

Supercritical fluid conditions to obtain a chan (Hyptis suaveolens (L.) Poit) seeds oil and its physicochemical characterization, spectroscopy and nutritional analysis.

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Abstract

The aim of this study was to obtain of chan seeds oil by supercritical fluids, and to evaluate its physicochemical properties and its quality. The optimal extraction conditions were 450 bar, 80°C, and 193 min with an extraction yield of 9.21% and 62.36% recovery. The viscosity of the oil was 104.13 mPa s, and the activation energy was 2.104 KJ/mol; primary and secondary oxidation was 1.681 and 0.662. The quality of the oils was evaluated with saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and unsaturated fatty acids (UFAs). They were also determined the atherogenicity (AI), (6.70–8.27) and thrombogenicity indices (TI), (0.075–0.094); and the hypocholesterolemic/hypercholesterolemic (HH) ratio, (19.12–33.84). The oils presented high content of fatty acids ω 3, 6, and 9, and the oxidizability was from 6.70 to 8.27, indicating good stability. The characteristics of the oil extracted were compared with the oil obtained conventionally.

Introduction

Currently, the world has approximately 7.75 billion inhabitants, and it is forecast that, before 2050, it will reach 10 billion. Therefore, it is necessary to have products that have food and nutraceutical functionality for consumption (ONU 2019).

In Mexico, various studies have been carried out on seeds of pre-Columbian use, such as amaranth, quinoa, chia, and chan. These seeds were used as food and medicine by Mesoamerican inhabitants, and they present important potential for human consumption because they are a source of carbohydrates, fats and oils, proteins, oligosaccharides, and minerals among others (Acuña-Gutiérrez et al. 2019).

The chan (*Hyptis suaveolens* (L.) Poit) has a worldwide distribution in temperate zones, particularly in the Mediterranean and Central Asia. In Mexico it is distributed in mountainous areas and it belongs to the Lamiaceae family (Martínez-Gordillo et al. 2013). This family has economic importance because some species are used as condiments (*Origanum*, *Thymus*, *Mentha*) to obtain essential oils (*Lavandula*, *Pogostemon*, *Salvia*) and ornamental oils (*Coleus*, *Salvia*, *Scutellaria*). The most frequent genera in the country are *Salvia*, *Scutellaria*, *Stachys*, and *Hyptis*. Chan seeds have a significant oil content, and their extraction and characterization have not been fully studied. The predominant fatty acids in the seeds are unsaturated fatty acids (UFAs), such as oleic, linoleic, and linolenic acids (Acuña-Gutiérrez et al. 2019).

Oils of vegetable origin are essential in the human diet, due to their energy intake and fat-soluble vitamins, as well as nutritional and functional properties. Currently, there has been special interest in polyunsaturated fatty acids (PUFAs) due to their benefits for health, prevention of cardiovascular diseases, and anti-inflammatory properties (Zamani Ghalesahi et al. 2020). Other studies have found that monounsaturated fatty acids (MUFAs) help improve insulin sensitivity; furthermore, their consumption is associated with healthier serum lipid profiles (Qiu et al. 2019).

Various diseases are related to the deficiency or imbalance of ω 3 and ω 6 fatty acids (Aremu et al. 2015). Seed oils are the main source of polyunsaturated fatty acids, especially α -linoleic and α -linolenic

acids. Chan seeds oil is predominantly composed of PUFAs, 76–86%, and MUFAs, 6–13.6%. Therefore, this type of oil can be considered for food applications (Sahu et al. 2020).

Recently, to evaluate the nutritional quality of vegetable oils, various indices have been developed that allow different groups of fatty acids to be related to each other to determine their potential for the prevention of some diseases. The hypocholesterolemic/hypercholesterolemic (HH) ratio assesses the effect of fatty acid (FAs) composition on cholesterol, by the ratio between hypocholesterolemic FAs (cis- $C_{18:1}$ and PUFA) and hypercholesterolemic FAs ($C_{12:0}$, $C_{14:0}$ and $C_{16:0}$). The atherogenicity index (AI) provides information on the degree of atherogenicity of fatty acids. Low AI can reduce the levels of total cholesterol, phospholipids and esterified fatty acids and therefore reduce the incidence of coronary heart disease (Munhoz et al. 2018; Chen and Liu 2020). On the other hand, the thrombogenicity index (TI) refers to the tendency to form clots in the blood vessels.

Alternately, different physicochemical and rheological properties of vegetable oils are considered quality parameters that are important in industrial processing, stability, useful life of the product, and cost. Such is the case for the acidity, iodine and peroxide values, refractive index, and oxidative stability (Zahir et al. 2017). Physical properties such as surface tension, density, and viscosity are important in transportation, processing, food manufacturing, and heat transfer (Sahasrabudhe et al. 2017).

The specific extinction coefficients, K_{232} and K_{270} , and the peroxide index are used to determine the degree of oxidation and degradation of the oils (Bordón et al. 2019; Muangrat and Jirarattanarangsri 2020). K_{232} is indicative of the formation of peroxides and hydroperoxides during the primary and intermediate oxidation process of the oil. Later, the secondary stage of fatty acid oxidation occurs with the formation of conjugated triene, aldehydes, ketones, and acids. These functional groups have the characteristic of absorbing at 268–274 nm, which allows them to be related to the specific extinction coefficient K_{270} (Timilsena et al. 2017).

Vegetables are a source of a wide variety of bioactive compounds, mixtures of triglycerides, fatty acids, flavonoids, terpenoids, phytosterols, fatty alcohols, and tocopherols. The extraction of these compounds from plant matrices, mainly seeds, leaves, roots, flowers, rhizomes, and bark represent important challenges in obtaining ingredients for food, cosmetics, and pharmaceutical products (Belbaki et al. 2017).

Supercritical fluids extraction (SFE) is friendly to the environment compared to conventional extraction methods; it maintains the quality and safety of the products, reduce energy consumption, toxic waste, and by-products. SFE uses fluids above its supercritical temperature and pressure as solvents. Under these conditions, the fluid has solvent properties typical of a liquid and a viscosity like that of a gas, which facilitates mass transfer (Mwaurah et al. 2020). Supercritical carbon dioxide ($scCO_2$) is widely used in FAs because they are thermodynamically stable, non-flammable, non-carcinogenic or non-mutagenic effects, and is inexpensive, it has low toxicity and high availability (Knez et al. 2019). In addition, it has low viscosity and high diffusivity, which allows it to have a better ability to penetrate

porous solid matrices than conventional solvents and can generate a much faster mass transfer, resulting in faster extractions (Kulazynski et al. 2016). The SFE process consists of pressurization, temperature adjustment, extraction, and separation. These extraction processes depend on factors such as particle size, mass flow rate, temperature, pressure, and extraction time. Industrial applications of SFE are increasing because it uses less process time, has high selectivity, and does not require a product recovery stage; among which are the extraction of antioxidants, spices, aromas, and nutraceutical components (Mwaurah et al. 2020). On the other hand, the extraction of vegetable oils through SFE processes has been studied in different materials (Sodeifina et al. 2018).

Currently, Fourier transformed infrared spectroscopy (FTIR) and Raman spectroscopy techniques have seen increased development in the food industry; in edible oils, they are used in characterization, detecting adulterations, quality analysis, degradation, and identifying oils (Rohman 2017; Zahir et al. 2017; Matwijczuk et al. 2019; Qiu et al. 2019). FTIR has been used as an alternative to conventional analytical methods, with the advantage of being a fast, nondestructive method (Wójcicki et al. 2015; Rohman 2017). Raman spectroscopy is used for the authentication of edible oils, determination of impurities, proportion of *cis/trans* isomers, and degree of unsaturation (Jiménez-Sanchidrián and Ruiz 2016); the technique is based on the change in polarization of the distribution of electrons in the molecule when it vibrates. The nonpolar groups present intense scattering bands, as is the case of multiple bonds ($\text{-C}=\text{C-}$) and the main chain of carbon atoms (-C-C-) of lipids. Alternately, the vibrational modes of the polar groups ($\text{C}=\text{O}$ and OH-) are weaker, contrary to what happens in infrared spectroscopy. These two spectroscopies provide complementary information for the characterization of vegetable oils (Samyn et al. 2012).

The aim of the present study was the extraction of chan seed oil by supercritical fluids as a function of pressure, temperature, and extraction time, as well as evaluate its physicochemical characteristics and nutritional quality.

Materials And Methods

Materials

The chan seeds was acquired at the “La Habanera” seed distributor in Colima, Mexico and kept at room temperature in airtight containers. The seeds were manually separated from foreign material such as stones, broken seeds, and leaves; to later, be ground in a blade mill (model A10, Ika-Werke. DE) to a particle size $> 75\ \mu\text{m}$. All reagents used in this study were analytical grade and were purchased from J. T. Baker (USA). Analytical grade HPLC solvents (Burdick and Jackson, USA) were used to determine fatty acids.

Supercritical Fluid Extraction (Sfe)

The oil extraction from the chan seeds was performed in SFE equipment, model SFT-250 (Supercritical Fluid Technologies, Inc. Newark, Delaware, USA), which consists of a stainless-steel chamber capable of containing supercritical fluids at pressures up to 550 bar and includes a pump driven by compressed air that produces high pressures. Under typical operating conditions, the flow ranges from 1 to 330 mL/min (250 g/min) of liquefied CO₂, which circulates through a recirculation bath (Mac Heto Lab Equipment, San Diego CA, USA) with cooling, which is subsequently pressurized. Pressures, pump flow, temperatures, and safety limits are controlled by PID (proportional, integral, and derivative) from the front panel of the equipment. A restriction valve regulates extraction pressure and flow rates (Fig. 1). Batches of 50 g of ground chan seeds (moisture of 8.94 ± 0.03%), contained in a cell that is 3 cm in diameter and 12 cm in height and manufactured with stainless steel, and were used for extraction. The extraction was carried out conditions at a constant flow of CO₂ (0.881 ± 0.056 kg/h). The samples were collected at atmospheric pressure in a stainless-steel container and kept in amber flasks until analysis. A Box-Behnken experimental design was used to determine the highest oil recovery yield, evaluating the effect of pressure (150, 300 and 450 bar), temperature (40, 60 and 80°C) and extraction time (60, 150 and 240 min).

Oil Extraction With Solvent

Chan seeds oil was obtained by extraction with hexane (Hex) at 80°C for 16 h in a Soxhlet Lab-Line multiunit extractor (Lab-line Instruments, USA), 50 g of ground seeds and 300 mL of hexane were used per batch. The yield was determined gravimetrically.

Extraction Yield

The amount of oil extracted by SFE was weighed to determine the yield of oil extracted with Hex according to Eq. 1.

$$Y = \frac{W_{SFE}}{W_{Hex}} \times 100 \quad (1)$$

where Y = yield (%), W_{SFE}=weight of oil extracted by SFE (g), and W_{Hex}=weight of oil extracted by Hex (g).

Primary And Secondary Oxidation

Oxidation, represented by the absorptivity coefficients K₂₃₂ and K₂₇₀ was determined using 50 mg of oil degassed in an ultrasound bath (Transsonic 460/H. Elmar, DE) dissolved and brought to a volume of 25 mL with hexane. The absorbance was determined at 232 and 270 nm in a UV/Vis WPA Biowave II spectrophotometer (Biochrom, USA), using quartz cells with a 1-cm light beam; hexane was used as a

blank. The specific absorptivity coefficient was calculated with the following equation (Timilsena et al. 2017).

$$K_{\lambda} = \frac{A_{\lambda}}{CL} \quad (2)$$

where K_{λ} =the specific extinction coefficient at a wavelength of λ [(g/100 mL)⁻¹cm⁻¹], A_{λ} =the absorbance value at a wavelength of λ , C = the concentration (g/100 mL), and L = the length of the cell (cm).

Determination Of The Physical Properties Of The Oil

Density, surface tension, pH, and refractive index

The density and surface tension of chan seeds oil were determined at 25°C in surface tension equipment (Analite 2141, Mc Van Instruments, Australia). The pH of the chan seed oil was determined in a potentiometer (Mod. HI 110 Hanna Instruments. MA, USA). The refractive index was determined at 25°C in an ABBE refractometer (American Optical Corporation. Buffalo, NY. USA).

Acidity, Iodine, and Peroxide indices

The oil dissolved in ethanol and added with 1% phenolphthalein as indicator was titrated with an alcoholic solution of 0.1N KOH. The result is expressed in percent oleic acid (O'Keefe and Pike 2010).

An oil sample dissolved in chloroform is reacted with excess iodine bromide (IBr) in glacial acetic acid. Excess IBr reacts with potassium iodide, forming reduced iodine which is titrated with 0.1N sodium thiosulfate (Na₂S₂O₃) and starch as an indicator. Chloroform is used as a blank, (O'Keefe and Pike 2010; Zahir et al. 2017).

An oil sample was dissolved in an acetic acid-chloroform solution, and then saturated KI solution was added. The peroxides present oxidize KI, releasing iodine, which is quantified by titration with a Na₂S₂O₃ solution and starch as an indicator (Zahir et al. 2017). The results are expressed as milliequivalents of oxygen per kilogram of sample.

Viscosity

The determination of the viscosity of the chan oil was carried out in a Paar Physica low- stress rheometer (model LS 100, Germany) using a CP20-4 plate cone geometry with a cone diameter of 20 mm, angle of 4°, and gap of 50 µm. The rheometer is located on a Kinetics System Vibraplane platform (model 2212-01-11. MA, USA). The temperature was 25°C ± 0.1, provided by an automatic system coupled to the rheometer (Julabo water recirculation bath, model F10, USA) controlled by Paar Physica software ver. 2.06.

Activation energy

The activation energy is related to the effect of temperature on viscosity. As the temperature increases, the viscosity of the oil decreases and allows a greater flow. This process requires some energy that can be described by the Arrhenius equation (Hlavác et al. 2017):

$$\eta = Ae^{-E_a/RT} \quad (3)$$

where η = the apparent viscosity, A = the pre-exponential factor, E_a = the activation energy, R = the universal gas constant, and T = the absolute temperature.

Fatty Acid Composition

The fatty acids of the oil of the chan seeds were determined by gas chromatography with the previous formation of the methyl esters of the fatty acids. The separation and quantification were carried out in a gas chromatograph with a flame ionization detector (Perkin Elmer Autosystem, USA), using a 30 m x 0.25 mm x 0.25 μ m ZEBRON ZB-Wax column. Nitrogen at a pressure of 8.4 psi was used as the carrier gas. The column temperature was kept at 170°C for 3 min, then it was increased from 170°C to 230°C at 5°C/min, and finally it was kept at 230°C for 5 min. The identification of the peaks was made by comparison of retention time of fatty acid standards (Supelco Inc, USA). The quantification was carried out in duplicate.

Nutritional Quality Indices And Calculated Oxidizability Value (Cox)

The nutritional quality of the oils was estimated according to their composition of fatty acids, for which the following parameters were evaluated: The ratio fatty acids (FAs) between hypocholesterolemic and hypercholesterolemic (HH) (Eq. 4), according to Ratusz et al. (2018).

$$HH = \frac{C_{18:1} + \sum PUFAs}{C_{14:0} + C_{16:0}} \quad (4)$$

Atherogenicity index (AI) related the main SFAs ($C_{12:0}$, $C_{14:0}$ and $C_{16:0}$) considered proatherogenic and the sum of UFAs (antiatherogenic), according to Eq. 5, as reported by Chen and Liu (2020).

$$AI = [C_{12:0} + (4 \times C_{14:0}) + C_{16:0}] / \sum UFAs \quad (5)$$

Thrombogenicity index (TI) evaluates the thrombogenic potential of AFs $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ (prothrombogenic) in relation to MUFAs ω -3 and ω -6 (antitrombogenic) according to Eq. 6, described by Munhoz et al. (2018).

$$TI = \frac{C_{14:0} + C_{16:0} + C_{18:0}}{(0.5 \times \sum MUFA) + (0.5 \times \sum \omega 6) + (3 \times \sum \omega 3)} \quad (6)$$

The calculated oxidizability value (COX) of the oils was determined based on the content of C₁₈ unsaturated fatty acids (Eq. 7), as described by Ratusz et al. (2018).

$$COX = \frac{C_{18:1} + (10.3 \times C_{18:2}) + (21.6 \times C_{18:3})}{100} \quad (7)$$

where PUFA = polyunsaturated fatty acids, MUFA = monounsaturated fatty acids, and UFA = unsaturated fatty acids.

Fourier Transform Infrared Spectroscopy

The FTIR characterization of the oil samples was performed on a spectrometer (Cary 630 Agilent, USA). A drop of the oil was placed on the detection surface of the diamond ATR (attenuated total reflectance) crystal, the spectra from 4000 to 500 cm⁻¹ were determined at a resolution of 4 cm⁻¹, and 4 simultaneous scans were made of each sample. Before each measurement, 4 background spectra were obtained with the empty ATR surface using the same sample acquisition parameters. The data were evaluated with Microlab FTIR Software (Agilent Technologies, USA).

Raman Spectroscopy

The Raman spectra of the oils were obtained by confocal Raman spectroscopy and atomic force microscopy (WITec, Denmark), with 4 objectives of 10, 20, 50, and 100 µm, in addition to a 633-nm-wavelength red laser coupled with fiber optics. 100 µl of oil was placed in a sample holder to be focused and obtain the Raman spectrum. Data acquisition was performed with Control software ver. 4.0 (WITec, Denmark).

Statistical analysis

An analysis of variance (ANOVA) was performed to evaluate significant differences between the results obtained for the chan seeds oil extracted by supercritical conditions and with solvent. Tukey's test was used to determine the difference between means with a confidence level of 95%. The analysis was performed by the software Statistical Minitab ver. 17 (Minitab Inc., State College. PA, USA).

Results And Discussion

Physicochemical characterization of chan seeds oil

The results obtained for the oil extracted under the different SFE conditions (Table 1) show that the lower pressure (150 bar) and higher temperature (80°C) of extraction induce the instability of the oil, and favor the primary and secondary oxidation, expressed as K_{232} (7.798) and K_{270} (2.268). The viscosity decreased to 82.91 mPa s, with respect to the maximum value of 144.20 mPa s determined for the oil extracted at 300 bar, 240 min extraction, and 40°C. This is attributed to the progressive breakdown of fatty acids caused by oxidation, which subsequently form low-molecular-weight compounds such as free fatty acids, alcohols, ketones, and aldehydes (Zahir et al. 2017). The refractive index varies from 1.4716 to 1.4762, and these values agree with those reported by Salinas et al. (2020) for walnut, almond, and pecan nut oils, as well as sunflower seeds and sesame oils (Godswill et al. 2018). The refractive index is related to the degree of oxidation and the composition of the fatty acids; it rises as the length of the fatty acid chains and the degree of unsaturation increase (Timilsena et al. 2017). The chan seeds oils obtained in this study have a refractive index of 1.475, higher than those reported for flaxseed oils, basil seeds oil (Zamani Ghalesahi et al. 2020) and watermelon seeds oil (Karrar et al. 2019).

Table 1

Viscosity, refractive index and primary and secondary oxidation of chan (*Hyptis suaveolens* (L.) Poit) seeds oil at experimental conditions

Pressure (bar)	Temperature (°C)	Extraction time (min)	Viscosity (mPa s)	Refractive index	K ₂₃₂	K ₂₇₀
150	40	150	88.52	1.4749	4.804	0.799
450	40	150	85.11	1.4759	3.985	0.811
150	80	150	82.91	1.4755	7.798	2.268
450	80	150	94.16	1.4762	2.486	0.653
150	60	60	94.82	1.4737	5.873	0.884
450	60	60	101.10	1.4732	3.802	1.108
150	60	240	107.00	1.4733	3.582	0.486
450	60	240	104.80	1.4721	3.348	0.433
300	40	60	91.28	1.4758	3.100	0.878
300	80	60	90.29	1.4757	2.412	0.721
300	40	240	144.20	1.4746	3.797	0.690
300	80	240	103.70	1.4716	3.092	0.812
300	60	150	103.10	1.4757	3.641	0.858
300	60	150	109.20	1.4754	5.830	1.022
300	60	150	131.80	1.4732	4.443	0.872
300	60	150	105.10	1.4739	2.677	0.809
300	60	150	101.90	1.4734	2.930	0.825
K ₂₃₂ = specific extinction at 232 nm, K ₂₇₀ = specific extinction at 270 nm.						

The chan seeds oils obtained was evaluated by their physicochemical properties (Table 2). The maximum extraction yield under supercritical conditions was 62.36% (n = 4) at a pressure of 450 bar, a temperature of 80°C and an extraction time of 193 min, and 14.77% by Hex; these data coincide with the obtaining of grapeseed oil by SFE (Fiori et al. 2014). However, the solvent extraction process is not selective and requires high temperatures, which cause the thermal decomposition of bioactive compounds, longer process times, and solvent recovery (Karrar et al. 2019).

Table 2
Physicochemical characterization of chan (*Hyptis suaveolens* (L.) Poit) seeds oil obtained by different extraction methods

Parameters	SFE	Hex
Extraction yield (%)	9.21 ± 0.128	14.77 ± 0.50
pH	6.277 ^a ± 0.023	6.093 ^b ± 0.021
K ₂₃₂	1.681 ^b ± 0.059	2.211 ^a ± 0.022
K ₂₇₀	0.662 ^a ± 0.026	0.671 ^a ± 0.053
Refractive index at 25°C	1.475 ^a ± 1.00x10 ⁻⁴	1.475 ^a ± 5.77x10 ⁻⁵
Surface tensión (mN/m)	26.600 ^b ± 0.100	30.533 ^a ± 1.012
Density at 25°C (g/cm ³)	0.987 ^a ± 0.016	0.9813 ^a ± 0.002
Acidity index (% Oleic acid)	2.202 ^a ± 0.096	2.2305 ^a ± 0.161
Iodine index (g I ₂ /100g)	110.630 ^b ± 0.431	123.225 ^a ± 0.413
Peroxide index (meq O ₂ /kg)	7.254 ^b ± 0.418	13.081 ^a ± 0.226
Viscosity (mPa s)	104.13	108.5
Activation energy (KJ/mol)	2.1041	5.477
SFE = supercritical fluid extraction, Hex = hexane extraction, K ₂₃₂ = specific extinction at 232 nm, K ₂₇₀ = specific extinction at 270 nm. The analysis was performed in duplicate. Different letters indicate minimal significant difference (p < 0.05).		

Table 2 shows that the oil obtained by SFE presents lower primary oxidation of unsaturated fatty acids, as indicated by the values of K₂₃₂ and the peroxide index of 1.681 and 7.254 meq O₂/kg, respectively, than those presented by the oil extracted with Hex. The acidity index and K₂₇₀ values do not present a statistically significant difference (p < 0.05), and due to their value (0.662–0.671), they indicated minimal formation of conjugated trienes. Currently, there are no reports in the literature on the K₂₃₂, K₂₇₀, and peroxide values of chan seeds oil. However, the peroxide index values obtained in this study are minors for oils extracted by cold pressing of 15 meq O₂/kg (Bordón et al. 2019) and avocado oil, which has a peroxide index of 10 meq H₂O₂/kg of oil (Espinosa-Alonso et al. 2017) that are similar with the values obtained for safflower seed oil under supercritical conditions (Bala et al. 2016). The K₂₃₂ values for chan seeds oil obtained by SFE are comparable with those obtained for chia seeds oil (Timilsena et al. 2017), pistachio oil (Rabadán et al. 2017), and purslane seed oil under supercritical conditions (Sodeifina et al. 2018).

The viscosity of the oils depends on the nature of the fatty acids, such as the length of the chain and the degree of unsaturation, and their arrangement in the molecules that make up the triglycerides of the oils. In addition, it is known that the density and viscosity of oils increase with the degree of saturation and polymerization of fatty acids, and with increasing temperature, these properties decrease (Sahasrabudhe et al. 2017). The density values of the chan seeds oils obtained by SFE and Hex were 0.987 and 0.981 g/cm³, with viscosities of 104.13 and 108.5 mPa s, respectively. The viscosity determined in chan seeds oils agrees with those reported for oils with a high content of unsaturated fatty acids (Zahir et al. 2017), and the density is higher than those reported for conventional oils (Karrar et al. 2019), which is explained by the predominant presence of high-molecular-weight fatty acids of 94.16–96.39% (Table 3).

Table 3
Fatty acid composition and nutritional quality index of chan (*Hyptis suaveolens* (L.) Poit) seeds oil extracted by different methods

Fatty acids (%)	SFE	Hex
Palmitic acid (16:0)	4.79 ^a ± 0.011	2.85 ^b ± 0.081
Palmitoleic acid (16:1)	2.62 ± 0.331	ND
Stearic acid (18:0)	1.05 ^a ± 0.075	0.76 ^b ± 0.018
Oleic acid (18:1) (-9)	25.67 ^a ± 0.716	17.87 ^b ± 0.711
Linoleic acid (18:2) (-6)	59.93 ^b ± 0.148	78.51 ^a ± 0.810
Linolenic acid (18:3) (-3)	5.93 ± 0.173	ND
Total SFA	5.84 ^a ± 0.064	3.61 ^b ± 0.100
Total MUFA	28.30 ^a ± 0.385	17.87 ^b ± 0.711
Total PUFA	65.86 ^b ± 0.321	78.51 ^a ± 0.810
Total UFA	94. 16 ± 0.065	96.39 ± 0.100
PUFA/MUFA	2.38 ± 0.043	4.40 ± 0.220
UFA/SFA	16.12 ± 0.188	26.69 ± 0.764
COX	6.70 ± 0.001	8.27 ± 0.076
HH	19.12 ± 0.0381	33.84 ± 1.100
AI	0.051 ± 0.0001	0.030 ± 0.0008
TI	0.094 ± 0.0004	0.075 ± 0.0022
Hex = hexane extraction, SFE = supercritical fluid extraction, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids, COX = calculated oxidizability value, HH = ratio of hypocholesterolemic to hypercholesterolemic, AI = atherogenicity index, TI = thrombogenicity index, ND = not detected. The analysis was performed in duplicate. Different letters indicate minimal significant difference (p < 0.05).		

The acid indices obtained for oils by SFE and Hex were 2.202 and 2.230% respectively, without finding a significant difference (p < 0.05). These values are increased in oils with a higher degree of unsaturation due to their sensitivity to oxidation and the formation of free fatty acids (Muangrat and Jirarattanarangsri 2020). The iodine value obtained for both oils were relatively high, 110.63 for SFE and 123.225 for Hex, with a significant difference (p < 0.05). The iodine value is commonly used to determine the content of unsaturated fatty acids, and higher levels are related to a high content of UFAs (unsaturated fatty acids), (Karrar et al. 2019). The refractive index, acid index, and density values reported in this study are higher than those determined for oil from chan seeds grown in India (Rai et al. 2013).

Surface tension is a molecular phenomenon where attractive forces intervene between molecules. In oils, it is important during frying because of its effect on the frequency of bubble formation on the surface of the food that drives convection and can contribute to the absorption of oil in food by capillary action. In addition, it can affect processed foods due to its influence on crystal nucleation rates and foaming (Xu et al. 2017). The surface tension of the oil obtained by SFE is less than that of the Hex oil, 26.600 vs. 30.533 mN/m, respectively, and they are significantly different $p < 0.05$.

Effect of temperature on the viscosity and activation energy of chan seeds oils extracted by SFE and Hex

The viscosity of an oil depends on the molecular weight and the degree of unsaturation of the fatty acids, and temperature. Generally, the viscosity decreases with increasing temperature, and its behavior can be described by the Arrhenius model (Diamante and Lan 2014). The flow curves of the oils extracted by SFE and Hex; showed a constant relationship between τ and γ , at the temperatures 15 at 65°C. This behavior corresponds to Newtonian fluids and coincides with that of other vegetable oils; this is due to the increase in kinetic energy and the decrease in intermolecular cohesion forces, generating lower oil viscosity. The activation energy and viscosity of vegetable oils are important for absorption, frying, cooking, and transport processes (Hlavác et al. 2017). The viscosity of chan seed oils decreases exponentially with increasing temperature, obtaining activation energies of 2.1041 and 5.477 KJ/mol with coefficients of determination (R^2) of 0.9243 and 0.9819 for the oil extracted by SFE and Hex, respectively. Therefore, the change in viscosity with temperature in the oils studied can be described with an Arrhenius-type model, and the activation energy can be determined.

Fatty Acid Composition Of The Oils Obtained

The fatty acid composition of chan seeds oil obtained by SFE and Hex (Table 3), presented a statistically significant difference ($p < 0.05$), with the predominant presence of UFAs from 94.16 to 96.39%, which are higher values than those reported for other oils (Bachheti et al. 2015). Both oils have C-16 and C-18 fatty acids. The UFA/SFA (unsaturated fatty acids/saturated fatty acids) ratio from 16.12 to 26.69 is higher than those reported for oils like, safflower (Bala et al. 2016), chia (Timilsena et al. 2017), and flaxseed (Zamani Ghalesahi et al. 2020). This parameter is a criterion for evaluating the functional and nutritional properties of vegetable oils (Sodeifina et al. 2018).

The PUFA/MUFA (polyunsaturated fatty acids/monounsaturated fatty acids) ratio for the oil extracted by SFE was 2.38, while it was 4.4 for that extracted by Hex. The UFAs of the oil extracted by SFE were oleic acid (C-18) (25.67%), linoleic acid (C-18) (59.93%), and linolenic acid (C-18) (5.93%). Fatty acids C-18 and C-18 are important for the cardiovascular system and for the development of the retina and nervous system; however, humans cannot synthesize them, and they must be supplied through the diet (Karrar et al. 2019).

On the other hand, HH ratio, atherogenicity index (AI), and thrombogenicity index (TI) are considered indices of nutritional quality (Chen and Liu 2020). HH evaluates the specific effect of fatty acids on

cholesterol metabolism. High HH values indicate the predominance of hypocholesterolemic fatty acids, as is the case of the studied chan seed oils (Table 3), where HH values of 19.12 and 33.84 are observed for the oil obtained by SFE and Hex, respectively. These values are higher than those found for other vegetable oils (Ratusz et al. 2018), which can be explained by the absence of myristic acid and low content of palmitic acid, which are the main hypercholesterolemic fatty acids (Munhoz et al. 2018). AI lists the main SFA (anti-atherogenic) and the UFA (pro-atherogenic). The consumption of foods with lower AI values reduces the risk and incidence of cardiovascular diseases; can lower total cholesterol and LDL-C in the blood (Munhoz et al. 2018). AI values of 0.051 and 0.030 were found for chan seeds oil by SFE and Hex, respectively. That coincide with those reported for other vegetable oils (Chen and Liu 2020). TI is an index that assesses the potential risk of clot formation in blood vessels due to fatty acids. A lower IT reduces the degree of thrombogenicity. In the oils studied, TI values ranged from 0.075 to 0.094, which are lower than those found for certain seaweeds, dairy products, and other vegetable oils (Chen and Liu 2020). The high calculated oxidizability value (COX) indicates the presence of a high content of UFAs and greater stability of the oil. The values found of 6.70 for oil obtained by SFE and 8.27 for oil obtained by Hex agree with those obtained for oils with high UFAS content, such as flaxseed oil (Moknatjou et al. 2015) and other vegetable oils (Abril et al. 2019).

Analysis Of Chan Seed Oils By Spectroscopy

FTIR

The FTIR spectra of the chan seeds oils obtained by SFE and Hex are shown in Fig. 2, where it is observed that both follow a similar spectroscopic pattern. The functional groups and types of vibration corresponding to each absorption frequency are presented in Table 4.

Table 4

Frequency of the main bands of the FTIR and Raman absorption spectrum of chan seed oils and type of vibration of related functional groups

FTIR Spectroscopy		Raman Spectroscopy	
Frequency (cm ⁻¹)	Functional groups and type of vibration	Frequency (cm ⁻¹)	Functional groups and type of vibration
3011.7	<i>cis</i> -olefinic double bonds $\nu(=C-H)$	3009	Stretching vibration in C-H $\nu_{as}(-C=C-H)$
2924.1	<i>cis</i> -bonds of aliphatic groups in triglycerides $\nu_{as}(-C-H_{vst}, -CH_2)$.	2958	Stretching vibrations of C-H bonds, $\nu(-C-H)$ in methyl (CH ₃) and methylene (CH ₂) groups
2855.1	<i>cis</i> -bonds of aliphatic groups in triglycerides $\nu_s(-C-H_{vst}, -CH_2)$	2917	Aliphatic C-H stretch (CH ₂) $\nu_{as}(-C-H)$
1744.4	Aliphatic ester carbonyl bonds $\nu(-C=O_{vst})$	2897	Stretching vibration in CH ₃ group $\nu_s(-C-H)$
1653.1	<i>cis</i> -olefinic bonds, $\nu_w(-C=C-)$	2874	Stretching vibration in methylene (CH ₂) group bounds $\nu(-CH_2-CH=CH-)$
1457.4	-C-H bonds of CH ₂ and CH ₃ aliphatic groups, $\nu_w(-C-H)$. -C-H <i>cis</i> -distributed in olefins, $\delta_w(-C-H)$.	2850	Aliphatic C-H stretch $\nu_s(-CH_2-)$
1377.3	-C-H bonds of CH ₃ groups, $\nu_{as}(-C-H, -CH_3)$	1660	Stretching vibration - <i>cis</i> $\nu(-HC=CH-)$
1237.5	C-O bond, $\nu(-C-O)$ or -C-H bonds of CH ₂ groups, $\delta(-CH_2-)$.		
1161.1	C-O bond, $\nu(-C-O)$	1440	-C-H bonds of CH ₂ groups, $\delta(-CH_2-)$.
1099.6	C-O bond, $\nu_{sw}(-C-O)$	985	- <i>cis</i> Stretching vibration $\nu(-C-C-, -C=C-, -C=O-)$ $\delta(-CH_2-)$
961.65	<i>trans</i> -bonds out-of-plane $\delta_w(-HC=CH-)$		
915.06	<i>cis</i> -unsaturated bonds out-of-plane, $\nu_w(-HC=CH-)$		
v = vibration, δ = deformation, s = symmetric, as = asymmetric, st = strong, w = weak			

FTIR Spectroscopy		Raman Spectroscopy
846.11	-CH bonds of aliphatic groups CH ₂ , δ (-CH ₂ -)	
724.97	Overlap methylene group δ (-CH ₂ -), and (-HC = CH-, <i>cis</i> -) out-of-plane in disubstituted olefins,	
ν = vibration, δ = deformation, s = symmetric, as = asymmetric, st = strong, w = weak		

The characteristic absorption peak due to the stretching vibration (ν C-H) of hydrogen atoms bound to sp^2 -type carbon atoms, which are related to *cis*-double bonds, occurs at 3010 cm^{-1} . In oils with higher content of PUFAs, the intensity of this peak tends to increase and shift at higher frequencies, as in the case of chan seed oils, which occurs at the studied oils (3011.7 cm^{-1}), as observed in Fig. 2. Vibrations corresponding to in alkyl-C-H bonds of methylene groups in the -*cis* position are characteristic of aliphatic groups in triglycerides vibrations asymmetric and symmetric, respectively (Samyn et al. 2012; Matwijczuk et al. 2019); the degree of unsaturation can be determined by the intensity of the bands close to 3010 and 2856 cm^{-1} . The carbonyl group bonds of saturated aliphatic esters (Table 4) vibrate at frequencies close to 1745 cm^{-1} (Wójcicki et al. 2015). Chan seeds oils present an acute band of high intensity and without shoulder is presented at 1744.4 cm^{-1} . This indicates that there are no inter or intramolecular interactions with other components of the oil and the null or low formation of dimers or monomers of carboxylic acid due to its degradation (Timilsena et al. 2017).

A weak band at 1653.1 cm^{-1} , which belongs to stretching vibrations of olefinic -*cis* bonds, of the -C = C- group and the band that appears at 3011.7 cm^{-1} indicate the degree of unsaturation (Matwijczuk et al. 2019). The band at 1457 cm^{-1} is characteristic of the bending vibration -C-H in the aliphatic groups CH₂ and CH₃. When the degree of unsaturation increases, a band appears adjacent to the main band, as in the case of chan seeds oils (Fig. 2); which is attributed to the rocking deformation of the -*cis* -C-H- bonds distributed in the olefins (Samyn et al. 2012). Stretching and deformation vibrations of asymmetric -C-H- bonds linked to a methyl group (CH₃), are observed at 1377 cm^{-1} (Matwijczuk et al. 2019). At 1237 cm^{-1} , a combination of two asymmetric stretching vibrations of the ester bond occurs, one of them of O-C-C and the other of C-C(=O)-O. In addition, it can be associated with the out-of-plane bending deformation of methylene groups (Matwijczuk et al. 2019). The contiguous band (1161.1 cm^{-1}) is of greater intensity and is formed by the stretching vibration of C-O (Zahir et al. 2017). A weak band at 1100 cm^{-1} , which decreases as the degree of unsaturation increases, corresponds to the vibration of O-C-O of esters originating from secondary alcohols and forming part of triglycerides. The intensity of this band decreases with greater unsaturation of the fatty acids of an oil (Samyn et al. 2012). The next band at 961.7 cm^{-1} is associated with the bending vibration of *trans*-CH = CH olefinic bonds out of plane (Wójcicki et al. 2015), and the changes by oxidation increase this type of bonding. In Fig. 2, it is observed

that this band is tenuous, which confirms the low or nonexistent oxidation of the oils studied. Alternately, the band at 915 cm^{-1} is related to stretching vibrations of cis-bonds of olefinic groups. The last vibration appears at 725 cm^{-1} , which constitutes the superposition of the rocking vibration of methylene groups ($-\text{CH}_2$) and the out-of-plane vibration of -cis disubstituted olefins ($-\text{CH}=\text{CH}-$) (Zahir et al. 2017).

Raman

Table 4 shows the frequencies, type of vibration, and assignment of corresponding functional groups. The Raman spectrum is shown in Fig. 3a, in which it is observed that the oils extracted by SFE and Hex show similar behavior. The region from 1300 to 800 cm^{-1} presents a maximum absorption at 985 cm^{-1} . In this area, the vibration bands that are characteristic of vegetable oils were not defined (Carmona et al. 2014). In this interval, there are stretching vibrations of -C-C- bonds of the methylene group $(\text{CH}_2)_n$ and C = O- bonds, twisting bending of -C-H- bonds of methylene groups, and deformations in the plane of = C-H- groups in a -cis ($-\text{C}=\text{C}-$) bond that is not conjugated (Qiu et al. 2019).

In Fig. 3b the region from 3100 to 2700 cm^{-1} is observed in detail, where 6 bands are seen. The first occurs at a frequency of 3009 cm^{-1} , which is generated by the asymmetric stretching vibration of -C-H- bonds in C = C-H groups (Carmona et al. 2015). The band at 2958 cm^{-1} corresponds to the stretching vibration of -C-H- bonds in methyl and methylene groups (Carmona et al. 2014). A weak band at 2897 cm^{-1} that corresponds to a symmetric stretching vibration of methyl groups (CH_3) is related to the asymmetric stretching vibration of methylene groups (CH_2) at a frequency of 2917 cm^{-1} (Jiménez-Sanchidrián and Ruiz 2016). A characteristic stretching vibration of methylene groups linked to an unsaturated carbon atom ($-\text{CH}_2-\text{CH}=\text{CH}-$) in unsaturated fatty acid chains occurs at a frequency of 2874 cm^{-1} . The last band identified at 2850 cm^{-1} is related to C-H- aliphatic bonds of methylene groups (CH_2) that present symmetric stretching vibrations (Carmona et al. 2014; Jiménez-Sanchidrián and Ruiz 2016).

In the $1800 - 1200\text{ cm}^{-1}$ region (Fig. 3c), -cis bond stretching vibrations ($\text{RHC}=\text{CHR}$) are distinguished at frequencies close to 1660 cm^{-1} . The ratio of the cis/trans isomers present in the oils determines the exact position of this band; at 1440 cm^{-1} , there is a band related to the scissoring bending deformation of the -C-H- bonds of methylene groups in fatty acid chains (Carmona et al. 2015).

Conclusions

Chan seeds had 14.77% oil and the highest recovery by SFE was 62.36% at 450 bar pressure, 193 min of extraction, and 80°C . The oil obtained by SFE shows less oxidation and degradation than the oil obtained with Hex. Unsaturated fatty acids are predominant, from 94.16 to 96.39%. In both cases, PUFAs are found in a higher proportion. Linoleic acid (-6) was the most abundant, greater than 60%. Analysis by FTIR and Raman spectroscopy showed that both oils have a similar structure, highlighting the presence of cis-olefinic double bonds and aliphatic bonds of methyl and methylene groups. These results confirm that chan seeds oil is mainly made up of unsaturated fatty acids. Its high nutritional quality was

demonstrated by AI and TI values that were less than 0.1 and HH values of 19.2 (SFE oil) and 33.84 (Hex oil). The lipid composition and nutritional quality of the oil can help reduce the risk of diseases, and the low calculated oxidizability value (6.70 and 82.27) indicate the good stability of the oils. Supercritical fluid extraction technology is a good alternative for obtaining chan seeds oil with potential for food uses, because it allows preserving its characteristics and quality in a more environmentally friendly way. Chan seed oil may have future applications in the Food and Pharmaceutical Industry.

Declarations

Author contribution to the article. The authors responsibilities they were the following:

MDDC: Experimental development, Methodology and Analysis.

EGRR: Results validation, Writing, Review and Edition manuscript.

MGS: Experimental supervision, Methodology.

JASM: Conceptualization, Supervision, Writing, Review and Edition of manuscript.

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Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest statement

The authors declare no conflict of interest.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Availability of Data and Materials Not applicable.

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Figures

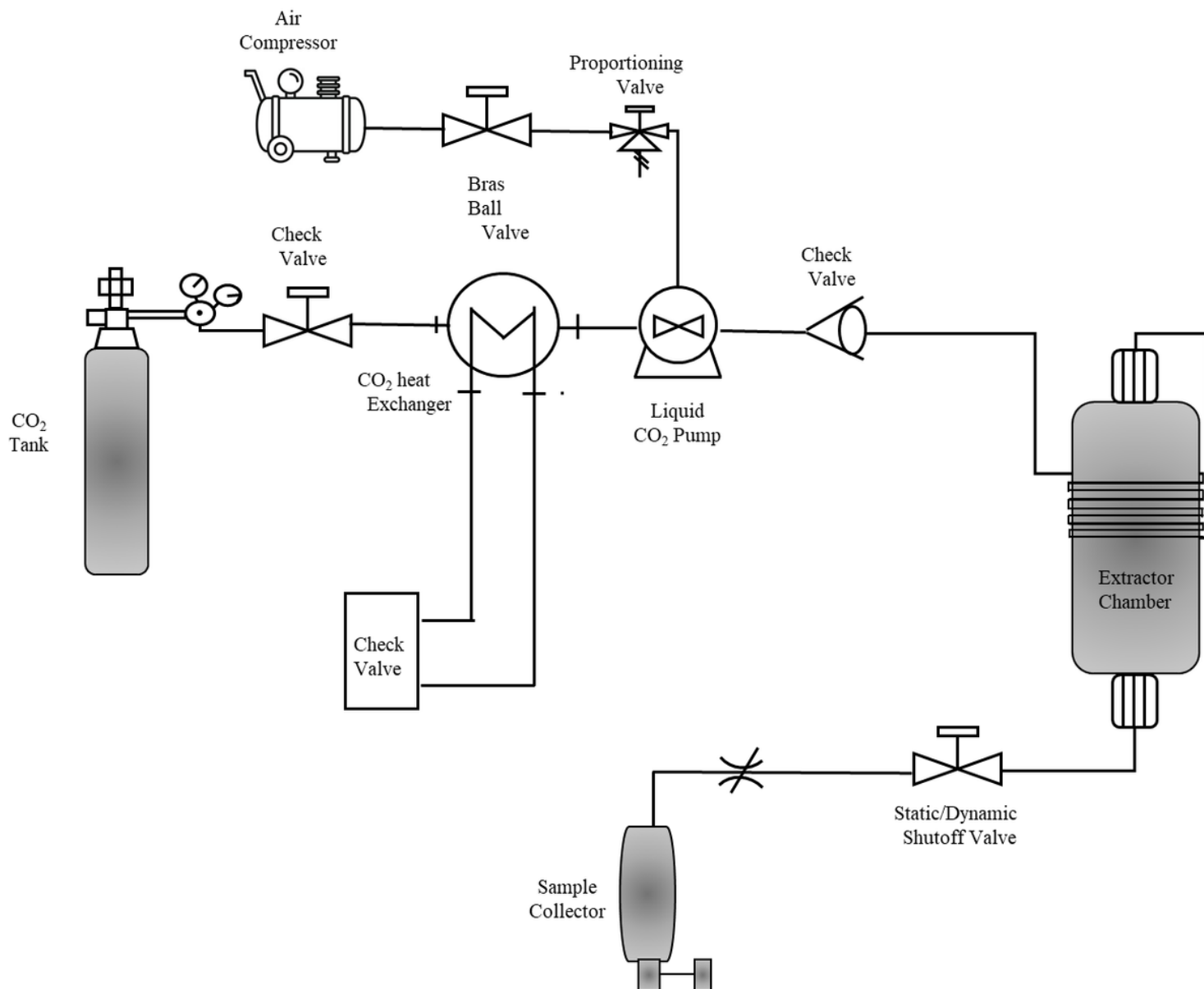


Figure 1

Block diagram of supercritical extraction system

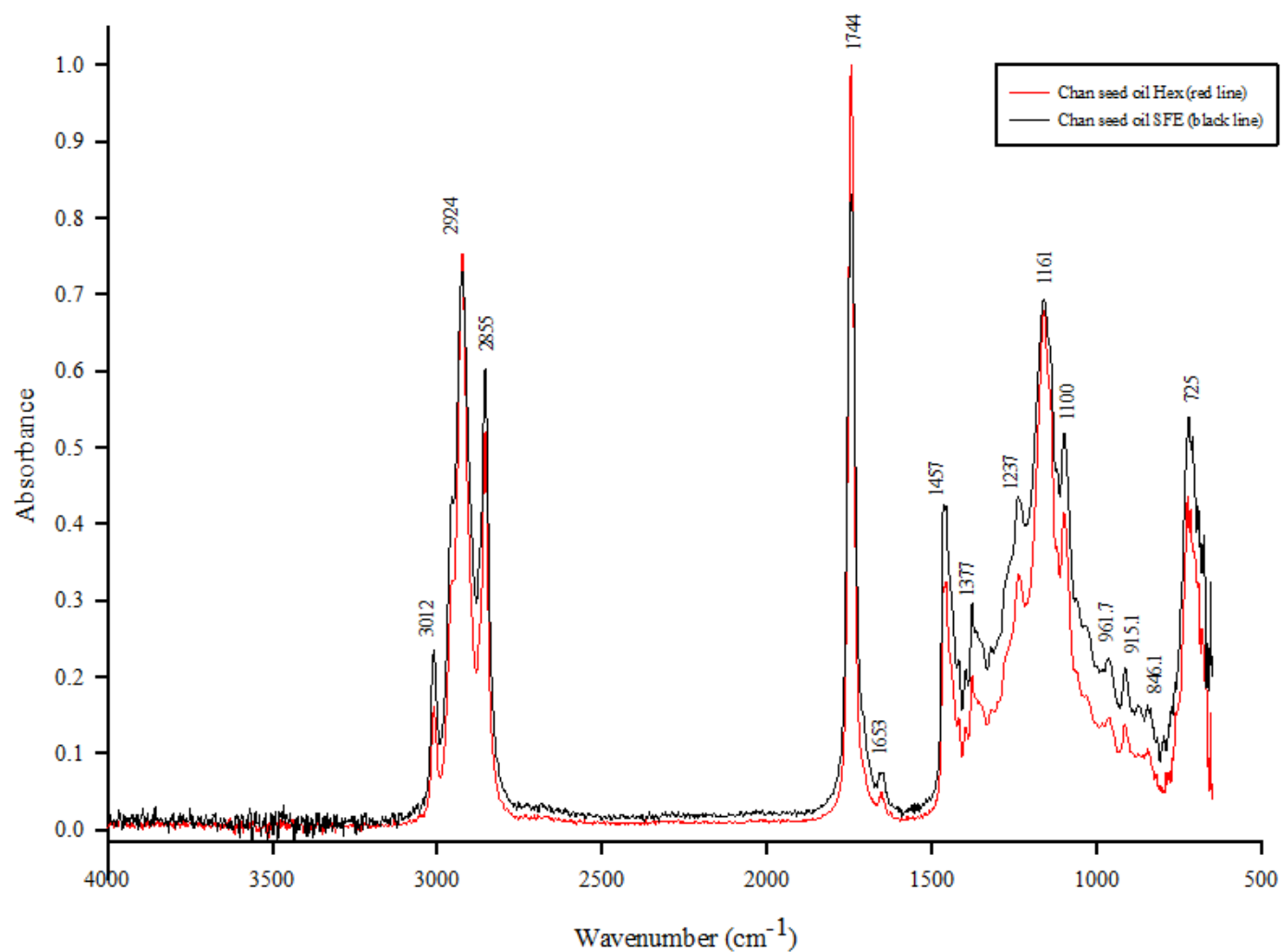


Figure 2

Comparative FTIR spectrum of chan (*Hyptis suaveolens* (L) Poit) seeds oil extracted under supercritical conditions and hexane extraction

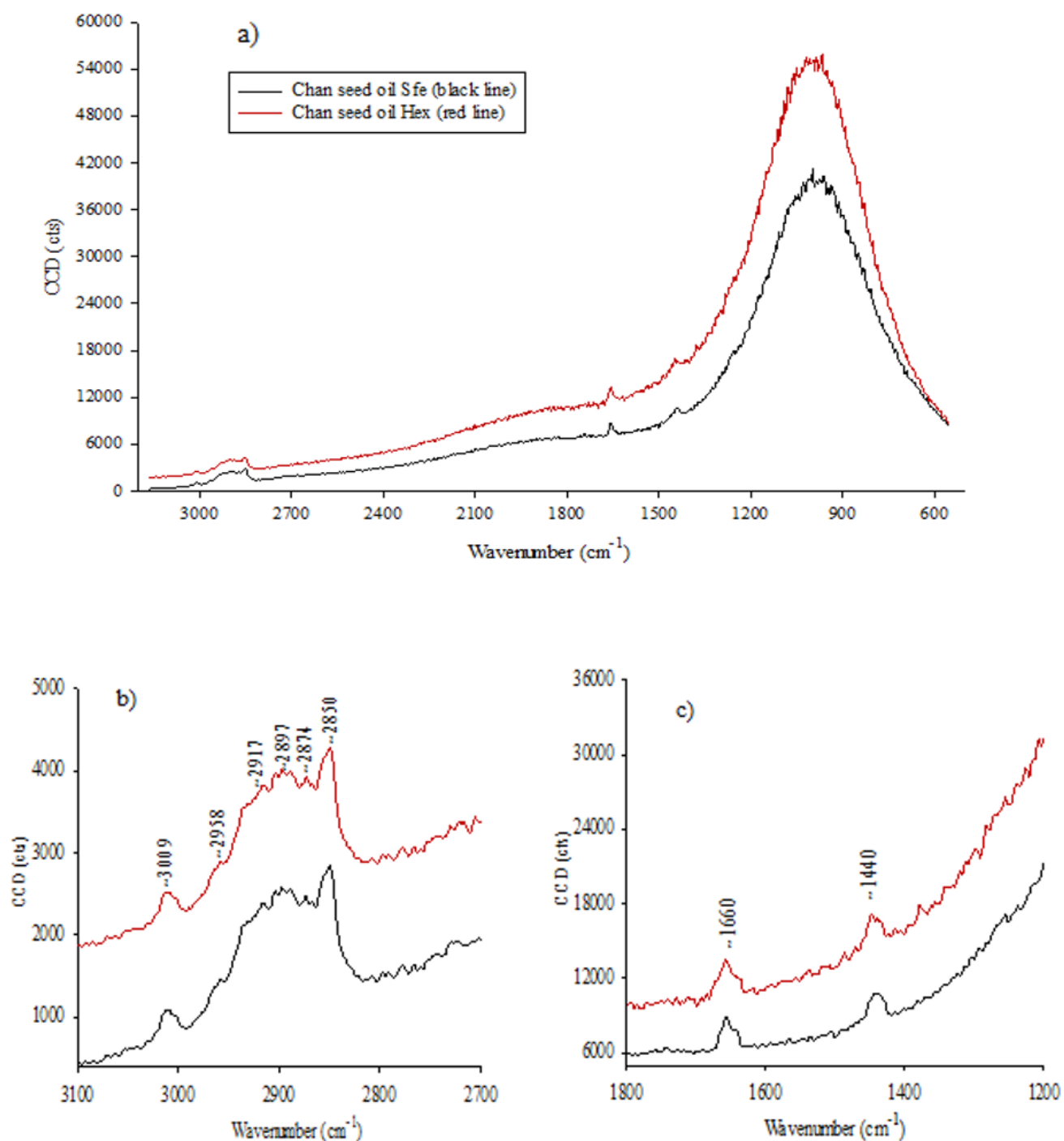


Figure 3

Comparative Raman spectrum of chan (*Hyptis suaveolens* (L) Poit) seed oil extracted under supercritical conditions and extraction with hexane. a) total spectrum,

b) detailed band at 3100 to 2700 cm⁻¹ region, c) detailed band at 1800 to 1200 cm⁻¹ region.