



Research article

Enhancement of omega-3 content in sacha inchi seed oil extracted with supercritical carbon dioxide in semi-continuous process

Sarawut Jitpinit^a, Chaiyapop Siraworakun^a, Yanyong Sookklay^a, Kamchai Nuithitikul^{b,*}^a Department of Chemical and Materials Engineering, Faculty of Engineering, Rajamangala University of Technology Thanyaburi, Pathum Thani, 12110, Thailand^b Biomass and Oil Palm Center of Excellence, School of Engineering and Technology, Walailak University, Nakhon Si Thammarat, 80160, Thailand

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ABSTRACT

Sacha inchi seed oil is a promising substance for applications in food, pharmaceutical, and nutraceutical industries because of its valuable components, particularly omega-3. In this research, sacha inchi oil was extracted from the seed kernels using supercritical carbon dioxide (CO₂) extraction compared with Soxhlet extraction. The influences of extraction time, type of solvents (hexane, ethanol, butanol, and *i*-propanol), and solvent volume on the oil yield and compositions were investigated in the Soxhlet. In the supercritical CO₂ extraction, the effects of extraction time, temperature, and pressure were evaluated. The physicochemical properties of sacha inchi oils extracted with supercritical CO₂ were characterized. Scanning electron microscopy (SEM) equipped with energy dispersive X-ray analysis (EDX), Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) were also carried out. The results showed the advantage of using supercritical CO₂ extraction to increase the omega-3 content in the extracted oil within a shorter extraction time. The omega-3 content of 46.08% was obtained from the supercritical CO₂ extraction at 400 bar and 60 °C. Supercritical CO₂ extraction is a safe and environmentally friendly method that yields a toxic-free oil.

1. Introduction

Sacha inchi (*Plukenetia volubilis* L.), commonly known as Inca nut, is an oily plant of the Euphorbiaceae family. The plants grow widely in rain forests and produce star-shaped fruits. When they are ripe, the seeds inside turn to be dark brown. Sacha inchi seeds are the source of healthy lipids and proteins because they are rich in oils (35–60 wt%) and proteins (25–30 wt%) [1]. They also provide macronutrients and bioactive compounds, which decrease the cholesterol level and the risk of certain types of cancer [2, 3]. Sacha inchi seed oil is traditionally used for the treatment of muscular and rheumatic problems [4]. It can be said that sacha inchi oil has a great potential for applications in the food, pharmaceutical, and nutraceutical industries [5, 6].

The major compositions of sacha inchi oil are polyunsaturated fatty acids, about 85–93% of the total [5, 7]. Among these, linolenic acid (omega-3) and linoleic acid (omega-6) are mostly found [5, 8]. Both fatty acids are essential for humans and only obtained from food. They could prevent hypertension and cardiovascular diseases, decrease the risk of cancers and other chronic diseases, and reduce cholesterol when used as food supplements [3, 7]. Moreover, oleic acid (omega-9) is reported as another nutrient in sacha inchi oil. However, the compositions of sacha

inchi seeds could vary depending on the cultivar, the growing condition, and the seed processing [2, 9].

Sacha inchi oil could be extracted from the seeds by various methods, i.e., cold pressing, conventional extraction using polar and non-polar solvents, and enzymatic extraction. Cold pressing process produces mainly crude oil; therefore, further purification processes, i.e., sedimentation of impurities and filtration, are necessary. Cold pressing is mostly used for the production of virgin sacha inchi oil, and the low oil yield is usually obtained, e.g., 35.4% [9]. Solvent extraction provides higher oil yields than cold pressing method but extracting solvents are toxic and flammable. Quality of oil could deteriorate by high temperature in solvent extraction process. In the food industry, the use of solvents is facing restrictions due to the high consumption of toxic solvents, the subsequent process of solvent removal, and the concern of solvent residues in final products [3]. The solvent residues must be reduced to very small concentrations, causing higher cost.

Supercritical carbon dioxide (CO₂) extraction is a promising method for extracting essential oils and valuable substances in food, pharmaceutical, and nutraceutical industries [10]. The benefits of supercritical extraction rely on the solvation and transport properties of the supercritical CO₂ to selectively separate required substances by varying

* Corresponding author.

E-mail addresses: kamchai_fius@yahoo.co.uk, kamchai.nu@wu.ac.th (K. Nuithitikul).

pressure and temperature that alters CO₂ density and solute solubility in the CO₂ [11]. The liquid-like density, gas-like viscosity, and diffusion coefficients of supercritical fluids make them penetrating more into the solid structure inaccessible to liquids due to negligible surface tension and viscosity [10]. Compared to traditional extraction, supercritical CO₂ extraction is a clean solvent-free technology [12] because there is no need to separate organic solvents from final products. Low-to-moderate operating temperatures prevent undesired reactions and deterioration of products. Supercritical CO₂ extraction at relatively mild operating conditions led to high selectivity to active principles and a large reduction in the content of undesirable compounds, allowing it to be used on an industrial scale [13]. Oil extraction with supercritical CO₂ was also found to generate nearly pure products with high yields [3]. The most advantage of using supercritical CO₂ technology is to possibly extract heat sensitive compounds without utilization of any hazardous solvent. CO₂ is a cheap, nonflammable, and green solvent. It is readily available since it is as part of the environment.

The study of supercritical CO₂ extraction of sacha inchi seed oil is limited. Most previous studies focused on the solvent extraction method and the nutritional properties and stability of sacha inchi seed oil. The maximum oil yield (50.1%) was obtained from a small-scale batch extraction of ground seeds (only 5 g) using supercritical CO₂ at 400 bar and 60 °C [8]. In a more recent study, a lower oil yield (26.81%) was obtained from continuous extraction of sacha inchi seeds (200 g) using supercritical CO₂ at 450 bar and 60 °C [11]. The experiment was conducted at only 60 °C without a detailed analysis of the oil composition. It is noted that the yield and quality of supercritical CO₂ extracted oils vary with the extraction conditions and could differ from solvent extracted oils. Supercritical CO₂ extraction was found to give flaxseed oil a higher content of omega-3 than solvent extraction method due to minimal degradation and higher recovery of the omega-3 with supercritical CO₂ [14]. Several researchers compared supercritical fluid extraction with conventional extraction methods and reported equal or better quality of the extracted products [15]. Therefore, this research aims to investigate the yields and compositions of oil extracted from sacha inchi seed kernels using supercritical CO₂ at various conditions in comparison with solvent extraction using Soxhlet. The supercritical CO₂ extraction was performed in a semi-continuous process at various extraction times, temperatures, and pressures. In the Soxhlet extraction, both polar and non-polar solvents (ethanol, *i*-propanol, butanol, and hexane) were employed. The properties of sacha inchi seed kernels and oil were also determined in this research.

2. Materials and methods

2.1. Preparation and characterization of sacha inchi seed kernels

Sacha inchi seeds (Figure 1) were purchased from a local producer in the central region of Thailand. The seeds were peeled to remove shells

and ground by a milling machine. The seed kernels were sieved to a required size between 12 and 16 mesh, which was equivalent to the mean diameter between 1.00 and 1.41 mm. Finally, they were packed in vacuum packages and stored in a refrigerator at 4 °C before use.

The ground seed kernels were analyzed in terms of moisture and ash contents according to the AOAC methods 925.10 and 923.03, respectively [16]. Scanning electron microscope (SEM) equipped with energy dispersive X-ray analysis (EDX) was performed on Zeiss Merlin VP Compact operated at 2 kV for the analysis of sacha inchi seed kernels before and after extractions with Soxhlet and supercritical CO₂ apparatus. An attenuated total reflectance Fourier transform infrared (FTIR) spectrometer (PerkinElmer-Frontier) was used to determine the functional groups of sacha inchi seed kernels before and after extractions. The spectra of samples were recorded in the wavenumber range of 400–4000 cm⁻¹ with a resolution of 4 cm⁻¹.

2.2. Solvent extraction of sacha inchi oil using Soxhlet

Four organic solvents: *n*-hexane (99.0%, RCI Labscan), ethanol (99.9%, Daejung Chemical & Metal), *i*-propanol (99.5%, Ajax Finechem), and *n*-butanol (99.5%, Daejung Chemical & Metal) were used for Soxhlet extraction of sacha inchi oil. In each experiment, 15 g of ground seed kernels was extracted with 150, 300, or 450 mL of a required solvent in a Soxhlet extractor for 0.5, 1, 3, 6, and 9 h. The heater temperature was maintained at 250 °C during the extraction process. After the extraction, the mixtures of extracted oil and solvents were separated using a rotary evaporator (BUCHI Rota vapor R-200). The oil yield was calculated based on Eq. (1). The compositions of extracted sacha inchi oils were analyzed using gas chromatography (GC) technique.

$$\text{Oil yield (\%)} = (m/m_0) \times 100 \quad (1)$$

m was the mass of extracted sacha inchi oil, and m_0 was the initial mass of ground sacha inchi seed kernels.

2.3. Extraction of sacha inchi oil using supercritical CO₂ apparatus

Supercritical CO₂ extraction was carried out with a Helix apparatus from Applied Separations, Inc. Figure 2 represents the schematic diagram of the apparatus. The extraction of sacha inchi oil was performed in a 100 mL extraction vessel. CO₂ (99.9%, Praxair) from an external cylinder was pressurized and cooled to the liquid state using a high-pressure pump (Model 7409, Applied Separations) and a recirculating coolant bath (Model 7401, Applied Separations) connected to the pump head. Compressed air was needed to drive the pump. CO₂ was subsequently heated in a CO₂ heater to become supercritical CO₂ before entering the extraction vessel. In the extraction of sacha inchi oil, ground seed kernels (70 g) were loaded into the extraction vessel. The vessel was then assembled and heated by a band heater to a desire temperature. Extraction

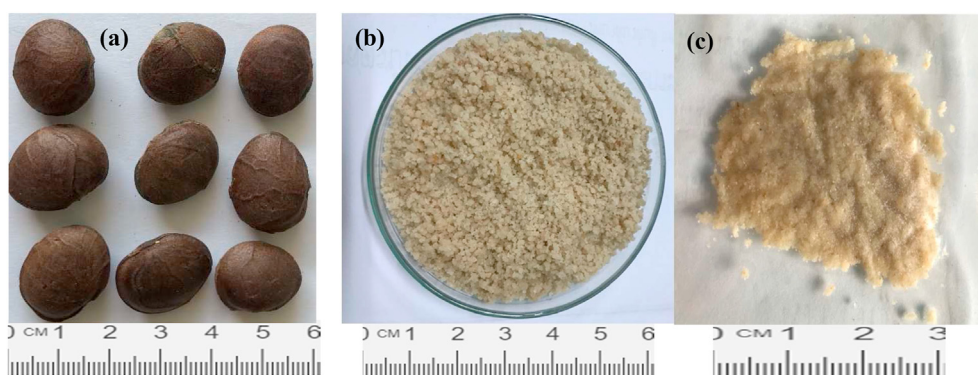


Figure 1. (a) Sacha inchi seeds; (b) ground seed kernels; (c) vacuum-packed ground seed kernels.

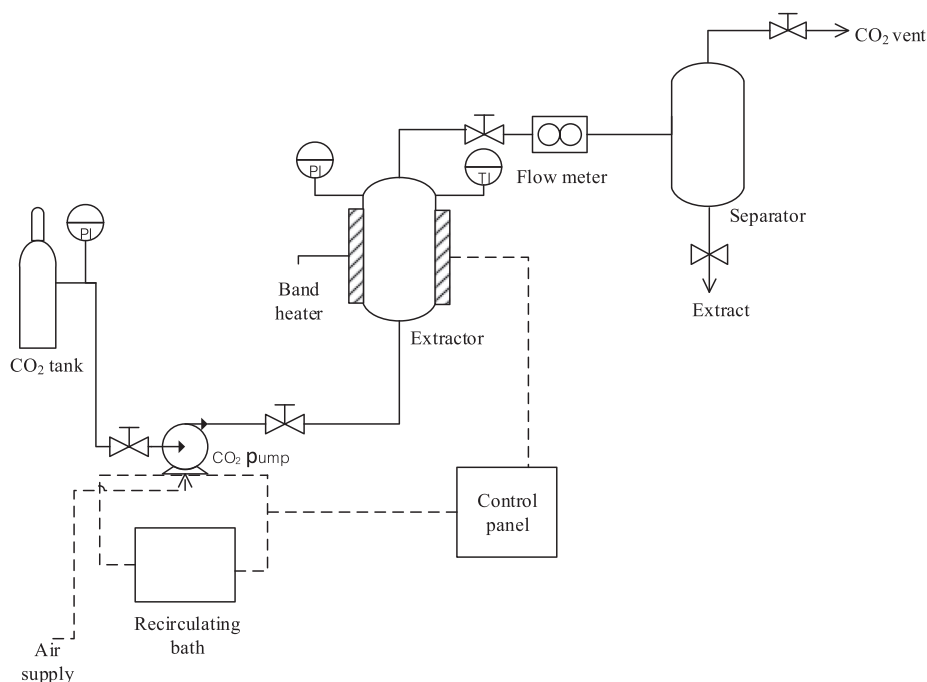


Figure 2. Schematic diagram of the apparatus used for supercritical CO₂ extraction.

temperature was monitored and controlled by a control panel. Semi-continuous extraction was performed by feeding supercritical CO₂ into the vessel bottom. Thereafter, supercritical CO₂ and dissolved extract flowed out the top outlet valve of the extraction vessel to a separator. The high-pressure pump continuously supplied CO₂ to the extraction vessel to maintain the pressure. In the separator, supercritical CO₂ depressurized to a gas, and the extract was separated and collected through the bottom outlet valves. The gaseous CO₂ was then directed to a gas flow meter (Alicat Scientific) and vented to the atmosphere. To investigate the effects of temperature and pressure, supercritical CO₂ extraction of sacha inchi oil was carried out at various temperatures (40, 50, and 60 °C) and pressures (300 and 400 bar). The flow rate of CO₂ was kept constant at 1.5 L/min, and the extraction process continued for up to 6 h. All experiments were carried out in triplicate. The density and viscosity of supercritical CO₂ under the extracting conditions was calculated using Aspen HYSYS program, version V8.8 with Chao Seader correlation.

2.4. Analysis of the extracted sacha inchi oil

The lipid compositions of sacha inchi oils extracted with solvents and supercritical CO₂ were analyzed by GC (Agilent 6890N model, USA) equipped with a flame ionization detector and a Supelco capillary column (SPTM-2560, 100 m × 0.25 mm i.d. × 0.2 μm). Helium (1.0 mL/min) was used as the carrier gas with a split ratio of 1:100. In-house method TE-CH-208 based on the AOAC method 996.06 [17] was used with the injection volume of 1 μL. The column temperature was maintained at 140 °C for 5 min, then increased to 250 °C at the ramp rate of 5 °C/min, and finally maintained at 250 °C for 17 min. The injection and detector temperatures were 250 °C.

The density and refractive index of sacha inchi oil extracted with supercritical CO₂ were determined by AOAC Methods 920.212 and 921.08 using a pycnometer and refractometer, respectively [18]. The iodine and saponification values of sacha inchi oil were evaluated based on the AOCS methods Cd 1c-85 and Cd 3-25, respectively [19]. The thermogravimetric analysis (TGA) of sacha inchi oil extracted with supercritical CO₂ was performed on a Thermogravimetric Analyzer (TGA8000, PerkinElmer, USA) from 25 to 1000 °C with the heating rate of 10 °C/min. The melting characteristics and specific heat capacities of

sacha inchi oil were determined using a differential scanning calorimeter (DSC 3+ model, Mettler Toledo, Switzerland). Nitrogen gas was used to purge the system at the flowrate of 20 mL/min. Calibration of DSC was carried out using indium (melting point 156.6 °C, ΔH_f 28.45 J/g). Samples (~1.6 mg) were weighed into an aluminium pan, cooled at -50 °C and held for 5 min, and finally heated to 40 °C with the heating rate of 5 °C/min. FTIR spectroscopy of sacha inchi oil was performed in the same manner as that of sacha inchi seed kernels.

2.5. Statistical analysis

A Statistical Product and Service Solution software program (version 16.0, SPSS Inc., USA) was employed to analyze the extraction data. Experimental results from the effects of extraction time, volume, temperature, and pressure were analyzed by *t*-test and one-way analysis of variance (ANOVA). Differences at $p < 0.05$ were considered as significant.

3. Results and discussion

3.1. Extraction of sacha inchi oil using Soxhlet: effects of solvent volume and time

The effects of solvent volume and extraction time on the yield of sacha inchi oil using Soxhlet are shown in Figure 3. At a constant volume, increasing extraction time clearly promoted the yields of sacha inchi oil. Rapid extractions were observed during the first hour for butanol, hexane, and *i*-propanol (see Figure 3a,c). The yields gradually increased after 1 h. The easily accessible oil covering the superficial layer of the seed particles is rapidly extracted at the beginning, and mass transfer resistance is negligible. At this stage, the solubility of oil in the solvents is the determining step. When the amount of oil at the external surface decreases, the oil inside the particles begins to transfer. At this stage, diffusion of oil from the interior to the surface (or intraparticle diffusion) plays an important role, resulting in a long slow extracting of oil. The extraction time of 9 h was observed to completely extract the oil from sacha inchi seeds with butanol and hexane. However, more extraction time was required for *i*-propanol and ethanol. One-way ANOVA was used

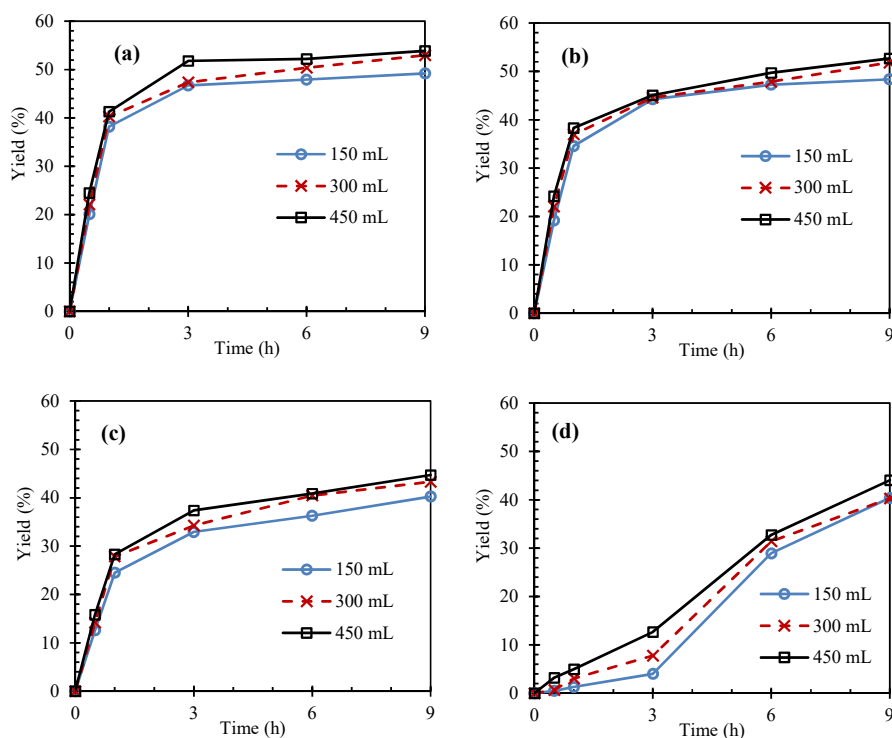


Figure 3. Effects of solvent volume and extraction time using Soxhlet extraction: (a) butanol; (b) hexane; (c) *i*-propanol; (d) ethanol.

to test the effect of extraction time on the oil yield at a confidence level of 95%. The result showed that there was a significantly positive effect (p -value = 0.028).

Unlike other solvents, slow extraction of sacha inchi oil was observed using ethanol as an extracting solvent (Figure 3d). This is likely due to the low boiling point of ethanol (78.4 °C). The extraction of oil with ethanol occurred at the lowest temperature compared with other solvents. In the Soxhlet extraction apparatus, the solvent is principally refluxed to extract solutes presented in the feedstock using a condenser. Extraction with ethanol gave low yields during the first 3 h, and the yields considerably increased and were similar to those obtained from *i*-propanol extraction after 9 h. This was probably due to the fact that at the beginning of the extraction process, moisture evaporated from sacha inchi seeds increased the water content in the ethanol. A mixture of ethanol and water is generally known to form an azeotrope. The increasing water content in turn caused a decrease in the solubility and diffusion of oil in ethanol. With a longer extraction time, however, water in the ethanol gradually evaporated and escaped from the Soxhlet extraction unit, leaving lower water content in the ethanol and promoting a higher extraction rate. Another possible reason for the different trends in oil yields obtained from ethanol extraction compared with *i*-propanol extraction is the difference in the rate-controlling step. In soybean oil extraction using pure *i*-propanol, the oil close to the particle surface is quickly removed, and after the initial stage, internal diffusion becomes the limiting step [20]. Therefore, the extracted oil yield considerably increases with time and begins to level off afterwards, which is in agreement with our results shown in Figure 3c. However, the mass transfer to the external liquid phase becomes more significant and limits the overall process when the extracting solvent of soybean oil is changed to ethanol [20]. In this case, the extraction rate has been slow since the beginning, and the yield has gradually increased until it is finally similar to that of *i*-propanol, which corresponds to our results in Figure 3d.

When considering the effect of solvent volume, the oil yields slightly increased when the volume of extracting solvents was increased from 150 to 450 mL. The plots of oil yields were nearly identical during the first hour (Figure 3a,c). At this stage, the solubility of oil in the solvents is a

limiting factor. When a sufficient amount of solvent was used (in this case, 150 mL), the oil yields could not increase with volume any further. Moreover, one-way ANOVA was tested to verify the effect of solvent volume on the oil yield. It was found that there was no significant difference in the oil yields when different volumes were used (p -value = 0.689). Based on these findings, an initial volume/mass ratio of 10:1 is recommended for the solvent extraction of sacha inchi oil using Soxhlet.

3.2. Extraction of sacha inchi oil using Soxhlet: effect of solvent type

The influence of solvent type on the yield of sacha inchi oil using Soxhlet extraction is presented in Figure 4. Butanol was the best extracting solvent, giving the maximum yields of 51.8, 52.2, and 53.9% at 3, 6, and 9 h, respectively. Compared to other solvents, butanol has the

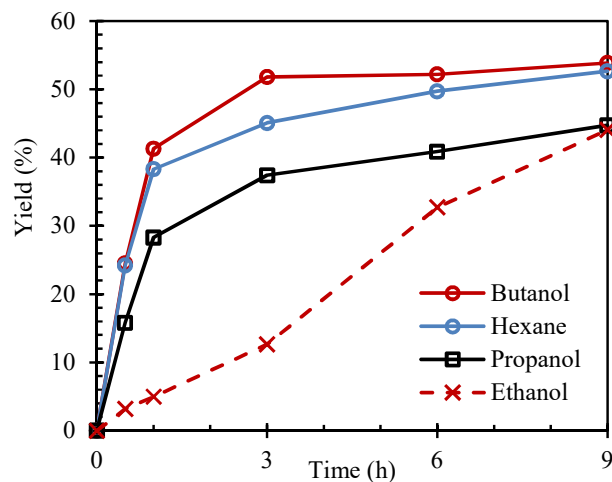


Figure 4. Comparison in sacha inchi oil yields using different solvents in Soxhlet extraction.

highest boiling point (117.7 °C), causing its condensing liquid having the highest temperature in the Soxhlet extraction apparatus. The high temperature promotes the solubility and extraction of oil from the seed kernels. As a result, the greatest yields were obtained. After butanol, using hexane gave higher oil yields than other solvents despite the lowest boiling point of hexane. This can be explained by the non-polarity of hexane. Hexane is a non-polar solvent which is similar to the property of sacha inchi oil components (mostly non-polar lipids). Ethanol was the poorer extracting solvent than *i*-propanol due to the lower molecular weight and higher polar than *i*-propanol. In summary, it can be postulated that a suitable polar alcohol for extracting sacha inchi oil should be long chain alcohols since their structures are similar to the fatty-acid components in the oil. For non-polar solvent, hexane was found to be a promising extractant.

By considering the oil yields, butanol and hexane are promising solvents to extract most of the oil using Soxhlet because sacha inchi seeds typically have the oil content between 35% and 60%, depending on the cultivar, the growing conditions, and the seed processing [2]. Compared with previous studies, the yields obtained in this research were comparable to 52.3–56.3% [8], but higher than 40.0–43.1% [1, 11]. The difference in the oil yields is attributed to the various oil contents in the feedstocks. Different cultivars, edaphoclimatic conditions, and harvesting time affect the oil content in sacha inchi seeds. Since the oil yields extracted from sacha inchi seeds were comparable with those from other seeds, e.g., sesame seeds (53%) [21], flaxseeds (35–41%) [22], and canola seeds (39–48%) [23], the production of sacha inchi oil could be commercially viable, considering the further requirement of healthy oils with a high content of unsaturated fatty acids.

Although both butanol and hexane gave very high yields, the extraction with butanol occurring at the higher boiling point temperature than hexane might affect the compositions of the extracted oil. Therefore, the compositions of sacha inchi oil extracted with butanol and hexane were determined and compared in Figure 5. The oil compositions varied in the range of 42.07–42.53% for omega-3, 38.71–39.20% for omega-6, 9.80–9.96% for omega-9, and 8.76–8.86% for other substances (i.e., palmitic acid, stearic acid, and free fatty acids). Omega-3 was the major component in the extracted oil, corresponding to previous studies: 50.8% [1], 50.51% [8], 42.4–48.61% [9], and 45.2% [24]. Omega-6 was also found in sacha inchi oil but with a slightly lower amount than omega-3. Both omega-3 and omega-6 are important in the prevention of coronary heart disease and hypertension. The ratio of omega-6/omega-3 in this research was in the range of 0.91–0.93. As shown in Figure 5, the compositions of oil extracted with butanol and hexane were similar. Therefore, one-way ANOVA was performed. The calculated significance values

were greater than 0.05, suggesting no difference in the compositions of oil extracted with butanol and hexane.

Although Soxhlet extraction using hexane and butanol provided the good yields of sacha inchi oil, the technique requires large consumption of toxic solvents and long extraction time. As indicated in Figure 3a,b, the oil yields began to level off at 9 h. The longer extraction time consumes more energy and decreases the production capacity. Therefore, the extraction of sacha inchi oil with supercritical CO₂ was further investigated and compared.

3.3. Extraction of sacha inchi oil using supercritical CO₂

Figure 6a shows the influence of extraction temperature on the oil yields using supercritical CO₂ at constant pressure of 300 bar. The yields considerably increased during the first 1.5 h and then gradually increased at all temperatures. The initial rapid extraction is due to the easy accessibility of supercritical CO₂ to the solute molecules deposited on the external surface of the seed particles. Regardless the extraction temperature, the yields began to level off after 3 h. Similar trends showing the effect of extraction time on the essential oils yield were found in the supercritical CO₂ extraction of walnut oil [25], soybean oil [26], grape seed oil [27], and *Swietenia mahagoni* seed oil [28]. After 6 h, the maximum oil yields were 19.3%, 20.7%, and 22.3% for the extractions at 40, 50, and 60 °C, respectively. Therefore, the extraction temperature significantly affected the yield of sacha inchi oil extracted with supercritical CO₂ under constant pressure.

The initial extraction rates calculated from the slopes of the plots (Figure 6a) during the first 1 h were 37.6, 45.5, and 52.3 g-oil/kg-CO₂/h for the extractions at 40, 50, and 60 °C, respectively. There was no mass transfer resistance in this period; therefore, the slopes directly related to the oil solubility in CO₂. The increase in the initial rates with temperatures is due to the increased oil solubility in CO₂ with elevated temperatures. The solubility of sacha inchi oil was previously reported to increase when the temperature was raised from 40 to 60 °C at constant pressure of 400 bar [8]. Moreover, the extraction temperature affects both interparticle and intraparticle diffusions. The increase in temperature raises the kinetic energy of CO₂ molecules and promotes the diffusion of CO₂ from bulk solution to the external surface and further the diffusion into the structure of the seed particles. The viscosity of supercritical CO₂ was found to decrease with elevated temperatures (see Table 1). This in turn increases the distribution and accessibility of CO₂ molecules, promoting the interaction between the solvent and solute.

The influence of extraction temperature on the oil yields at constant pressure of 400 bar is illustrated in Figure 6b. Similar to the result at 300

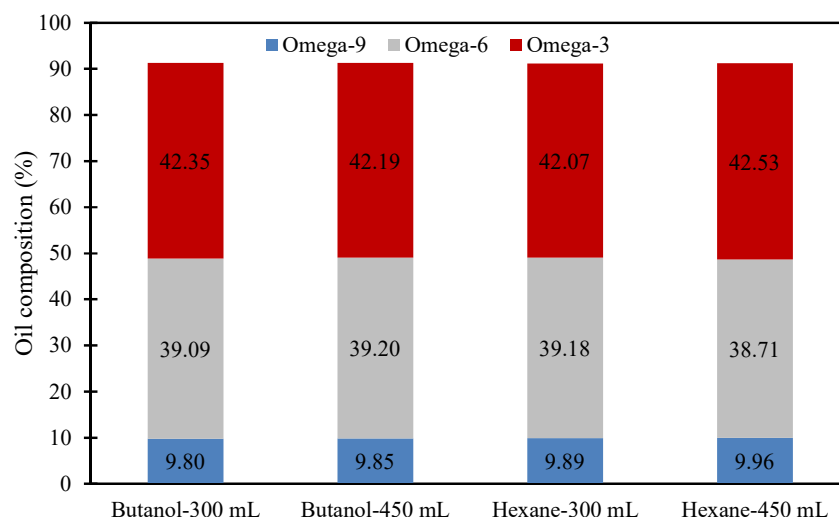


Figure 5. Compositions of sacha inchi oil extracted with butanol and hexane (300 and 450 mL) in Soxhlet for 9 h.

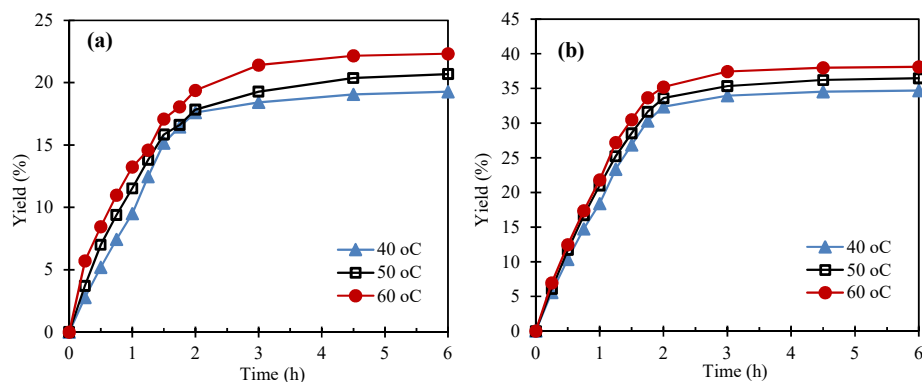


Figure 6. Effect of extraction temperature and time on the yield of sacha inchi oil extracted with supercritical CO₂ at (a) 300 bar; (b) 400 bar.

Table 1. Density and viscosity of supercritical CO₂ at various extraction conditions*.

Pressure (bar)	Temperature (°C)	Density (kg/m ³)	Viscosity (μPa·s)
300	40	840.5	8.17
300	50	806.9	7.60
300	60	773.1	7.08
400	40	896.5	9.39
400	50	868.7	8.83
400	60	840.9	8.31

* Calculated from Aspen HYSYS software.

bar, the yields substantially increased during the first 1.5 h and began to level off at about 2 h. All extraction temperatures gave similar trends. The oil yields were found to increase with the temperatures. The maximum oil yields after 6 h were 34.7, 36.5, and 38.1% for the extractions at 40, 50, and 60 °C, respectively. The calculated initial extraction rates increased with the extraction temperatures and were 72.9, 83.2, and 86.3 g-oil/kg-CO₂/h at 40, 50, and 60 °C. Compared to the results presented in Figure 6a, the initial rates at 400 bar were higher than those at 300 bar at the same temperatures due to the larger solvating power of CO₂ at higher pressures. The optimum extraction time was 3 h. Shorter extraction time leads to incomplete extraction. However, longer extraction time results in higher consumptions of CO₂ and energy.

The results in Figure 6a,b suggested that there were three periods of oil extraction with supercritical CO₂. The first rapid rate of extraction (about 1.5 h) is due to the easy accessibility to the solute molecules deposited on the superficial layer of the seed particles. In this period, the particle surface is completely covered by oil; therefore, mass transfer resistance is negligible. In other words, the oil solubility in CO₂ is the limiting factor. The amount of oil to be extracted is solely controlled by the oil solubility, leading to a linear relationship. About 75% of the total sacha inchi oil was recovered in the constant rate period. In the second period when the amount of solute available on the external surface decreases, the intraparticle diffusion of the solute from inside the particles to the external surface takes place and becomes more important. The observed extraction rate at the second stage was lower than the first one. The overall extraction process is governed by the oil solubility and intraparticle diffusion. Finally, there is a deficit of the solute on the external surface, and a small amount of solute slowly diffuses from inside the particles. Hence, the extraction rate substantially decreased. It was found that the decreased extraction rates in the last two periods recovered the remaining 25% of the total sacha inchi oil.

In supercritical CO₂ extraction, temperature has a positive or negative effect on oil yield because it alters both the density of CO₂ solvent and the vapor pressure of oil solute, depending on which one dominates the other [29, 30, 31]. The decrease in CO₂ density with increased temperature causes the reduction in the solvent power to dissolve the solute. In

contrast, the temperature elevation increases the vapor pressure of oil solute, thus promoting the solubility of oil in supercritical CO₂ [32]. The predominance of solvent density or solute vapor pressure depends on the extraction conditions, particularly the operating pressure. The solvent density was found more important than the solute vapor pressure at low operating pressures and became less important than the vapor pressure at high pressures [25]. The yield of essential oil extracted from *Swietenia mahagoni* seeds decreased with elevated temperatures at constant pressures of 20 and 25 MPa and increased with elevated temperatures at constant pressure of 30 MPa [28]. In our study, raising the extraction temperature from 40 to 60 °C increased the sacha inchi oil yield at constant pressures of 300 and 400 bar. Despite the decrease in supercritical CO₂ density (see Table 1), the higher oil yields with the increased temperatures are due to the more pronounced effect of the solute vapor pressure than the solvent density. Similar to our result, the increase of oil vapor pressure was more important than the decrease of CO₂ density with elevated temperatures in supercritical CO₂ extraction of wheat bran oil [33].

The highest temperature for supercritical CO₂ extraction in this study was 60 °C, which is not high enough to affect the oil properties. Triana-Maldonado and co-workers found that the sacha inchi oil quality (fatty acid composition, acidity, and iodine value) was not affected by prolonged exposure at a temperature of 60 °C and pressure of 450 bar [11]. Moreover, the effect of pressing temperatures (60, 75, and 90 °C) on the properties of sacha inchi oil extracted with a screw press machine was previously studied and the results revealed no effect of the pressing temperatures on the peroxide, saponification, and iodine values of the oil [34].

By comparing the results from Figure 6a,b, the oil yields increased with the CO₂ pressure at constant temperatures. When the pressure was raised from 300 to 400 bar, the final oil yields at 40, 50, and 60 °C increased from 19.3 to 34.7%, 20.7–36.5%, and 22.3–38.1%, respectively. The improvement in the oil yields with elevated pressures resulted from the increased density of CO₂ (see Table 1). Consequently, the solvating power and intermolecular interaction strength were improved [28, 35], leading to the increased solubility of sacha inchi oil in CO₂. Similar results of the positive impact of pressure on oils yield were described in supercritical CO₂ extraction of sacha inchi [8] and *Swietenia mahagoni* seeds [28], wheat bran [33], and *Microula sikkimensis* seeds [36].

The supercritical CO₂ density was found to increase with pressure, resulting in a larger solvent power to extract active principles as well as other unwanted compounds [13]. Based on the calculation using the Aspen HYSYS program, the density of CO₂ at 200 bar and 60 °C was quite low (662.5 kg/m³). Since the liquid-like density of supercritical CO₂ is an important solvating property for extracting desired compounds, higher pressure should be used to promote rapid extraction. The solubility of sacha inchi oil in supercritical CO₂ was found to increase considerably when pressure was raised from 200 to 300 bar at constant temperatures:

the solubilities increased from 4.39 to 9.16 g-oil/kg-CO₂ at 40 °C and from 1.67 to 7.95 g-oil/kg-CO₂ at 60 °C [8]. Similarly, the solubilities of other oils such as apricot kernel oil [37], argan oil [38], and grape seed oil [39] in supercritical CO₂ improved significantly at pressure above 300 bar, leading to higher extraction rates. It is possible that selectivity might be affected by operating at higher extraction pressures since other compounds could be also extracted. However, the operation pressures of 300 and 400 bar were used for this study in order to obtain a high yield of sacha inchi oil in a short time while sacrificing selectivity.

In this research, the maximum oil yield (38.1%) was obtained from the supercritical CO₂ extraction at 400 bar and 60 °C. The yield was lower than that obtained from the Soxhlet extraction using butanol, corresponding to 70.7% recovery if butanol was presumed to extract 100% of the oil. However, the supercritical CO₂ extraction consumed shorter extraction time than the Soxhlet extraction. Similar results have been reported for other vegetable oils. At 300 bar and 40–60 °C, 64–78% of oil contained in soybean seeds was extracted with supercritical CO₂ [40]. The yields of sacha inchi and soybean oils obtained from supercritical CO₂ extraction was lower than those from Soxhlet extraction using hexane [8, 26]. Our result from the hexane extraction also showed higher oil yields than the supercritical CO₂ extraction. This was probably because that hexane was less selective than CO₂ and thus produced oil containing other unwanted compounds [41].

The compositional analysis of oil extracted with supercritical CO₂ was performed, and the result is presented in Figure 7. Overall, the main components were omega-3 in the range of 45.57–46.27%, omega-6 of 37.42–37.74%, and omega-9 of 8.45–8.99%. The statistical analysis of the influences of extraction temperature and pressure on the oil composition found the non-variation of the omega-3, omega-6, and omega-9 contents in the extracted oils obtained from different temperatures and pressures. The omega-3 proportion in sacha inchi oil extracted with supercritical CO₂ in a previous study was 50.45% [8], which was higher than that obtained in our study. This is likely due to changes in crops that affect the biosynthesis of polyunsaturated fatty acids in plants. Different sources of sacha inchi seeds could provide different contents of omega-3 [8,11].

Compared with the solvent extraction (Figure 5), the compositions of sacha inchi oil extracted with supercritical CO₂ (Figure 7) were different. Interestingly, extraction with supercritical CO₂ gave the oils a higher content of omega-3 and lower contents of omega-6 and omega-9 statistically. The ratio of omega-6/omega-3 was in the range of 0.81–0.83,

which was lower than that using the solvent extraction (0.91–0.93). Similarly, the ratio of omega-9/omega-3 in the range of 0.18–0.20 was lower than the range of 0.23–0.24 found in the solvent extraction. These findings illustrate the advantage of using supercritical CO₂ for promoting the extraction of omega-3 while retarding the extractions of omega-6 and omega-9. This might be associated with the lower operating temperatures. In the Soxhlet extraction, higher temperatures caused more evaporation of omega-3 component. Thermal condition also accelerated the deterioration of the unsaturated C=C double bonds in the omega-3 structure more easily than those in the omega-6 and omega-9 structures.

Table 2 compares the omega-3 content of sacha inchi seed oil extracted with supercritical CO₂ in this research with that from other methods in the literature. The omega-3 content in the supercritical CO₂ extracted oils of this study was slightly lower than that obtained from previous supercritical CO₂ extractions [8, 11] due to the different sources of the seeds. The omega-3 and oil contents in sacha inchi seeds vary with

Table 2. Comparison of the omega-3 content in the supercritical CO₂ extracted oil of this research with previous studies.

Extraction method	Origin of sacha inchi seeds	Extraction condition	Omega-3 content in the extracted oil (%)	References
Supercritical CO ₂	Thailand	40–60 °C/ 300–400 bar	45.57–46.27	This study
Supercritical CO ₂	Peru	40 °C/300 bar	50.45	[8]
Supercritical CO ₂	Colombia	60 °C/450 bar	48.31	[11]
Subcritical <i>n</i> -propane	Colombia	30–60 °C/ 80–120 bar	44.0–44.3	[3]
Solvent	Thailand	Butanol, Hexane	42.07–42.53	This study
Solvent	Colombia	Ethyl ether + Petroleum ether	42.53	[3]
Solvent	Colombia	Hexane	50.8	[1]
Solvent	Peru	Petroleum ether	37.3–44.2	[2]
Mechanical pressing	Peru	100–110 °C	44.3	[42]
Mechanical pressing	Ecuador	Room temperature	38.84	[43]

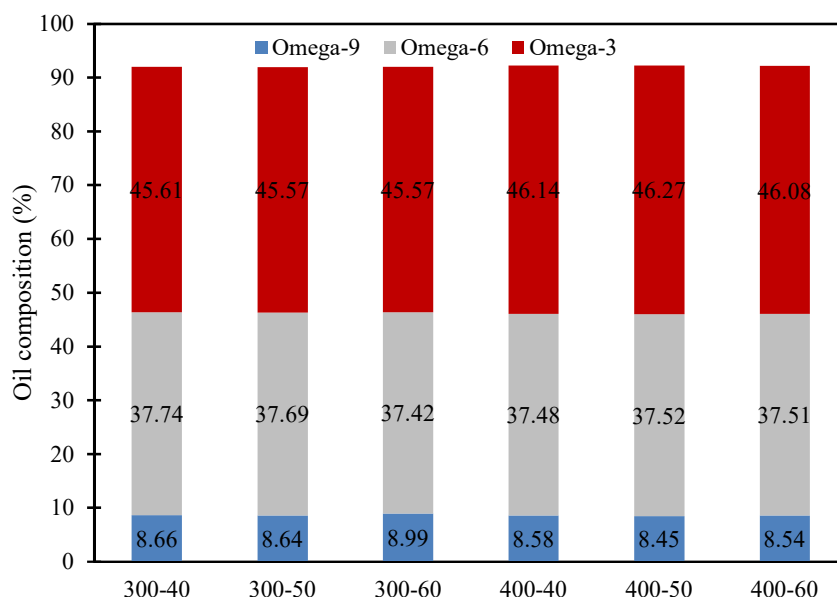


Figure 7. Compositions of sacha inchi oil extracted with supercritical CO₂ at 300–400 bar and 40–60 °C.

cultivars, climate, growing conditions, geographical location, and harvesting time. These parameters could affect the biosynthesis of polyunsaturated fatty acids in plants. Even in the same country (Peru), the omega-3 content in 16 different sacha inchi cultivars was found to be significantly different in the range of 37.3–44.2% [2]. Compared with solvent extraction using butanol or hexane, supercritical CO₂ extraction provided the oil with a higher content of omega-3 for the same sources of sacha inchi seeds. A *t*-test analysis showed a significant effect of the extraction method on the omega-3 content in the extracted oil ($p < 0.05$). The higher content of omega-3 could be attributed to the greater selectivity of supercritical CO₂. Due to the poor selectivity of the solvent, polar substances could be dissolved along with lipophilic substances from the raw material in a typical solvent extraction method [15]. Low operating temperatures in supercritical CO₂ extraction could also prevent the deterioration of the unsaturated C=C double bonds in the omega-3 structure. Similar to our results, subcritical *n*-propane extraction at high pressures and low temperatures gave the oil a higher content of omega-3 than that obtained from solvent extraction [3], keeping the same source of the seeds. The omega-3 content of the supercritical CO₂ extracted flaxseed oil was found higher than that obtained from solvent extraction due to minimal degradation and higher recovery of the omega-3 with supercritical CO₂ [14]. The omega-3 content in the solvent extracted oil in this research was comparable to that obtained from solvent extraction and mechanical pressing methods [1, 2, 3, 42, 43], regarding different sources of the seeds.

3.4. Characterization of sacha inchi seed kernels and oil

As shown in Table 3, the moisture content of sacha inchi seed kernels was 4.38 wt%, which was closed to 4.30% [8] and within the range of 3.3–8.32% [9]. The moisture content is acceptable for preserving and processing without microorganism degradation of the triglycerides [44]. The ash content of sacha inchi seeds was 2.36 wt%, which was closed to 2.7% [45]. The variation in the compositions of sacha inchi seeds is due to the differences in cultivars, climate, growing condition, and geographical location.

SEM images of sacha inchi seed kernels before and after extractions with butanol and supercritical CO₂ are presented in Figure 8. The initial seed kernels were entirely covered with oil (Figure 8a). After the Soxhlet extraction with butanol, the oil was completely removed and the structure was destroyed. Consequently, the particles were disintegrated. Some flakes and many round and oval shaped particles (<10 μm) were observed (see Figure 8b). Similarly, the most particles after the supercritical CO₂ extraction were round and oval (Figure 8c). The particles clumped together with the attachment of flakes. Overall, the supercritical CO₂ extraction did not give different results from the solvent extraction. At 5000x magnifications, however, the surface of the particles after the solvent extraction appeared more shrunken than that after the supercritical CO₂ extraction. This implied that there was more oil remained in sacha inchi seeds after the supercritical CO₂ extraction. The finding was

in agreement with the lower oil yields obtained from the supercritical CO₂ extraction in comparison with the solvent extraction.

EDX analysis of sacha inchi seeds after butanol and supercritical CO₂ extractions revealed similar results as shown in Figure 9. After the solvent extraction, the seeds consisted of carbon (61.8%) as the main component, followed by oxygen (28.6%), nitrogen (8.1%), potassium (1.2%), and magnesium (0.3%). Similarly, the seeds after the supercritical CO₂ extraction had 72.5% carbon, 19.2% oxygen, 5.5% nitrogen, 2.1% potassium, and 0.7% magnesium. The results in this research were in agreement with previous studies. In addition to the organic components, potassium, magnesium, and calcium were found as the main minerals in sacha inchi seeds [1]. After cold pressing, the sacha inchi pressed-cake contained potassium, phosphorus, magnesium, and calcium [46].

The FTIR spectra of sacha inchi oil and seed kernels are presented in Figure 10. The spectrum of sacha inchi oil is similar to the spectra of other edible oils [47]. The sharp peaks at 3010, 2924, and 2854 cm⁻¹ result from the vibrations of the *cis* olefinic CH double bonds (=C-H), the methylene (-C-H) asymmetrical and symmetrical stretchings, respectively. These vibrations are in agreement with those of oil extracted from roasted sacha inchi seeds [48]. The proportion of polyunsaturated fatty acids in edible oil could be indicated by these vibrations. The degree of unsaturation was determined from the ratio of absorptions of peaks at 3010 and 2854 cm⁻¹. The ratio of ~0.6 was obtained, indicating the high degree of unsaturation compared with other edible oils, i.e., sunflower, olive, and rapeseed oils that gave lower values in the range of 0.1–0.3 [48, 49, 50]. The very sharp peak at 1743 cm⁻¹ is due to the carbonyl (-C=O) stretching of esters. Other major peaks at 1463, 1160, and 721 cm⁻¹ result from the -C-H bending (scissoring) of methylene and methyl groups, the -C-O stretching of ester groups, and the methylene (-HC=CH-) rocking of long-chain linear aliphatic structures, respectively. The stretching vibration of -C-O bonds also generates small peaks at 1099 and 1238 cm⁻¹. Moreover, a small peak at 1377 cm⁻¹ is due to the symmetrical -C-H bending vibration of methyl groups.

The FTIR spectra of sacha inchi seeds before and after extractions with butanol and supercritical CO₂ exhibited the same functional groups but different peak intensities (See Figure 10a–c). The lowest peak intensity belonged to the seeds after butanol extraction because the oil was extracted to the greatest extent. This was in agreement with the higher yields of sacha inchi oil obtained from butanol extraction in comparison with supercritical CO₂ extraction (Figure 4 vs. Figure 6). The broad bands at 3278–3279 cm⁻¹ result from the -OH stretching in the polysaccharides and/or lignin structures of sacha inchi seeds. Compared with the spectrum of oil, sacha inchi seeds revealed the different peaks at about 1635, 1534, and 1051 cm⁻¹, which could be attributed to the C=C stretching of alkenes, the C=C stretching of aromatics, and the C-O stretching of primary alcohols, respectively.

Figure 11 illustrates TG/DTG curves of sacha inchi oil extracted with supercritical CO₂. The TG curve was flat (negligible weight loss) up to ~150 °C, indicating the absence of water in the extracted oil. Supercritical CO₂ could selectively extract sacha inchi oil, thus negating the requirement for further purification. The oil started losing weight at ~190 °C and almost completely decomposed at 540 °C, leaving a residue of 2%. The behavior of the DTG plot suggested several stages of thermal degradation of the various components in the oil, mainly polyunsaturated, monounsaturated, and saturated fatty acids. A slight weight loss (2.8%) from ~190 to 300 °C with a broad peak on the DTG curve indicated the decomposition of antioxidant compounds such as tocopherols. These compounds were reported to be thermally stable up to ~230 °C [51].

As suggested by the TG/DTG curves, two main weight-loss steps were observed at the temperature ranges from 300 to 420 °C and from 420 to 490 °C, representing a complex degradation process of sacha inchi oil. The first step from 300 to 420 °C (29.4% weight loss) with a peak on the DTG curve at 410 °C was attributed to the beginning of the decomposition of polyunsaturated and monounsaturated fatty acids. The decomposition continued during the second stage (420–490 °C), recording for

Table 3. Properties of sacha inchi seed kernels and their extracted oil.

	Mean ± SD
<i>Seed kernels</i>	
Moisture content (wt%)	4.38 ± 0.08
Ash content (wt%)	2.36 ± 0.05
<i>Sacha inchi oil obtained from supercritical CO₂ extraction</i>	
Color	Bright yellow
Density at 25 °C (g/cm ³)	0.916 ± 0.018
Refractive index at 25 °C	1.4784 ± 0.0009
Iodine value (g-I ₂ /100 g)	191.8 ± 1.7
Saponification value (mg-KOH/g)	190.2 ± 3.2

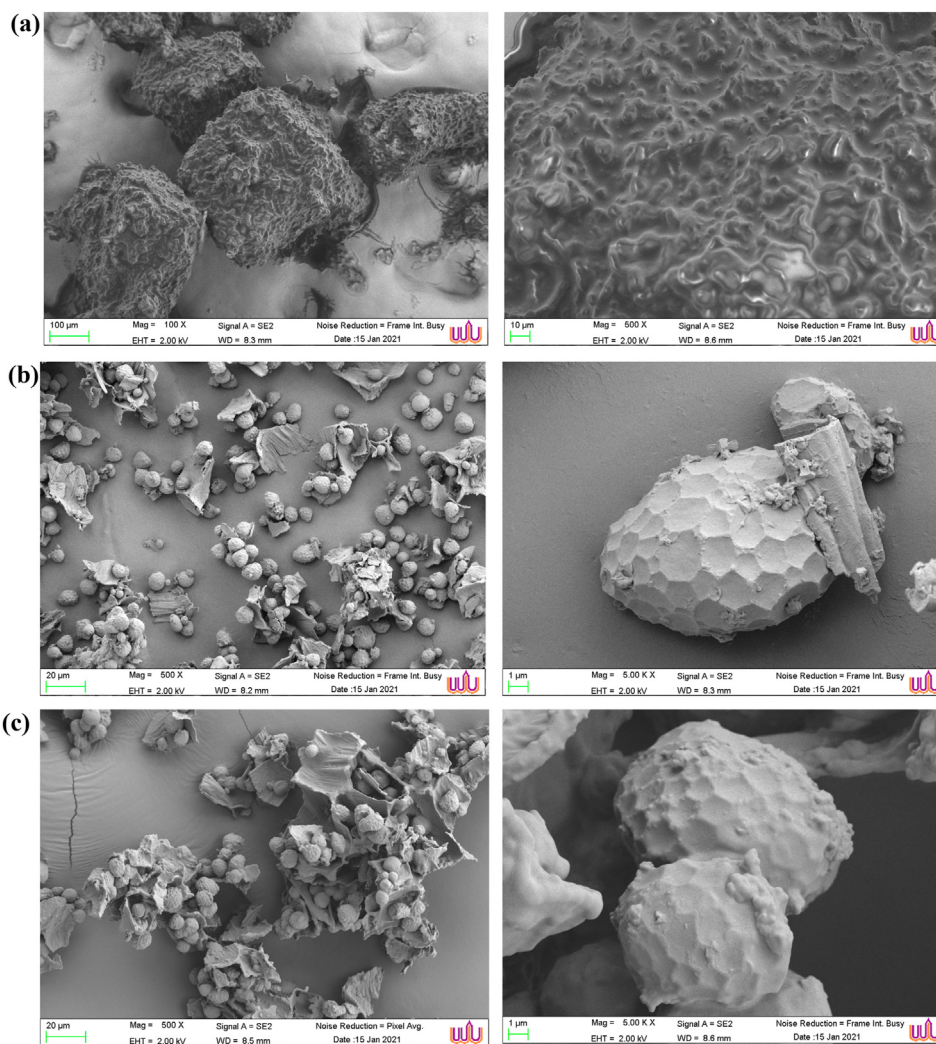


Figure 8. SEM images of sacha inchi seed kernels: (a) before extraction at 100x and 500x magnifications; (b) after extraction with butanol at 500x and 5000x magnifications; (c) after extraction with supercritical CO₂ at 500x and 5000x magnifications.

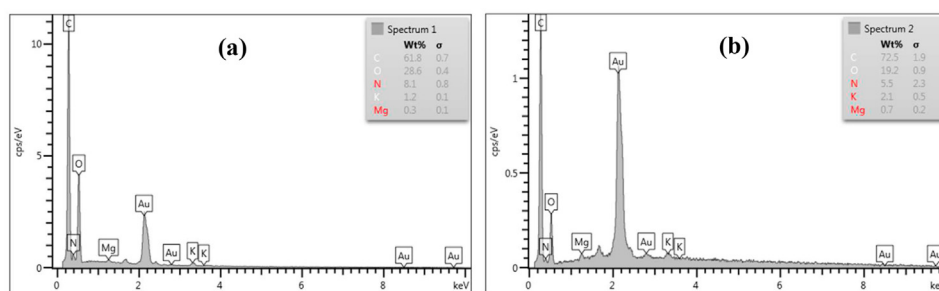


Figure 9. EDX spectra of sacha inchi seeds after extraction with: (a) butanol; (b) supercritical CO₂.

65.7% weight loss. In the second stage, two overlapping steps were observed with the peaks at 439 and 473 °C. During this stage, unsaturated fatty acids further decomposed with the increasing temperature and overlapped with the decomposition of saturated fatty acids being noticeable at 473 °C. This phenomenon was similarly found in the decomposition of grape seed oil [52]. The accumulated degradation of unsaturated and saturated fatty acids from 300 to 490 °C indicated that they constituted more than 95% of sacha inchi oil, corresponding to the compositional analysis of sacha inchi oil (Figure 7). Other saturated fatty acids in sacha inchi oil were palmitic and steric acids. A final, small

decomposition step at high temperatures (>490 °C) was attributed to the slow decomposition of carbonaceous residue. The remaining was tar, accounted for ~1% of the initial oil weight.

The melting behavior of sacha inchi oil was characterized by the presence of two overlapping prominent peaks with a preceding shoulder peak over the temperature range from -34 to -10 °C (Figure 12a). The overlapping peaks are due to the different melting points of the oil components (polyunsaturated, monounsaturated, and saturated fatty acids). The melting of two or more triglyceride structures could occur simultaneously, generating broad or overlapping melting transitions

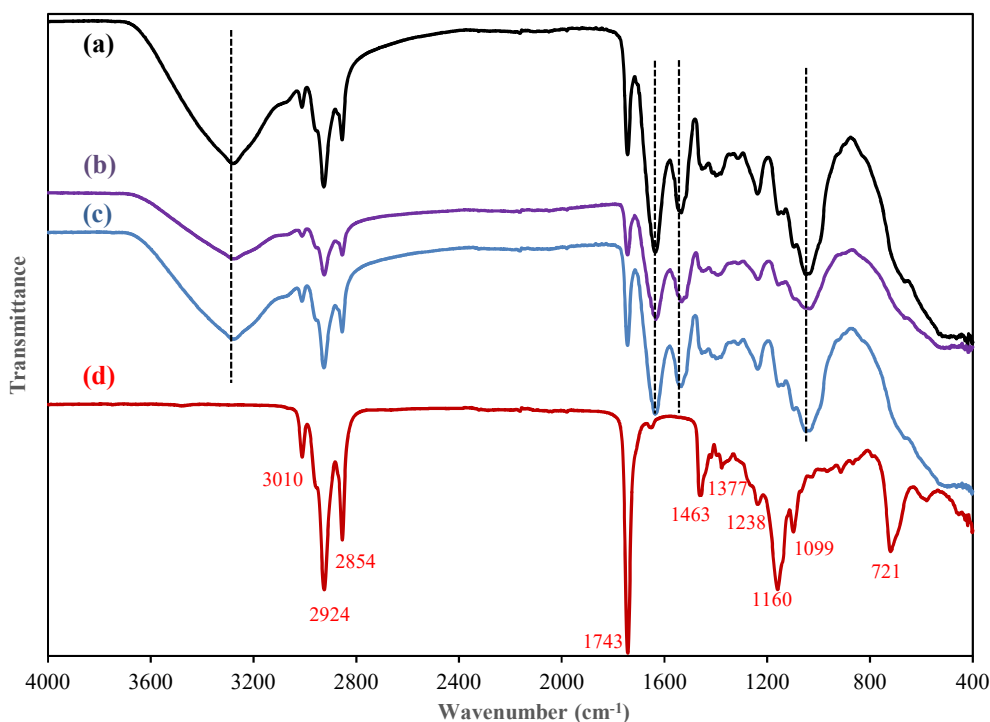


Figure 10. FTIR spectra of: (a) sacha inchi seed kernels before extraction; (b) seed kernels after extraction with butanol; (c) seed kernels after extraction with supercritical CO₂; (d) seed oil after extraction with supercritical CO₂.

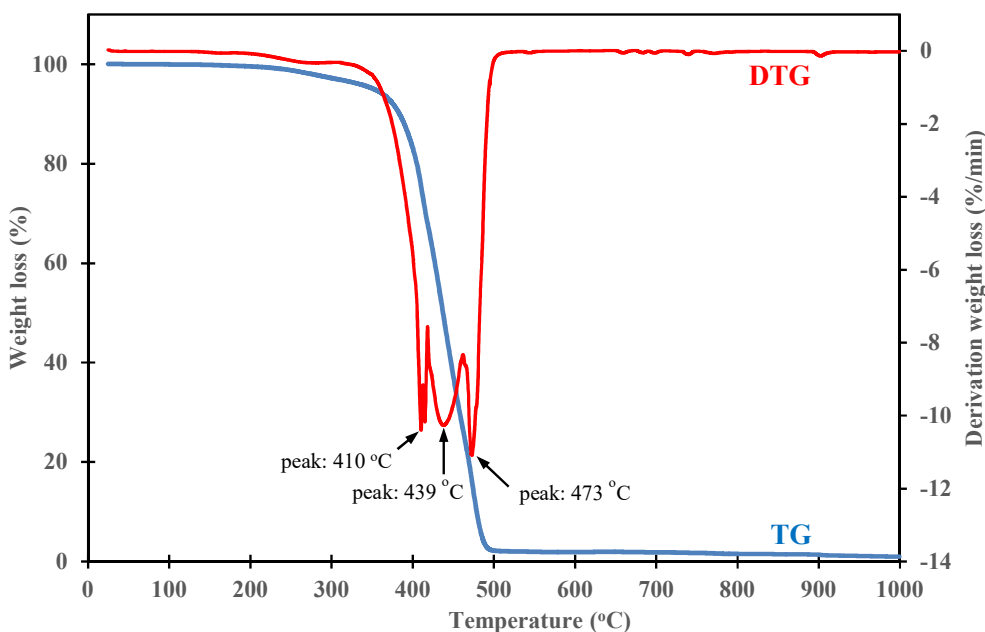


Figure 11. TG/DTG curves of sacha inchi oil extracted with supercritical CO₂.

[53]. The highest endothermic peak was found at about $-24.8\text{ }^{\circ}\text{C}$ with a melting enthalpy of 79.5 J/g (see insert of Figure 12a). The melting enthalpy of sacha inchi oil falls into a typical range of $60\text{--}80\text{ J/g}$ for vegetable oils [27, 53].

The specific heat capacity of sacha inchi oil varied from 1.9 to $3.3\text{ J/g}^{\circ}\text{C}$ in the investigated temperature range from -50 to $40\text{ }^{\circ}\text{C}$ (Figure 12b). This is in agreement with the specific heat capacities of sacha inchi oil, ranging from 1.1 to $3.2\text{ J/g}^{\circ}\text{C}$ [1]. The heat capacities of the edible vegetable oils depended on the concentrations of unsaturated

and saturated fatty acids and ranged from 1.8 to $3.9\text{ J/g}^{\circ}\text{C}$ in the DSC analysis of sunflower, rapeseed, rice, corn, soybean, and olive oils [54].

The density of sacha inchi oil extracted with supercritical CO₂ in this study was 0.916 g/cm^3 (Table 3), which was very close to that extracted with hexane (0.9187 g/cm^3) [1]. The density of sacha inchi oil was slightly higher than those of other vegetable oils such as canola, corn, peanut, and soybean oil [55] because the density increases with the increasing degree of unsaturation. The refractive index of sacha inchi oil in this research was 1.4784 , which was close to 1.4791 [1]. The

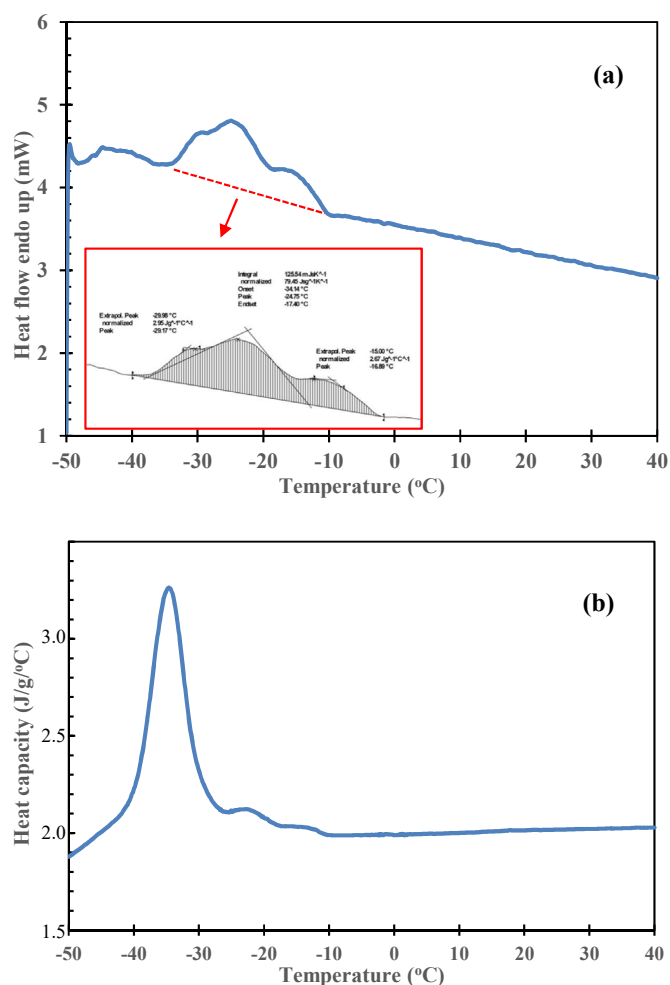


Figure 12. Thermal properties of sacha inchi oil extracted with supercritical CO₂: (a) DSC melting curve; (b) heat capacity as a function of temperature.

refractive index of sacha inchi oil was higher than those published for other vegetable oils such as corn, soybean, and sunflower oils [56] because the refractive index has a positive relationship with the number of double bonds. Other physicochemical properties of sacha inchi oil included: iodine value 191.8 g-I₂/100-g and saponification value of 190.2 mg-KOH/g.

4. Conclusions

In the solvent extraction, butanol and hexane were promising solvents since they gave very high and comparable yields (53.9% and 52.7%) of sacha inchi oil after 9 h of extraction. There was no significant difference in the oil yields when the solvent volume to feedstock mass ratio was higher than 10:1 mL/g. The oil compositions obtained from butanol and hexane extractions were statistically indifferent and within the ranges of 42.07–42.53%, 38.71–39.2%, and 9.85–9.96% for omega-3, omega-6, and omega-9, respectively. For the supercritical CO₂ extraction, the maximum sacha inchi oil yield was 38.1%, using the extraction temperature of 60 °C and the pressure of 400 bar. The optimum extraction time was 3 h. Both extraction temperature and pressure positively affected the extraction rate and the oil yield but unaffected the oil compositions. The omega-3, omega-6, and omega-9 contents of the extracted oil were 45.57–46.27%, 37.42–36.74%, and 8.45–8.99%, respectively. Despite a lower oil yield, the content of omega-3 in the extracted oil using the supercritical CO₂ was higher than that using the solvent extraction, indicating an advantage of using supercritical CO₂ to extract sacha inchi oil with a higher content of omega-3. Moreover, the

supercritical CO₂ extraction consumed shorter extraction time than the solvent extraction.

Declarations

Author contribution statement

Sarawat Jitpinit: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Chaiyapop Siraworakun & Yanyong Sookklay: Contributed reagents, materials, analysis tools or data.

Kamchai Nuithitikul: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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