection of edible oils against lipid oxidation (Faugno et al., 2019; Ghazani & Marangoni, 2013). The amount of tocopherols in hempseed oil varies between 80 and 150 mg 100 g^{-1} of oil; the predominant homologue is v-tocopherol, which reaches 85%-91% of the total. Several factors influence the amount of these compounds in hempseed oils, such as the cultivar, technical crop management adopted, processing methods, and storage conditions (Izzo et al., 2020). Carotenoids are natural pigments produced by the chloroplasts in most plant cells and in some other photosynthetic organisms. Two classes of carotenoids are generally present in vegetable oils, xanthophylls, as yellow pigments in oils, and carotenes. The latter, especially β-carotene, have the function of protecting chlorophylls from degradation and preventing any color change during storage, acting essentially as an antioxidant in reducing the risk of degenerative diseases (Izzo et al., 2020). Also, linseed oil is known for its exceptionally high content of tocopherols $(20-70 \text{ mg } 100 \text{ g}^{-1}, \text{ almost } 170 \text{ mg } 100 \text{ g}^{-1} \text{ for Railinus genotype})$ with a prevalence of y-tocopherol, and also a high level of carotenoids (about 57 mg kg⁻¹) (Matthäus & Özcan, 2017; Prevc et al., 2015; Suri et al., 2020). Among different methods for removing pigments, a whole range of impurities and considered to be of critical importance in determining quality and stability of the final product, oil bleaching is the most important step in the edible oil refining, which could also be adopted at farm level. Generally, the industrial bleaching process is carried out at temperatures between 90 and 120°C for 20-30 min, using 0.5%-2% up to 4% bleaching earth on a weight basis (Abbasi et al., 2017; Zschau, 2001). Specific bleaching earths such as earth silicates, like bentonite, but also sepiolite, montmorillonite, and palygorskite are commonly used in bleaching of vegetable oils. Attempts have been made to use ultrasound waves (Abedi et al., 2015; Su et al., 2013) as an alternative to earth in vegetable oil bleaching and recently, ultrasound treatment in combination with earths and referred as ultrasound bleaching, could be considered an effective alternative to conventional industrial bleaching processes (Aachary et al., 2016). In fact, ultrasound in oil bleaching is an interesting and environmentallyfriendly application, because it can improve bleaching efficiency by reducing the amount of earth needed (Liang et al., 2018). However, the application of ultrasound waves in the oil generates cavitation, that is, the formation, growth and, sometimes, the implosion of microbubbles created in a liquid when ultrasound waves propagate through it, which affects structural and functional components up to lipid oxidation and deterioration. During cavitation, very high temperatures are achieved that could not only reduce the pigments, but also have an adverse impact on the bioactive compounds and hence on the oil quality.

The main objective of this work was to compare the efficiency of different bleaching methods in removing the chlorophyll in hempseed and linseed cold-pressed oils, and at the same time evaluate the impact of the same bleaching treatments on antioxidant compounds, mainly tocopherols, and carotenoids, naturally present in both vegetable oils, which exhibit beneficial effects on human health.

2 | MATERIALS AND METHODS

2.1 | Materials, standards and chemicals

The seeds of the two different species were obtained at the "A. Servadei" agricultural farm of the University of Udine (46°04′N, E13°23′, 109 m a. s. l. and 0% slope). The monoecius cultivar Fedora for hemp and the oil cultivar Kaolin for linseed were utilized. The plants were harvest manually at seed maturity and in particular, in correspondence of the code 2306 by Mediavilla et al. (1998) for hemp and at the stage 89 adjusted to the extended BBCH-scale, as reported in the Italian Official Gazette (MiPAAF, 2012), for linseed. After the harvest, the seeds were immediately separated from the inflorescence and the pods by means of a threshing machine and oven-dried at 40°C until 8%–10% of seed moisture (Marszałkiewicz et al., 2020). After that, the seed was air cleaned and stored at 2°C for about 30 days, until the activity and analysis established began.

Hempseed and linseed oils were obtained by using a single screw mechanical press "Oleo 60" (Gen.Tec, Parma), powered by a 5.0 kW motor, with a maximum squeezing capacity of 30 kg of seed per hour. The output oil, which in both species never exceeded a temperature of 50°C, was placed for 120 hours in special containers to facilitate decantation of the suspended particles in dark conditions at a temperature of about 20°C. Subsequently it was filtered, by using bag filters with a mesh diameter of 5 μ m (Envirogen). Standards of α , γ and δ tocopherols, 3,5-Di-*tert*-4-butylhydroxytoluene (BHT), High Performance Liquid Chromatography (HPLC) grade methanol (MeOH), ethanol, cyclohexane and ethyl acetate were purchased from Merck (Darmstadt, Germany). The water was purified by a Milli-Q[®] IQ 7000 water purification system from Millipore (Merck, Darmstadt). Two commercial bleaching earths were utilized: E1, a non-activated bentonite and E2, an acid-activated calcium bentonite.

2.2 | Ultrasound treatment

The ultrasound treatment was carried out following the procedures reported by Aachary et al. (2016), with suitable modifications. A SONOPLUS Ultrasound Homogenizer HD 4400 system (BANDELIN electronic GmbH & Co. KG, Heinrichstrabe) consisting of Ultrasound Convertor UW 400, probe TS 413 and Ultrasound Generator- GM 4400 was used to produce high-power ultrasound with high amplitudes and frequency (20 kHz \pm 500 Hz). Power of ultrasound treatment was the ultrasound amplitudes expressed as a percentage. Linseed and hempseed oil samples (100 \pm 0.1 g, in duplicate) were

placed in 200 mL centrifuge tubes and treated with 20% ultrasound power under a pulsing ultrasound operating mode with an active and passive interval of 2 and 3 s, respectively. Where required by the treatment, the bleaching earth acid-activated (E2) in a quantity of 40 g kg⁻¹ was added to the oil sample, as utilized in the Canadian refining industry (Su et al., 2013) and by Aachary et al. (2016) in hempseed oil, resulting the most efficient concentration. The ultrasound probe was immersed halfway into the sample and treated for 20 min (four cycles of 5 min each with a 1 min cooling time) (Aachary et al., 2016). The oil temperature was recorded at the end of each of the four 5 min cycles (Figure S1). After sonication of the samples in this condition, filtration of each oil/earth sample was performed as reported below, in order to separate the bleaching earth from the bleached oil.

2.3 | Earth bleaching treatment

Two-hundred milliliters of filtered hempseed oil were poured in a 500 mL conical Erlenmeyer flask (bleaching vessel) inserted in a 1000 mL pyrex beaker with 400 mL of water and subjected to a water bath by using a thermostatic heating plate (420 W FALC F60). The oil was heated, while the stirring of the liquid was ensured by means of the magnetic stirrer (8 \times 55 mm, 80 rpm) and the temperature controlled by an immersion laboratory mercury thermometer (0-100°C, precision 0.5°C). Upon reaching the temperature required for the treatment, the bleaching earth was added slowly and at the same time the magnetic stirrer speed was brought to 1000 rpm for 1 min to allow a fast and uniform distribution of each earth (see Section 2.5 for detailed information about earth type, amounts and temperature). The rotation speed was then adjusted and maintained at 400 rpm, while the treatment temperature was constantly monitored and maintained at ±2°C from the treatment target for the duration of 20 min. This contact time between the oil and the bleaching earth was decided in agreement with previous experience (Brooks et al., 2021), which indicated that 20 to 30 min is the most common contact period. Afterwards the heating was stopped, but stirring continued for another 30 min. When the temperature of the oil reached 50°C, the clear oil was separated from the bleaching earth by pre-filtration with a paper filter with 15 µm of porosity and subsequently, filtered with a syringe filter with 0.7 µm of porosity and a diameter of 25 mm (GVS). The oil samples thus obtained were stored in dark containers at 4°C for subsequent analyses.

2.4 Chemical characteristics of oil

The chlorophyll and carotenoid contents of bleached oil samples were determined by the method described by Minguez-Mosquera et al. (1991). Briefly, 7.5 g of oil were dissolved in cyclohexane up to the final volume of 25 mL. These concentrations of oil samples were then measured by a UV–Vis spectrophotometer (Evolution OnePlus, ThermoFisher Scientific) at 670 and 470 nm, respectively, according to the following equations:

Chlorophyll $\left(\mu g g^{-1}\right) \!=\! A_{670} \times 10^6/613 \times 100 \times d,$

Carotenoid $(\mu gg^{-1}) = A_{470} \times 10^6 / 2000 \times 100 \times d$,

where A is the absorbance and d is the spectrophotometric cell diameter (1 cm).

The PV was assessed according to the official method ISO 3960 (AOCS Cd 8b-90) and expressed as mEq kg^{-1} of oil. For the tocopherols determination and quantification, a stock standard solution was diluted in ethanol in presence of BHT 0.1%, to prevent tocopherol loss, and was stored at -20° C in dark bottles for up to a month. Working standard solutions, instead, were obtained after dilution of stock standard solution with ethanol every batch of analysis. All stock and working standard solutions were stored at -20° C until analyses. One gram of oil was weighed into 15 mL polypropylene tubes, then 9 mL of ethyl acetate were added; the sample was briefly vortexed and filtered through syringe filters (PTFE 0.22 um, 25 mm, DTO Servizi Srl). The filtrate was transferred into an autosampler vial and 20 µL were injected into HPLC. The HPLC system included a Shimadzu LC-20AT pump, a vacuum degasser, a Prominence SPD-M20A photodiode array detector, a Prominence SIL-20 AC HT autosampler (20 µL loop), and a Prominence CTO-20 AC column oven set at 25°C (Shimadzu Corporation). The HPLC separation was achieved using a Zorbax Eclipse Plus C18 column (4.6 \times 150 mm, 5 μ m particle size, Agilent Technologies). Equipment control, data acquisition and integration were performed with Shimadzu LabSolutions (Ver. 5.54 SP2) Software. An isocratic elution system of MeOH:H₂O (96:4) as a mobile phase was utilized at a flow rate of 1 mL min⁻¹ and the detector was set at 292 nm; full spectra were recorded in the range 190-400 nm. Each analysis was conducted in duplicate. Chromatograms of a standard solution (α -, δ -, and γ -tocopherol at a concentration of 100, 100, and 30 mg L^{-1} respectively), a sample of hempseed oil and a sample of linseed oil, as controls, have been included in Data S1 (Figures S2-S4).

2.5 | Experimental description and statistical analysis

The first experiment (Experiment 1) was conducted with oil types and bleaching methods as main factors, adopting a completely randomized factorial design with three replications. Data obtained were submitted to two-way analysis of variance (ANOVA). In particular, the treatments for the main factors were: two oil types (hempseed and linseed oil) and three bleaching methods, consisting of ultrasounds, ultrasounds with earth addition (E2 at 4% on oil weight basis) and earth alone (E2 at 4% on oil weight basis and 80°C as processing temperature), compared with the filtered oils of both species as controls. The hempseed oil was also utilized for a second experiment (Experiment 2) adopting a completely randomized design with three replications. One-way ANOVA was performed in order to compare the filtered oil as control with eight bleaching treatments resulting from a complete factorial combination of the following treatments: two bleaching earth type (E1 – natural bentonite and E2–acidactivated calcium bentonite), two processing temperatures (60 and 80°C) and two earth concentrations (2% and 4% on oil weight basis). The statistical software R (Version 4.0.2) (R Core Team, 2020) was used to analyze the data obtained and the Shapiro–Wilk normality test was performed to test normality condition. The mean differences were analyzed by Duncan's multiple range test.

3 | RESULTS AND DISCUSSION

Table 1 reports the mean values of the components analyzed in the cold-pressed and filtered oil of the two species. Both PV obtained (10.5 and 4.4 mEq kg⁻¹) for cold-pressed hempseed and linseed oils, respectively (Table 1), fall within the standard of the Codex Alimentarius Commission for cold-pressed oils (the maximum PV level is up to 15 mEq kg⁻¹) (Codex Alimentarius Commission, 2001). However, the values obtained were higher than the values of 3.7 and 7.2 mEq kg⁻¹ obtained by Liang et al. (2018) and Borhade (2013) respectively, for hempseed oil and also higher than the 2 mEq kg⁻¹ found by Teh and Birch (2013) for linseed oil. The cause of the slightly high PV values is probably due to the extraction method used. In fact, although the seeds before pressing were not subjected to any heat and/or steam treatment, the oil output still had a temperature of about 50–55°C, due to the high internal pressure and temperature reached during the processing inside the press cylinder.

In the present experiment cold-pressed hempseed oil showed a mean level of total chlorophyll content (59.4 μ g g⁻¹) (Table 1) in agreement with the values (from 56.3 to 98.6 μ g g⁻¹) obtained by other authors (Aachary et al., 2016; Aladić, 2015; Liang et al., 2018). The linseed oil showed a much lower total chlorophyll content (3.6 μ g g⁻¹) than that obtained by other authors (Esmaeilifard et al., 2017; Teh & Birch, 2013). The total carotene content for the two types of oil is exactly the opposite of that observed for chlorophyll; the linseed oil showed a much higher value (41.2 μ g g⁻¹) than hempseed oil (25.8 μ g g⁻¹) (Table 1), in accordance with the results obtained by several other authors (Aladić, 2015; Esmaeilifard et al., 2017; Liang et al., 2018). The content of total tocopherols of hemp and linseed oils

 TABLE 1
 PV value and content of the studied components in cold pressed and filtered linseed and hempseed oil, without any bleaching treatment, and utilized as controls. Values are means ± SE.

Oil	PV (mEq kg $^{-1}$)	Carotenoids ($\mu g g^{-1}$)	Chlorophylls (µg g $^{-1}$)	Tocopherols (mg 100 g^{-1})
Linseed	4.4 ± 0.85	41.2 ± 2.21	3.6 ± 0.07	50.8 ± 1.22
Hempseed	10.5 ± 1.12	25.8 ± 1.88	59.4 ± 0.87	53.5 ± 1.43

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TABLE 2 Experiment 1—Result of analysis of variance (ANOVA) of the mean effect of factors oil type (linseed and hempseed oil) and bleaching treatment (Control, Ultrasound, Ultrasound + Earth, Earth alone) and their interaction on PV, carotenoids, chlorophylls and tocopherols level in the oil.

Source of variation	df	PV	Carotenoids	Chlorophylls	Tocopherols
Oil type (O)	1	*	**	***	**
Treatments (T)	3	*	*	**	**
OxT	3	**	**	***	**

Note: *, **, and *** significant at the p < 0.05, 0.01, and 0.001 levels, respectively.

FIGURE 1 Bleaching treatments effect on PV values expressed as % variation (bars represent \pm *SD*) with respect to the unbleached oils (controls). Different letters indicate statistically significant differences between treatments (*p* < 0.05 Duncan test).



obtained in the present study (Table 1) are consistent with the study of Siger and Gornas (2023) which place both species in the group of plants with an average/medium concentration (51–100 mg 100 g⁻¹ oil). In linseed oil, the total tocopherol content was 53.5 mg 100 g⁻¹, in agreement with the minimum value of the range (56.4–575 mg 100 g⁻¹) obtained by several previous experiments in different years and locations (Bozan & Temelli, 2008; Sun et al., 2022; Zhang et al., 2021). Similarly, the total content of tocopherols in hempseed oil (50.8 mg 100 g⁻¹) was within the range (41 to 111 mg 100 g⁻¹) obtained by Matthäus et al. (2006), but lower than the minimum amount obtained by Liang et al. (2015) (80 mg 100 g⁻¹) in a biennial trial, with γ -tocopherol as the predominant homologue.

Cold-pressed hempseed and linseed oils provide an adequate supply intake of bioactive minor components such as tocopherols and carotenoids, which in nutritional terms, are expressed as mg α -tocopherol equivalent (vitamin E) and µg retinol equivalent 100 g⁻¹ (RE, as vitamin A), respectively. In our experiment, linseed and hemp oils showed nine and six times more RE, respectively, than those of extra virgin olive oils (Table S1). These values represent 57 and 36% for linseed and hempseed oils respectively, of the EFSA-recommended percentage of vitamin A (EFSA NDA Panel, 2015). In the linseed oils, we found an average of 4.81 mg of α -tocopherol equivalent (Vit. E) and 9.13 mg in hempseed oils (Table S1), representing respectively 48% and 91% of the recommended daily amount of Vit. E (EFSA NDA Panel, 2015). Nevertheless, the levels of the above parameters in a cold-pressed and filtered oil can be affected by several factors such as cultivar, field conditions, time of harvest, extraction method and storage conditions as in the case of the PV that evidenced a gradual increase during storage at room temperature (Matthäus & Brühl, 2008).

3.1 | Experiment 1

Table 2 shows the results of the ANOVA analysis for Experiment 1. The interaction effect oil type x treatment was statistically significant for all the parameters considered. PV is commonly used to determine the primary oxidation products, mainly forms of hydroperoxides, and oil oxidative rancidity, which indicates the level of oxidative deterioration in heated oils (Gomathi et al., 2011; Mohdaly et al., 2010). The variation percentages with respect to the untreated control are shown in Figure 1. As had been expected, PV of both oils increased, mainly in linseed oil (+69.1%) due to the high temperature reached (up to 120°C, as reported in Figure S1) during ultrasound treatment, resulting in lipid oxidation and related production of free radicals by sonolysis (Chemat et al., 2004; Jahouach-Rabai et al., 2008). Considering the ultrasound treatment with earth, the increase in the PV in linseed oil was reduced to +35% when compared with the control. In hempseed oil a decrease of about 34% with respect to the control was observed.

Bleaching treatment with earths alone showed a significant and very similar reduction of PV compared with the hemp (-10%) and linseed (-14%) oils as controls (Figure 1). The mitigation of the negative



FIGURE 2 Bleaching treatments effect on carotenoids content in the oil expressed as % variation (bars represent \pm *SD*) with respect to the unbleached oil (control). Different letters indicate statistically significant differences between treatments (p < 0.05Duncan test).

FIGURE 3 Bleaching treatments effect on total chlorophylls content in the oil expressed as percentage variation (bars represent \pm *SD*) with respect to the unbleached oil (control). Different letters indicate statistically significant differences between treatments (*p* < 0.05 Duncan test).

ultrasound effect on PV obtained with the earth addition is in agreement with other experiments, where an adsorption of oxidated products by earths, probably enhanced by ultrasound power (Abedi et al., 2015), was observed. The higher oxidation susceptibility evidenced by the linseed compared with hempseed oil (Figure 1) is essentially due to the fatty acid profile. Both show a similar polyunsaturated fatty acids (PUFAs)/saturated fatty acids (SFAs) ratio, but the linseed oil has a higher peroxidizability index when compared with the hempseed oil, due to the high percentage (> 55%) of α -linolenic (ALA; C18:3 n-3) as the main component of the total PUFAs. The ALA is well known to be highly susceptible to oxidation, much more than linoleic acid (C18:2 n-6), which, in contrast, is the main component of total PUFA in hempseed oil (Grajzer et al., 2020; Razmaitė et al., 2021). Although the content of carotenoids and chlorophylls is very different between the two oils as controls (Table 1), the reduction percentage due to the different bleaching treatments adopted showed quite a similar behavior for both pigments and oil types, as shown in Figures 2 and 3. In the case of hempseed oil, carotenoid and chlorophyll content was essentially unaffected by ultrasound treatment alone. Conversely, a slight reduction (about 10%) of both pigments was observed in linseed oil, with respect to the control

(Figures 2 and 3). The ultrasound treatment with earth reduced the carotene content by 49% and 80% with respect to the control, in linseed and hempseed oil, respectively. The treatment bleaching with earths alone showed a reduction of carotenoids similar to the maximum obtained in the experiment and exactly -72% in linseed and - 77% in hempseed oil with respect to the control (Figure 2). With regard to the chlorophylls, the ultrasound with earths was more effective in reducing the content than earth alone in both oils. In hempseed oil the reduction was 87% and 62% and in linseed oil 45% and 35% for ultrasound with earths and earths alone, respectively (Figure 3). The magnitude of reduction of both pigment contents was comparable to results reported by Aachary et al. (2016). In hempseed oil especially, the chlorophyll removal efficiency of ultrasound was clearly improved by earths presence that may be effective, after thermal degradation, in pigment absorption and removal, as already reported by several authors (Abedi et al., 2021; Su et al., 2013). The level of tocopherols was just slightly negatively affected by ultrasound treatments in both oils (Figure 4). In hempseed oil the above result was confirmed by the earth addition to ultrasound, conversely, in the corresponding treatment with earths alone, a significant positive increase of tocopherol content of 7% was observed. In linseed oil, a

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FIGURE 4 Bleaching treatments effect on total tocopherols content in the oil expressed as percentage variation (bars represent \pm *SD*) with respect to the unbleached oil (control). Different letters indicate statistically significant differences between treatments (p < 0.05Duncan test).



TABLE 3 Experiment 2–Result of analysis of variance (ANOVA) of the mean effect of treatments obtained by the factorial combination of two earth types, two earth concentrations and two temperatures compared with the control (see material and methods section for treatments detail), on PV, carotenoids, chlorophylls and tocopherols level in the hempseed oil.

Source of variation	df	PV	Carotenoids	Chlorophylls	Tocopherols
Treatments	8	**	**	***	*

Note: *, **, and *** significant at the p < 0.05, 0.01, and 0.001 levels, respectively.

similar and significant reduction of about 24% and 26% of tocopherol content was observed compared with the control, with the utilization of earths with ultrasound and earths alone, respectively (Figure 4). Generally, the heat and cavitation obtained by the ultrasound treatment would destroy the bioactive compounds (Abedi et al., 2015), by inducing ultrasound oxidation that could change the structural and functional components of the oils, especially linseed oil, which is characterized by a particular fatty acid composition. The utilization of an acid-activated bleaching earth, as E2 in this experiment, could have acted as an acid catalyst, determining esterification or protonation of tocopherol to form oxonium ions in this very oxidative environment (Karabulut et al., 2005; Suliman et al., 2013; Zhu et al., 2016). However, the uncertain benefit of bleaching efficiency in pigment removing utilizing ultrasound with acid-activated earths can have detrimental side effects in the linseed oil, as the reduction of tocopherols (Vit. E), and an elevated PV value can occur probably due to an increased level of free fatty acids (List & Patterson, 2009).

Concerning the effect of the treatments adopted on the nutritional aspects of the oils, the acid earths used alone or in combination with ultrasounds have shown a high efficiency in pigment removal. In particular in the oil of hemp, the level of Vit. A (as μ g retinol equation 100 g⁻¹) was reduced by 78–80% compared with the control reducing the percentage of recommended safe intake of Vit. A from 36% to 7%–8% (Table S2). In linseed oil, the ultrasound + earth treatment resulted in a reduction of Vit. A (–50%) compared with the control, lowering the percentage of recommended safe intake from 57% to 29% (Table S2). In contrast, the effect of the bleaching treatments on the content of tocopherols (Vit. E in terms of α -tocopherol eq.) in both oils had little impact, substantially maintaining the level of RDA% of the controls (Table S2).

3.2 | Experiment 2

Table 3 shows the results of the ANOVA analysis for experiment 2. The treatment effects carried out in comparison with the hempseed oil as control resulted in a significant effect for all the parameters considered. Figure 5 indicates no significant increases in PV for all treatments adopted, showing a substantial difference from Figure 1, in which the negative effect of ultrasound on the oxidative stability of hempseed oil was evident. In contrast, a significant decrease (Figure 5) compared with the control, was observed corresponding to the lowest processing temperature (60°C), associated with the natural bentonite utilized (E1), irrespective of the amount of earth utilized. The slight increase in PV in the case of using the lowest concentration of acid-activated earth at the highest temperature (E2_2_T2, in Figure 5), can be explained by the associated degradation of oilsoluble antioxidants such as tocopherols, which could promote the decomposition of hydroperoxides into secondary oxidation products. In this case, these last products are, probably, not sufficiently absorbed by the limited amount of the earth utilized (Chew et al., 2017).



-36%

E2 2 T1

-42%

E2 2 T2

-69%

E2 4 T1

-77%

E2 4 T2

FIGURE 5 Earth bleaching treatments effect on PV values in hempseed oil expressed as percentage variation (bars represent \pm SD) with respect to the unbleached oil as control. Different letters indicate statistically significant differences between treatments (p < 0.05 Duncan test). E1 = earth non-activated bentonite:E2 = earth acid-activated calcium bentonite: 2 = 2% of earth concentration on oil weight basis; 4 = 4% of earth concentration on oil weight basis; $T1 = 60^{\circ}C$ process temperature; $T2 = 80^{\circ}C$ process temperature.

FIGURE 6 Effect of earth bleaching treatments on carotenoids content in hempseed oil expressed as percentage variation (bars represent ± SD) with respect to the unbleached oil as control. Different letters indicate statistically significant differences between treatments (p < 0.05 Duncan test). E1 = earth non-activated bentonite; E2 = earth acid-activated calcium bentonite; 2 = 2% of earth concentration on oil weight basis: 4 = 4% of earth concentration on oil weight basis; $T1 = 60^{\circ}C$ process temperature; $T2 = 80^{\circ}C$ process temperature.

In the case of carotenoids (Figure 6), a general significant decrease in content was observed for all treatments studied compared with the control. In particular, a reduction of about 34%-42% of the carotenoid content was obtained compared with the control using only 2% of earth concentration, regardless of temperature and type of earths used. The highest impact on carotenoid reductions (69% and 77%) was observed utilizing the highest concentration (4%) of acidactivated earth, at 60 and 80°C, respectively. This result confirms previous experiments in which the removal of carotenoids was increased by a high concentration of bleaching earths with elevated processing temperatures (Jiang et al., 2020; Rossi et al., 2001). The chlorophyll content (Figure 7) shows a significant reduction (70% and 62%), utilizing the E1 and E2 earth, respectively, combining the highest earth concentration (4%), and temperature (80°C). During the bleaching process, an elevated oil temperature may facilitate the interaction between the bleaching earth and color pigments (Chew & Ali, 2021; Rossi et al., 2001), as in the present experiment, in which 80°C during

-34%

E1_2_T2

-49%

E1 4 T1

-59%

E1_4_T2

-35%

E1 2 T1

-50%

-70%

-90%

the bleaching process impacted more than 60°C. The tocopherol content in hempseed oil was not significantly negatively affected by any of the treatments performed, with the exception of E2_4_T1 and E2 4 T2 (Figure 8). On the contrary, the treatments with natural earth (E1) at maximum concentration (4%) resulted in the highest (and significant) increase in the content of tocopherol compared with the control, irrespective of the processing temperature adopted which was in agreement with other results obtained in bleached kenaf and palm oils (Chew et al., 2017; Rossi et al., 2001). At the same time, the acidactivated earth, as reported in Figure 8, acting as acid catalyst for esterification of tocopherols, can explain its lower efficacy with respect to the E1, as mentioned above. Moreover, the utilization of an acid-activated earth can lead to the risk of formation of undesirable 3-monochloropropane-1,2-diol esters (MCPD), as reported by Ramli et al. (2011). Also in this experiment, all eight bleaching treatments, resulting from the factorial combination of two types of earth \times two concentrations \times two temperatures, significantly reduced the Vit. A

FIGURE 7 Effect of earth bleaching treatments on chlorophylls content in hempseed oil expressed as percentage variation (bars represent \pm SD) with respect to the unbleached oil as control. Different letters indicate statistically significant differences between treatments (p < 0.05 Duncan test). E1 = earth non-activated bentonite; E2 = earth acid-activated calcium bentonite; 2 = 2% of earth concentration on oil weight basis; 4 = 4% of earth concentration on oil weight basis; $T1 = 60^{\circ}C$ process temperature: $T2 = 80^{\circ}C$ process temperature.

FIGURE 8 Effect of earth bleaching treatments on tocopherols content in hempseed oil expressed as percentage variation (bars represent \pm *SD*) with respect to the unbleached oil as control. Different letters indicate statistically significant differences between treatments (*p* < 0.05 Duncan test). E1 = earth non-activated bentonite; E2 = earth acid-activated calcium bentonite; 2 = 2% of earth concentration on oil weight basis; 4 = 4% of earth concentration on oil weight basis; T1 = 60°C process temperature; T2 = 80°C process temperature.



content in hemp oil. In particular, the treatment involving the activated acid earth at the highest concentration and temperature reduced the Vit. A content by 77% compared with the control, lowering the % of recommended safe intake from 36% to 8% (Table S3).

Regarding the content of Vit. E in hemp oil after bleaching treatments, while the use of activated acid earth, regardless of the concentration and temperature adopted, caused a slight lowering of the content compared with the control, the use of the natural earth, in particular at the highest concentration, seems to increase the content of Vit. E, with an improvement of the RDA% (recommended daily allowance; Table S3).

4 | CONCLUSIONS

Regarding the cold-pressed linseed, any bleaching methodologies adopted must not include ultrasound treatment, as the high

temperatures reached encourage high levels of oxidation to the detriment of the oil. In contrast, the use of only acid-activated earths at the highest concentration and temperature, determines an effective reduction of the carotenoid content (which in this type of oil is mainly responsible for affecting the color, clarity and stability), without increasing the primary oxidation products and maintaining largely the tocopherol content.

Concerning the cold-pressed hemp oil, when only the efficiency of specifically eliminating the main pigments (carotenoids and chlorophylls) is considered, the best method is the ultrasound treatment combined with acid-activated earth at the highest concentration and temperature. However, at farm level where sustainability of the process and nutritional aspects of the oil have to be considered, the bleaching method cannot include the ultrasound process, because, in addition to being expensive from an economic, energy, and environmental aspect, it is also technologically complicated. 10 of 12 WILEY-

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Considering therefore a bleaching method that uses earths alone, the natural earth shows a satisfactory elimination of chlorophyll, when used at the highest concentration and the lowest temperature with significant improvement in stability and in nutritional aspects of the oil (Vit. E). Furthermore, the natural earths are the only bleaching clay option that meets the "organic" oil certification status because their manufacture does not involve any restricted chemical agent. Another aspect worthy of being investigated, as a future study, is the evaluation of the effect of ultrasound and different bleaching earths on the fatty acid profile and on the elimination of undesirable contaminants in the oil.

AUTHOR CONTRIBUTIONS

Claudio Ferfuia: writing - Original Draft Preparation (equal); Formal analysis (lead); review and editing (equal); Software (lead); methodology (equal); Fabio Zuliani: Investigation (lead); Methodology (supporting); formal analysis (equal); Barbara Piani: writing - review and editing (equal); writing - Original Draft Preparation (supporting); Inves-(equal); methodology (lead); Luisa tigation Dalla Costa writing - review and editing (equal); Mirco Corazzin: writing - review and editing (equal); Maurizio Turi Writing - review and editing (equal); Mario Baldini: writing - original draft (lead); review and editing (lead); Conceptualization (lead); Resources (lead); funding acquisition (lead): Project administrator.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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