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Volatile profile, phenolic content and antioxidant activity of chia seed (*Salvia hispanica* L.) essential oils obtained by different extraction methods

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SUMMARY: This investigation aims to reveal the physico-chemical properties and volatile profiles of essential oils derived from chia seeds (*Salvia hispanica* L.) by employing both hydrodistillation (HDE) and microwave-assisted hydrodistillation (MAHDE) approaches. Microwaves are preferred for seed pre-treatment in HDE, whereas MAHDE uses microwaves directly. Each extraction technique showed different effects on the oil yield, physico-chemical quality parameters, antioxidant capacity and total phenolic content, as well as the volatile profiles of the essential oils. Although chia essential oils extracted by HDE have three and five times more total phenolic (30.6 mg GAE/kg oil) and antioxidant capacity (62.7 μ M Trolox/100 g oil) values than these of MAHDE, chia essential oils extracted by MAHDE had a higher distinct effect on the yield (4.20%) of essential oils and prevented the loss of volatile compounds. Both essential oils were subjected to GC-MS analysis, which identified 40 and 48 volatile compounds (mainly as linalool, mesitylene, anethol, cumene, eugenol, β -ocimene, eugenol acetate) in HDE and MAHDE, respectively. The results of this study contribute to the potential use of chia essential oil as a valuable raw material in the food & nutrition, pharmaceutical and cosmetic industries. This research represents the first documentation of the volatile profile of chia seed essential oil.

KEYWORDS: Chia Seed; Essential Oil; Hydrodistillation; Microwave-Assisted Extraction; Volatile compounds

RESUMEN: *Perfil volátil, contenido fenólico y actividad antioxidante de los aceites esenciales de semilla de chía (Salvia hispanica L.) obtenidos por diferentes métodos de extracción.* Esta investigación tiene como objetivo mostrar las propiedades físico-químicas y el perfil de volátiles de los aceites esenciales derivados de las semillas de chía (Salvia hispanica L.) mediante el empleo de métodos de hidrodestilación (HDE) e hidrodestilación asistida por microondas (MAHDE). En la HDE se prefieren las microondas para el pretratamiento de las semillas, mientras que en la MAHDE se utilizan directamente las microondas. Cada técnica de extracción mostró efectos diferentes sobre el rendimiento de aceite, los parámetros de calidad físico-químicos, la capacidad antioxidante y el contenido fenólico total, así como sobre los perfiles volátiles de los aceites esenciales. En particular, el MAHDE tuvo un efecto significativo sobre el rendimiento de los aceites esenciales y evitó la pérdida de compuestos volátiles. Ambos aceites esenciales se sometieron a análisis mediante GC-MS, donde se identificaron 40 y 48 compuestos volátiles (principalmente linalol, mesitileno, anetol, cumeno, eugenol, β -ocimeno, acetato de eugenol) en HDE y MAHDE, respectivamente. Los resultados de este estudio contribuyen al uso potencial del aceite esencial de chía como valiosa materia prima en las industrias alimentaria, farmacéutica y cosmética. Esta investigación representa la primera documentación del perfil volátil del aceite esencial de semillas de chía.

PALABRAS CLAVE: Aceite esencial; Compuestos volátiles; Extracción asistida por microondas; Hidrodestilación; Semilla de chía

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1. INTRODUCTION

Chia (*Salvia hispanica*) is an annual graminaceous plant historically cultivated in Mexico, Guatemala, Argentina, Colombia, and Australia, with roots dating back to ancient Mayan and Aztec cultures (De Falco *et al.*, 2017). According to the European Union regulations (No 258/97), chia seeds include 20-25% protein, 30-35% oil, 25-41% carbohydrate, 18-30% crude fiber and 4-5% ash. The use of this new food ingredient is permitted in various breads and breakfast cereals at a maximum of 5 and 10%, respectively (EUC, 2009; Jeelani *et al.*, 2023).

Recently, chia seeds have gained global popularity, particularly among vegetarian and vegan consumers and individuals with celiac disease and non-celiac gluten intolerance. Formulations incorporating white, grey or black chia seeds as a natural supplement in bakery products or desserts are considered functional foods due to their beneficial health effects. Chia seeds are rich in functional components such as essential amino acids, α -linolenic acid, dietary fiber, vitamin E and minerals such as potassium (K) and magnesium (Mg), which have the ability to help lose weight and suppress the risk of hypertension, cholesterol, gastrointestinal diseases and also cancer (Khalid et al., 2023; Jeelani et al., 2023). Chia seeds also contain high levels of phenolic acids such as caffeic, rosmarinic and chlorogenic acids, as well as flavonoids such as myricetin, quercetin and kaempferol, which have the ability to neutralize free radicals, bind metalic ions and donate hydrogen atoms, and are the main contributors to the antioxidant activity of the seeds (Timilsena et al., 2017).

Black or white chia seeds are an important source of nutritious oil known as chia seed oil worldwide. Chia seed oil was approved as a novel food by Commission Implementing Regulation (2017/2470) and shows potential in various food industry applications It can enhance the quality of bakery products, be used as an ingredient in edible fats and oils (up to 10%), and serve as a functional ingredient (up to 2 g/ day) in dietary supplements, as defined in Directive 2002/46/EC (CIR, 2017; Derewiaka *et al.*, 2019)

Chia seeds are also very rich in polysaccharides that are able to form a gelatinous matrix when treated with water, and this matrix is called chia mucilage. Chia musilage can be suitable as well for generating an edible film in the food industry, as a biopolymer in the cosmetic sector, and for the controlled release of drugs in the pharmaceutical industry (Timilsena *et al.*, 2017; da Silveira Ramos *et al.*, 2021)

A recent study (Alejo-Jacuinde *et al.*, 2023) investigated the role of gene expression in chia seed mucilage metabolism and identified a total of 37 proteins, mainly seed storage proteins, that modulate lignan and lignin biosynthesis during seed growth and in response to biotics and abiotics.

Conventional techniques for extracting natural products have limitations, including poor extraction performance, low yield, excessive solvent usage, thermal degradation risk during long extraction times and high temperatures (Aydeniz et al., 2014). Microwave technology is able to generate heat through friction and molecular rewiring (cavitation) to increase the mass transfer coefficient. As a result, microwave-assisted techniques have gained prominence for the isolation and extraction of phytoconstituents such as phenolics, pigments, amino acids and vitamins due to their superior extraction efficiency compared to conventional methods. In particular, essential oils obtained by microwave-assisted hydrodistillation have enhanced antimicrobial activity, higher levels of oxygenated compounds and natural aroma components (Lucchesi et al., 2004). It also offers the possibility of producing the essential oil's natural aroma components much more precisely than hydrodistilled essential oil (Karakaya et al., 2014).

In addition to the direct extraction of fixed or volatile oils from oilseeds by microwave-assisted extraction, it has been reported that microwave pre-treatment of seeds prior to oil extraction can also support the oxidative stability of the oil by positively affecting the values of tocopherol, total phenolic matter and antioxidant capacity (Lucchesi *et al.*, 2004; Aydeniz *et al.*, 2014).

Essential oils, rich in volatile components, are highly valuable in food flavoring, traditional medicine, perfumes and cosmetic industries among the secondary metabolites in aromatic plants (Viuda-Martos *et al.*, 2009; Cherif *et al.*, 2019). Chia seeds, stems and leafs may have volatile component-rich essential oils with commercial importance to the flavor and fragrance industry. It was reported that volatile components such as β -caryophyllene, globulol, and β -pinene in essential oils have intense repellent action against insect species and an antimicrobial effect on plant pathogenic fungi and bacteria (Elshafie *et al.*, 2018). Based on these findings, the current study focuses on the volatile profile, phenolic compounds and antioxidant capacity values of essential oils extracted via hydrodistillation (HD) with microwave pre-treatment and microwave-assisted hydrodistillation techniques (MAHDE).

Hydrodistillation and microwave-assisted hydrodistillation were selected for extracting chia seed essential oil, and the effectiveness of both techniques was compared in terms of oil yield, extraction time, total phenolic contents, antioxidant capacity values, and volatile profile of chia essential oils. To the best of our knowledge, this study is the first to reveal the aroma profiles of chia seed essential oils obtained through hydrodistillation (HD) with microwave pre-treatment and microwave-assisted microwave-assisted hydrodistillation (MAHDE).

2. MATERIALS AND METHODS

2.1. Materials

Chia seeds and cold-pressed chia seed oil (CPCO) used as control oil were purchased from Yayla Gourmet Cereals (Mersin, Türkiye) and Botalife chia oil (Manolya Natural and Aromatic Products Ltd, Isparta, Türkiye), respectively. H_2SO_4 , NaOH, H_3BO_3 , Na₂SO₄, MetOH, Folin–Ciocalteu reagent, Na₂CO₃, Gallic acid, NHAC₄, CuCl₂ and other reagents were of analytical purity. All analytical grade chemicals and reagents used in this study were purchased from Merck (Darmstadt, Germany) and Sigma Chem. Co. (St. Louis, MO, USA).

2.2. Preparation of chia seeds

Prior to analysis, chia seeds (see Figure 1) were ground and homogenized using a high-speed blender (7011S model, Waring Laboratory, USA) and ground samples were kept at 4 °C until further use.

2.3. Physical properties of chia seeds

100 chia seeds were randomly collected, weighed on an analytical balance (Sartorius ED224S, Sartorius, Germany) and their weight was multiplied by ten. To determine the dimensional properties (length, width) of chia seeds, ten seeds were randomly chosen and measured with a calipper (CD-15CP, Mitutoyo Ltd., Andover, UK). All procedures were repeated at least four times.

Instrumental color values (L, a*, b*) of all chia seed samples were recorded using a Minolta colorimeter (CR-400, Osaka, Japan) calibrated with a white ceramic plate.

2.4. Proximate composition of chia seeds

The moisture content in ground chia seeds (%) was determined with an infrared moisture analyzer (Ohaus MB45, Switzerland) at 110 °C for 30 min. The contents of ash (%) and protein (%) in the chia seeds were analyzed using the official methods of the Association of Official Agricultural Chemists (AOAC, 2005) and American Association of Cereal Chemists (AACC, 2012). The total oil content in chia seeds (mainly composed of 6.4% palmitic, 2.9% stearic, 7.1% oleic, 18.1% linoleic and 62.8% α -linolenic acid) was measured by 6-h extraction with



FIGURE 1. Chia seeds (A: raw chia seed B: grounded chia seeds, C: grounded chia seeds after microwave treatment).

n-hexane in an automated Soxhlet apparatus (Gerhardt Soxtherm Manager SX, Germany).

2.5. Microwave pre-treatment and hydrodistillation procedure

The essential oil in chia seeds was extracted using a Clevenger-type apparatus (a glass hydro-distiller) (Uzkuç *et al.*, 2021). Prior to essential oil extraction from chia seeds via hydrodistillation, microwave pre-treatment was conducted. For this purpose, chia seeds were pre-roasted in a household microwave oven (Samsung, ME711K, Malaysia), based on the parameters of our previous work (Aydeniz *et al.*, 2014). In the microwave oven, 360 W power applied to the seeds for 6 min (3 minutes apply/3 minutes wait) for homogeneous heat distribution.

Subsequently, approximately 200 g of ground chia seeds were used for each extraction, and the seed-to-water ratio was set at 1:3 w/v (weight/volume). During the oil extraction process, heating was provided by an electric mantle (Termal Laboratory Equipments, Istanbul, Türkiye), and the condenser temperature was maintained at 8 °C using continuous cold water circulation. The distillation process continued until no further increase was observed in the amount of essential oil collected in the measuring cylinder (see Figure 2). The collected essential oils (HDE) were passed through anhydrous sodium sulfate, stored in amber-colored bottles at -18 °C until analysis, and the extraction procedure was repeated at least twice.

2.6. Microwave-assisted hydrodistillation procedure

A solvent-free microwave-assisted hydrodistillation method was applied with an advanced microwave extraction system (Milestone ETHOS X, Milestone, Italy) following minor modifications as described in Lucchesi et al. (2004). Before the extraction process, ground chia seeds (4% moisture content) were left to thaw (1:3 w/w, weight/weight) for 60 min in distilled water at room temperature. Excess water was then removed from the seeds, and they were transferred to a glass reactor. Thawing is a crucial step to ensure the initial moisture level required for the seed absorption of microwave irradiation. Hence, a preliminary study was conducted to determine the extraction conditions (Uzkuç et al., 2021). The extraction process was carried out at 750 W until no further essential oil could be extracted. (See Figure 3).

During microwave-assisted hydrodistillation, the software monitored the extraction parameters such as temperature, time, pressure, and power. The extracted essential oils (MAHDE) were dried with anhydrous sodium sulfate to separate them from the water. They were then collected in an amber vial with a PTFE-silicon septum and stored at -18 °C.

2.7. Essential oil analyses

Specific gravity values of HDE and MAHDE were determined by the ratio of the weight of 10 μ L



FIGURE 2. Essential oil extraction by Clevenger apparatus.

essential oil to the weight of 10 μ L distilled water according to Karakaya *et al.* (2014). The extraction yields for the essential oils were calculated (Karakaya *et al.*, 2014) and refractive index values were also recorded using a refractometer (Soif Optical instruments, DA 0158, China). Both measurements were made in triplicate at 23 °C.

2.7.1. Preparation of essential oil phenolic extracts

Phenolic extractions of the essential oils were performed according to the method described by Aydeniz *et al.* (2014). For this purpose, essential oil samples and solvent mixture (water:methanol, 60:40 v/v) were mixed (1:1 v/v) and shaken vigorously for 30 seconds (Heidolph Reax Top, Heidolph, Germany). The methanolic phases were removed following centrifugation (7500 rpm, 4 °C, 10 min, Sigma 2–16K, Postfach, Germany) and this procedure was repeated once more for each essential oil sample. All methanolic phases were combined, passed through 0.45 μ m filters, and the obtained extracts were utilized in assessing the total phenolic content and antioxidant capacity.

2.7.2. Determination of total phenolic content

The total polyphenol contents in the essential oil extracts were analyzed according to Chotimarkorn *et al.* (2008). Prepared phenolic extracts (250 μ L) were pipetted into a 10 ml volumetric flask. Folin–Ciocalteu reagent (500 μ L), distilled water (6 ml) and sodium carbonate (2 ml, 15% v/v, Na₂CO₃) were added to the volumetric flask, and the final volume was made up (10 ml) with distilled water.

The absorbance reading was recorded spectrophotometrically at 750 nm (Agilent 8453 UV-Visible spectrophotometer, Waldbrann, Germany) at the end of incubation in the dark (2 h, 23 °C). Total phenol contents were estimated quantitatively through a calibration curve prepared with gallic acid and expressed as mg gallic acid equivalent (GAE)/100 g essential oil.

2.7.3. Determination of cupric reducing antioxidant capacity (CUPRAC)

Experimental solutions of ammonium acetate buffer (NHAC₄, 1 M), neocuproine (Nc, 7.5 mM)



FIGURE 3. Essential oil extraction by microwave-assisted hydrodistillation.

and copper (II) chloride (CuCl₂,10 mM) were prepared for the CUPRAC method (Apak *et al.*, 2004). Phenolic extracts (100 μ l) and distilled water (1 ml) were added to a test tube containing 1 ml of each experimental solution and vortexed for 10 s. A test tube without phenolic extract was used as a blank sample, and the absorbance values were measured at 450 nm after a reaction time of 30 minutes at 25 °C. The results were expressed as μ M Trolox per 100 g essential oil.

2.7.4. Extraction, identification, and quantification of the volatile compounds from the essential oil samples

The solid phase microextraction technique (SPME) was employed to extract volatile compounds from the essential oil samples. Nearly 3 grams of oil sample were placed into a 40 mL SPME vial. The vial was then incubated in a water bath at 40 °C for 20 minutes to allow the volatiles in the vial's headspace to reach equilibrium. Afterward, an SPME needle (2 cm 50/30 µmdivinylbenzene/carboxen/polydimethylsiloxane stable flex, Bellafonte, USA) was placed into the vial, and the SPME fiber was positioned at a depth of 2 cm in the vial's headspace for 20 min to extract the volatiles. Then, the SPME needle was injected into the GC-MS in the splitless mode (HP 6890 and 7895C MS, Agilent, USA). Separation of volatile compounds was carried out using a HP5 MS column (30-m 9 0.25-mm id 9 0.25-lm film thickness; J&W Scientific, Folsom, CA, USA). The carrier gas consisted of Helium at a flow of 1.5 ml/min. The oven temperature was programmed from 40 °C up to 230 °C at 10 °C/min, with initial and hold times of 5 and 20 min at 40 °C, respectively. The mass spectra condition was electron impact mode and Ionization voltage was 70 eV in 33-300 amu mass range (Guneser et al., 2015). The tentative identification of the volatile components was based on comparison of the mass spectra of unknown compounds with those of the National Institute of Standards and Technology (NIST) and the Wiley Registry of Mass Spectrum Database. The relative amounts of identified volatile compounds was calculated by using percentages (%) of the peak area of the total ion chromatogram (peak normalization tecnique) and expressed as a percentage (%), (Guneser et al., 2015).

2.8. Statistical analysis

All essential oil extraction procedures were repeated twice. All physico-chemical analyses in chia seeds and seed essential oils were carried out twice for each replicate sample. All collected data were subjected to analysis of variance (ANOVA) using Minitab ver. 16.1.1 statistical package programs at 5% significance level (Minitab, 2010).

3. RESULTS AND DISCUSSION

3.1. Physical and chemical properties of chia seeds

The nutritional benefits of chia seeds, such as gluten-free, high fiber and protein contents, and isoflavone source, are promising characteristics for the development of innovative food and beverage formulations. As a result, the use of whole chia seeds, chia flour, hydrated chia or chia musilage in the food industry is increasing tremendously on a daily basis. Dairy and meat products, gluten-free bakery formulations, chia fresca and hydrocolloid agents containing chia are the best known and most popular products available in local markets (Zettel and Hitzmann, 2016).

Some physical and chemical properties and essential oil yields of chia seeds are listed in Table 1. The seed sizes and 1000-mass weight of chia seeds used for oil production are consistent with those cited in the literature (Porras-Loaiza *et al.*, 2014).

According to the findings stated by different researchers (Goyat et al., 2018; Timilsena et al., 2017), the basic nutritional composition of the seeds

Properties	Values
1000-seed mass (g)	1.25 ± 0.06
Dry matter (%)	92.73 ± 0.66
Ash (%)	4.56 ± 0.06
Oil content (%)	17.46 ± 0.94
Protein content (%)	23.45 ± 0.39
Oil yield (%)	
Hydrodistillation technique	2.17 ^b
Microwave-assisted hydrodistillation technique	4.20ª

Values are the mean \pm SD (n=4). a-b Different lower-case letters in the same column indicate significant differences (p < 0.05) according to One-way ANOVA/Tukey's test.

varies within a wide range of 30% fat, 18-30% dietary fiber, 15-21% protein, 25-42% carbohydrates and 5% ash. Crude oil content was also found to be lower than reported in the literature. This may be due to species and geographical differences.

Process parameters such as short extraction time, minimum solvent volume and environmental impact, low cost, oil rich in bioactive components and volatile compounds and maximization of oil yield were preferred in the selection of the extraction process. Considering all these process parameters, microwave-assisted hydrodistillation for essential oil extraction has many advantages (Aydeniz *et al.*, 2014).

The efficiency of essential oil extraction is influenced by several parameters such as the extraction method, pre-extraction, pre-treatment, etc. For example, microwave-assisted distillation had the highest oil yield content, which was significantly different (P < 0.05) from that of hydrodistillation (Table 1).

Direct microwave irradiation during the extraction process can cause structural changes in the seed wall. This can have a significant impact on oil yield. According to Lucchesi *et al.* (2004), microwave irradiation has a distinct affect on essential oil extraction because microwave beams allow the essential oil to be removed from the seed quickly without leading to major changes or loss in the volatile aromatic compounds. Thus, the main advantages of microwave-assisted extraction are higher oil yield (4.20%), shorter extraction time, minimum energy consumption (kW h/kg oil) and environmental impact (kg CO_2 rejected/kg oil).

The color characteristics of chia seeds are shown in Figure 1 and Table 2. L^* and b^* values were statistically significantly affected (P > 0.05) by the pre-treatments. Size reduction or grinding of seeds can significantly increase the measured redness and yellowness of seeds due to an increase in surface area.

3.2. Physical and chemical properties of chia seed essential oils

Relative density and refractive index are important quality parameters for detecting adulteration and also for purity control in essential oils. These criteria are also specified in the European Pharmacopoeia (Conseil de L'Europe, 1996). Table 3 represents the relative density and refractive index values for chia seed oils obtained by different techniques.

Although it is noteworthy that cold-pressed oils have higher relative density and refractive index values, the effect of the distillation technique on the physical properties of essential oils is not statistically significant. The fact that the cold pressing technique is based on a different extraction principle and the pressing temperature is limited to a maximum of 40 °C is thought to be responsible for the higher values.

Color value	Chia seed (control)	Grounded chia seed	Ground chia seed treated with microwave
L*	$40.25\pm0.27~b$	$43.01\pm0.67\ b$	45.96 ± 1.05 a
a*	2.16 ± 0.17	2.32 ± 0.14	1.95 ± 0.14
b*	$4.98\pm0.24~b$	7.28 ± 0.23 a	6.95 ± 0.42 a

TABLE 2. Instrumental color values of chia seeds

Values are the mean \pm SD (n=4). a-b Different lower-case letters in the same row indicate significant differences (p < 0.05) according to One-way ANOVA/Tukey's test.

Chia seed oil	Relative density	Refractive index
HDE	$0.97\pm0.05~^{\rm b}$	$1.34\pm0.15~^{\rm b}$
MAHDE	1.03 ± 0.03 ^{ab}	$1.34\pm0.03~^{\rm b}$
СРСО	1.42 ± 0.14 ^a	1.48 ± 0.12 ª

Values are the mean \pm SD (n=4). a-b Different lower-case letters in the same column indicate significant differences (p < 0.05) according to One-way ANOVA/Tukey's test. HDE: Chia seed essential oil extracted by hydrodistillation; MAHDE: Chia seed essential oil extracted by microwave-assisted hydrodistillation; CPCO: Cold-pressed chia seed oil.

Rokosik *et al.* (2020) reported that the relative density and refractive index values of cold-pressed chia seed oil were 0.929 and 1.4825, respectively. The relative density value mentioned by researchers was lower than that of essential oils and cold-pressed chia seed oil.

It is believed that the physical structures of all three oils (essential, cold press, solvent extracted) are completely different (Fathollahi *et al.*, 2021). Similarly, although this result is lower than the refractive index values found in our essential oils, it is similar to the index values of cold-pressed chia oil.

3.3. Total phenolic contents and antioxidant activity values of chia seed essential oils

The total phenolic content and antioxidant capacity data obtained in the current study are presented in Table IV. From the chia seed oils produced by different techniques, total phenolic content ranged from minimum to maximum concentrations of 11.37 mg GAE/kg oil (MAHDE) and 102.90 mg GAE/kg oil (CPCO), respectively (Table 4). In general, it was determined that the hydrodistillation technique was found to have three and five times more total phenolic and antioxidant capacity values than the microwave technique, respectively.

Notable statistical differences (P < 0.05) in the levels of total phenolic content and antioxidant capacity indicate that hydrodistillation is a superior method for obtaining higher levels of total phenolic content and antioxidant capacity. During the hydrodistillation process, the plant materials are immersed into the boiling water, which leads to heat-induced cell wall degradation and breakdown of phenolic-protein bonds in chia seeds, ensuring a faster and more effective transport of phenolic compounds (Bhalla *et al.*, 2023).

These results are consistent with previously reported studies on the effect of extraction methods

on the total phenolic and antioxidant capacity of chia seed oils. Bodoira *et al.* (2017) determined the total phenolic content of solvent-extracted chia seed oils to be 42 mg GAE/kg oil. Although this value is very close to the total phenolic content (30.56 mg GAE/kg oil) of the essential oil obtained by the hydro-distillation technique, it is 2.5 times lower than the total phenolic content of the cold-pressed chia oil we obtained from the market.

3.4. Volatile compound profile of chia seed essential oils

Although essential oils have a complex structure with more than a hundred components, the majority (95%) consists of monoterpenic hydrocarbons and volatile components (Viuda-Martos *et al.*, 2009).

A total of 90 volatile compounds were identified in cold-pressed chia seed oil and chia essential oil samples. The volatile compounds consisted of aldehydes, alcohols, esters, ketones, pyrazine and terpenes (Figure 4).

It was found that the percentage of many of the volatile compounds identified in the chia seed oil samples ranged between 0.5-5% except for some volatiles such as linalool, α -pinene, cumene, α -thujene, o-cymene, mesitylene (Table 5). Considering the volatile composition of the oil samples together, it can be seen that all the oil samples have different characteristics. These different characteristic can be attributed to preferred extraction method and working principles which can affect the volatile composition of the essential oil.

 α -pinene is the major component with the highest percentage of 34.56% in the volatile profiles of CPCO. Other major volatiles include hexanal, β -pinene, α -thujene and o-cymene with percentages of 7.15, 8.73, 10.60 and 19.21%, respectively. These volatiles accounted for 80.25% of the total

TABLE 4. Total phenolic content and antioxidant capacity values of chia seed oils

Chia seed oil	Total phenolic content (mg GAE/kg oil)	Antioxidant capacity (μΜ Trolox/100 g oil)
HDE	30.56 ± 0.98 ^b	62.71 ± 5.47 ^a
MAHDE	$11.37 \pm 1.01 \ ^{\mathrm{b}}$	12.88 ± 0.28 ^b
СРСО	102.90 ± 23.2 ^a	$22.10\pm3.56~^{ab}$

Values are the mean \pm SD (n=4). a-b Different lower-case letters in the same column indicate significant differences (p < 0.05) according to One-way ANOVA/Tukey's test. HDE: Chia seed essential oil extracted by hydrodistillation; MAHDE: Chia seed essential oil extracted by microwave-assisted hydrodistillation; CPCO: Cold-pressed chia seed oil.

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FIGURE 4. GC-MS chromatogram of chia seed essential oil.

volatile composition while other volatiles accounted of 19.75% with low percentages. In particular, α -pinene, o-cymene, β -pinene and α -thujene associated with woody, terpene, herbal, green pine aroma descriptions were detected in only CPCO.

The results obtained were found to be similar to those reported by Yin *et al.* (2020), who reported that hexanal, 1-hexanol and ocimene were responsible for the "fresh grass or green vegetable odor" of cold-pressed oils, and by Guneser and Yilmaz (2017), who reported that α -thujene and β -cymene were the main volatiles with the highest concentrations in cold-pressed oils.

In the case of the chia essential oil sample obtained by hydrodistillation (HDE), mesitylene (1,3,5-trimethyl benzene), anethole, cumene, eugenol and benzaldehyde were identified as the major volatiles with the percentages of 14.10, 9.03,7.48, 5.95 and 4.68%, respectively. Mesitylene, anethole and cumene are aromatic compounds that may be responsible for the sweet, anise oil-like and pungent odors of HDE (Pubchem, 2024). The remaining components were identified to range between 0.45-2.91%. Depending on the extraction methods, some seed oils contain different volatile compounds which can be directly affected by extraction conditions and are accepted as indicators of chemical reactions occurring during oil extraction. Especially furans, pyrazines and also pyrroles are formed by the thermal degradation of sugars and lipid oxidation (Kraljić *et al.*, 2018).

During hydrodistillation, thermal degradation caused by gradually increasing temperature explains the fact that some volatile compounds such as 2-furanaldehyde (3.82%), 2-acetylfuran (2.91%), 5-methylfurfural (2.59%), ethylpyrazine (2.02%) and 2-pentyl furan (1.73%) were identified only in HDE, unlike the other samples.

According to Jakab *et al.* (2018) the degradation of eucalyptol caused by cleavage of the ether linkage followed by elimination of water leads to the formation of D-limonene. During hydrodistillation, the thermal process leads to the degradation of eucalyptus by dehydration and the formation of d-limonene, which explains the fact that d-limonene and eucalyptol were only detected in HDE.

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No	Compounds	Concentration (%)a		
110	Compounds	СРСО	HDE	MAHD
1	(Z)-2-pentene	$1.84{\pm}0.12$	-	-
2	3-methylbutanal	_b	-	2.14±0.07
3	(E)-2-pentenal	4.60±0.09	-	-
4	Hexanal	7.15±1.05	-	7.25±0.27
5	2-furanaldehyde	-	3.82±0.09	-
6	4-methyl 2-hexanone	-	-	0.21±0.01
7	Ethylbenzene	1.01 ± 0.02	2.56±0.88	-
8	(E)-2-hexenal	0.25±0.01	-	0.50±0.23
9	Hexanol	-	-	8.53±0.04
10	2-heptanone	0.80 ± 0.84	-	1.31±0.01
11	Heptanal	-	-	1.67±0.20
12	α-thujene	10.60±0.75	-	-
13	α-pinene	34.56±1.24	-	-
14	Methyl caproate	-	-	0.56±0.01
15	1-acetyl-1- Cyclohexene	3.31±0.38	-	-
16	2-acetylfuran	-	2.91±0.62	-
17	Ethyl pyrazine	-	2.02±0.01	-
18	Benzaldehyde	-	4.68±0.81	2.96±0.05
19	(E)-2-heptenal	$0.69{\pm}0.25$	-	$0.24{\pm}0.04$
20	Cumene	-	7.48 ± 0.24	-
21	Propyl cyclohexan	-	2.65±0.55	-
22	4-carene	-	2.27±0.05	-
23	2-methyl-5-propyl Thiophene	-	2.57±0.26	-
24	Heptanol	-	-	1.67±0.03
25	Propyl benzene	-	1.95 ± 0.01	-
26	β-pinene	8.73±0.10	-	-
27	1-octen-3-ol	-	-	1.32±0.04
28	5-methyl furfural	-	2.59±0.80	-
29	Mesitylene	-	14.10±0.79	2.84±1.10
30	3-octanone	-	-	$1.96{\pm}0.08$
31	2-pentyl furan	-	1.73±0.25	-
32	β-myrecene	-	2.35±0.02	-
33	Decane	-	4.18±0.30	-
34	Hexyl acetate	-	-	4.09±0.02
35	2,4 heptadienal	1.87 ± 0.17	$0.74{\pm}0.01$	1.47±0.22
36	4-methyldecane	-	1.32±0.06	-
37	o-cymene	19.21±2.17	-	-
38	D-limonene	-	1.29±0.30	-

 TABLE 5. Volatile compound profile of chia seed oils

No	Compounds	Concentration (%)a		
110	Compounds	СРСО	HDE	MAHD
39	Eucalyptol	-	1.99±0.45	-
40	3-octen-2-one	-	-	$1.12 \pm .0.06$
41	α-ocimene	$0.40{\pm}0.07$	-	-
42	Benzeneacetaldehyde	-	1.71 ± 0.76	6.40±0.37
43	(E)2-octenal	-	-	1.46±0.03
44	Butyl benzene	-	$1.17{\pm}0.28$	-
45	γ-terpinene	$1.19{\pm}0.18$	$0.80{\pm}0.01$	-
46	3,5-octadiene-2-one	$0.80{\pm}0.18$	-	3.98±0.18
47	4-methyl benzaldehyde	-	0.68±0.16	-
48	4-methyl phenol	-	1.08±0.21	-
49	3-methyl benzaldehyde	-	1.73±0.26	-
50	Terpinolene	0.17±0.04	-	-
51	2-methoxy phenol	-	2.13±0.14	-
52	Linalool	$0.54{\pm}0.07$	-	27.99±0.32
53	1-octenyl-3-acetate	-	-	0.51±0.01
54	3,5-dimethyl-1,2,4-trithiolane	-	-	0.69±0.02
55	Benzyl alcohol	-	0.74±0.37	-
56	Camphore	0.19±0.06	0.86±0.32	3.35±0.12
57	(E)-2-nonenal	-	-	0.75±0.02
58	Borneol	-	0.61±0.01	0.42±0.01
59	4-terpineol	$0.06{\pm}0.01$	0.61±0.17	0.39±0.03
60	Azulene	-	0.79±0.10	0.24±0.01
61	Hexyl butanoate	-	-	0.97±0.06
62	α-terpineol	-	-	2.40±0.01
63	Safranal	-	-	0.20±0.01
64	Decanal		-	0.27±0.01
65	2,4-nonedienal	-	-	0.22±0.01
66	Geraniol	-	-	0.25±0.06
67	β-ocimene	-	-	3.89±0.12
68	Anethol	$0.09{\pm}0.01$	9.03±0.24	-
69	Lavandulil acetate	-	-	2.27±0.02
70	Carvacrol	-	1.37±.15	-
71	2,4 decadienal	0.07±0.01	-	0.38±0.01
72	Eugenol	-	5.95±2.24	-
73	Neryl acetate	0.02 ± 0.02	-	0.96±.02
74	Geranyl acetate	-	-	1.18±0.02
75	Nerol	-	-	0.21±0.01
76	β-demascenone	-	-	0.19±0.01
77	Methyl eugenol	-	0.73 ± 0.12	-

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No	Compounds	Concentration (%)a			
		СРСО	HDE	MAHD	
78	Longifolene	0.03±0.01	-	-	
79	Trans caryophyllene	0.03 ± 0.01	-	-	
80	Cinnamyl acetate	-	$0.44{\pm}0.08$	-	
81	Isoeugenol	-	0.64±0.32	-	
82	Nerolacetone	-	-	$0.08{\pm}0.02$	
83	β-myrecene	-	-	0.03±0.01	
84	β-ionone	0.03 ± 0.01	-	0.03±0.01	
85	Eugenol acetate	-	3.45±2.10	-	
86	Sathulenol	-	-	0.05±0.01	
87	Caryophyllene oxide	-	-	0.59±0.01	
88	Apiol	-	0.76±027	$0.04{\pm}0.02$	
89	Myricetin	-	0.45±0.21	-	
90	Nootkatone	0.04 ± 0.02	-	0.03±0.01	

a calculated by using % area peak normalization; b not detected. Values are the mean \pm SD (n=4). HDE: Chia seed essential oil extracted by hydrodistillation; MAHDE: Chia seed essential oil extracted by microwave-assisted hydrodistillation; CPCO: Cold-pressed chia seed oil.

The major compound identified in the chia essential oil sample obtained by microwave-assisted hydrodistillation (MAHD) was linalool with percentages of 27.99% and followed by hexanol (8.53%), hexanal (7.25%), benzene acetaldehyde (6.40%), hexyl acetate (4.09%) and 3,5-octadiene-2-one (3.98). The percentages of the remaining volatiles in these oil samples ranged from 0.04 to 2.96%.

Linalool is widely used in the perfumery and cosmetic industry for its floral and lavender-like fragrance potential. In addition, the calming and anxiolytic effects of linalool inhalation have been clinically demonstrated in animal models (Souto-Maior *et al.*, 2011). Based on these observations in animals, it is thought that MAHD, which contains linalool in about a third of its volatile compositions, may be used for similar purposes.

To the best of our knowledge, there are limited studies in the literature on the volatile composition of chia seed oil and chia seed essential oil. The results of the present study are consistent with previous studies on different Salvia species (Taârit *et al.*, 2014; Elshafie *et al.*, 2018). Wen *et al.* (2019) revealed that diisobutyl phthalate and phenylacetaldehyde were the major volatiles of chia seed aromatic water while chia seed oil obtained from supercritical CO_2 extraction had mainly aromatic hydrocarbons and alkanes

including toluene, nonanal and mesitylene. In another study (Taârit et al., 2014) on the volatile profile of the essential oil from Salvia species in addition to S. hispanica, α -thujone (14.77%), camphor (13.08%), and 1,8-cineole (6.66%) were determined as major compounds in the essential oils of S. officinalis, while the essential oils of S. verbenaca were characterized by camphor (38.94%), caryophyllene oxide (7.28%), and 13-epi-manool (5.61%) as major volatiles. It was also determined that the essential oil of S. sclarea was composed of linalool (24.25%), α -thujene (7.48%), linalyl acetate (6.90%), germacrene-D (5.88%), bicyclogermacrene (4.29%), and α -copaene (4.08%). Similarly, Tulukcu *et al.* (2019) determined that hexanal, sabinene, α -pinene, α -thujone, borneol, linalyl acetate, β-pinene, camphene, α -thujene, 2,4(10)-thujadien, β -myrcene, limonene, 1,8-cineole and camphor are major volatile compounds, which were similar volatiles determined in the chia seed oil and essential oil in our study.

Similar to our findings, Jung *et al.* (2021) reported that cold-pressed chia seed oil contained volatiles of acids, alcohols, aldehydes, ketones and terpenes. Hexanoic acid, hexanal, (3E, 5E)-3,5-oc-tadien-2-one, 2-ethylfuran, α -pinene and p-cymene were found in high concentrations in cold-pressed chia seed oil. The researchers reported that the levels

of these compounds were 1.23 , 4.74 , 42.6 , 1.06 , 4.42 and $1.55~\mu/kg,$ respectively.

5. CONCLUSIONS

This comprehensive study, delved into the physical and chemical properties of chia seeds and their essential oils, unveiling key insights that hold significant implications for both the food industry and scientific research.

Our investigation of the physical and chemical properties of chia seeds has provided valuable findings to previous literature. The size and 1000-mass weight of chia seeds used for oil production align with established norms. However, the basic nutritional composition of chia seeds exhibits variations within a wide range, which could be attributed to species and geographical factors. Regarding chia seed essential oils, we assessed their relative density and refractive index, which are crucial for quality control and purity evaluations. While cold-pressed chia seed oils exhibit higher values, the influence of distillation techniques on essential oil properties was not statistically significant.

The essential oils of chia seed were extracted using hydrodistillation and microwave-assisted hydrodistillation techniques and the effects of extraction parameters on phenolic content, antioxidant capacity and also the volatile profile of the essential oils were determined. Notably, microwave-assisted distillation outperformed hydrodistillation, achieving a significantly higher oil yield, shorter extraction time, and reduced energy consumption, which underscore its potential for sustainable and efficient oil extraction processes.

It is also noteworthy that the hydrodistilled essential oils have three times the total phenolic content and five times the antioxidant capacity of the microwave-assisted hydrodistilled oil. Additionally, the comprehensive analysis of volatile compounds in chia essential oils revealed a diverse range of components including aldehydes, alcohols, esters, ketones, etc. The unique volatile profile of each oil sample was influenced by the distillation method.

 α -pinene, β -pinene, o-cymene and α -thujene are the major volatiles in cold-pressed chia seed oil, while linalool, mesitylene, anethole and cumene were identified as the major volatile compounds for chia seed essential oils.

In conclusion, this study not only advances our understanding of chia seeds and their essential oils but also underscores their significance in both the food industry and scientific research. This research represents the first evidence in the literature of the volatile profile of chia seed essential oils.

The diverse applications of chia seeds, its potential as a source of bioactive compounds, and the optimization of essential oil extraction processes hold promise for innovative product development and health-related applications. These findings open doors for further research into the applications of chia seed essential oils across industries. Optimizing the extraction of essential oils from chia seeds as a potential source of bioactive compounds promises innovative approaches in the aroma, food, cosmetic and pharmaceutical industries, as well as further scientific research.

DATA AVAILABILITY

The data in this article is available on reasonable demand

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DECLARATION OF COMPETING INTEREST

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