

# Physicochemical Properties, Fatty Acid and Amino Acid Profiles, and Antioxidant Activity of Sacha Inchi (*Plukenetia Volubilis* L.) Seed Oil and Press Cake Extracted Using Hydraulic Press and Soxhlet Methods

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## Abstract

Sacha Inchi (*Plukenetia volubilis* L.) oil is increasingly recognized for its high polyunsaturated fatty acid content, particularly its balanced omega-3 and omega-6 ratio, making it a valuable ingredient in functional foods and nutraceuticals. However, the choice of extraction method significantly affects its physicochemical properties, bioactive compound retention, and oxidative stability. This study aims to compare the effects of hydraulic press (SIOP) and Soxhlet extraction (SIOS) methods to determine the most effective technique for preserving the nutritional integrity of Sacha Inchi oil and its by-products. The study assessed oil density, refractive index, moisture content, peroxide value, and acid value, employing gas chromatography for fatty acid composition and high-performance liquid chromatography for amino acid profiling. SIOP was obtained through mechanical pressing without solvents, while SIOS involved continuous solvent reflux. The press cakes (SIOPC and SIOSC) were analyzed for protein and fiber content. Results showed that SIOP exhibited superior oil quality, with lower peroxide values (2.31 mEq O<sub>2</sub>/kg), higher  $\alpha$ -linolenic acid content (46.77 %), and greater antioxidant activity (IC<sub>50</sub>: 104.05 mg/mL) compared to SIOS. Additionally, SIOPC retained more protein and antioxidants, highlighting its potential as a functional food ingredient. Our findings highlight the trade-offs between oil quality and yield. SIOP retains more polyunsaturated fatty acids and antioxidants, making it ideal for high-quality oil, while SIOS achieves higher yield but compromises bioactive retention. These insights help industries select the most suitable extraction method based on quality or efficiency priorities.

**Keywords:** Sacha Inchi oil, Physicochemical characteristics, Fatty acid profile, Amino acid profile, Antioxidant activity, Functional foods.

## Introduction

Sacha Inchi (*Plukenetia volubilis* L.), native to the Amazon rainforest, has gained significant global attention due to its rich nutritional profile and bioactive compounds, including essential fatty acids, proteins, and antioxidants. Traditionally consumed in its native regions, Sacha Inchi seeds are increasingly recognized for their potential applications in functional foods and

nutraceuticals [1]. For example, sacha inchi oil has been traditionally used in dermatological applications to enhance skin softness, promote wound healing, and alleviate symptoms associated with insect bites and skin infections [2]. This plant is now cultivated commercially outside of South America, particularly in Southeast Asia, with Indonesia emerging as one of the producers. In

recent years, research has focused on understanding the chemical composition and health benefits of Sacha Inchi seeds, particularly their oil content. This oil is reported to be rich in polyunsaturated fatty acids (PUFAs), such as alpha-linolenic acid (omega-3) and linoleic acid (omega-6), which are crucial for human health [3,4]. Several studies have analyzed the fatty acid composition of Sacha Inchi oil, highlighting its high PUFA content. The PUFA content of Sacha Inchi oil has been documented to range from 77.5 to 84.4 %, highlighting its significant health benefits, particularly its anti-inflammatory properties [5]. Another study provided an even higher estimate, indicating 48.5 % omega-3 and 34.8 % omega-6, with a total PUFA content of approximately 83.3 %, classifying it as a highly polyunsaturated oil [6]. Additionally, the omega-6 to omega-3 ratio of Sacha Inchi oil was reported to be approximately 1:1, which is considered optimal for human health [3]. These findings align with prior reports that Sacha Inchi oil contains 42.3 %  $\alpha$ -linolenic acid (omega-3) and 39.5 % linoleic acid (omega-6), demonstrating a well-balanced proportion of essential fatty acids [7].

Compared to other vegetable oils, Sacha Inchi oil has a well-balanced composition of omega-3 and omega-6 [3,5–7]. For instance, chia seed oil contains approximately 60 %  $\alpha$ -linolenic acid and 20 % linoleic acid, while flaxseed oil has 55 - 60 %  $\alpha$ -linolenic acid and 15 - 20 % linoleic acid [5,6]. These oils contain higher omega-3 levels than Sacha Inchi oil but have a lower proportion of omega-6. In contrast, fish oil is dominated by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with omega-3 levels ranging between 30 - 40 %, but with lower omega-6 content [5]. These comparisons position Sacha Inchi oil as a valuable plant-based alternative to traditional sources of omega-3, offering a balanced omega-3 to omega-6 ratio that aligns with dietary recommendations. These characteristics position Sacha Inchi oil as a promising ingredient for developing new food products and health supplements.

The nutritional and functional properties of Sacha Inchi oil are influenced significantly by the methods used during processing. For instance, thermal treatments such as roasting have been shown to affect the phenolic content and antioxidant activity of the seeds, leading to variations in the nutritional quality and efficacy of the

extracted oil [8]. Moreover, the choice of extraction methods, such as hydraulic press and Soxhlet extraction, determines the yield, purity, and physicochemical properties of the oil. Various studies have documented the impact of these methods on the fatty acid profile and antioxidant capacity of plant-based oils, indicating that optimizing extraction techniques is crucial to maximizing the oil's nutritional benefits [9,10]. Given these factors, further exploration into the best methods for processing Sacha Inchi seeds is necessary to fully harness their potential for use in health and cosmetic industries.

Despite the known benefits of Sacha Inchi oil, challenges remain in identifying the most effective processing method that optimizes its nutritional properties while maintaining its stability. Current research highlights that mechanical pressing (hydraulic press) and solvent extraction (Soxhlet) are the 2 dominant methods for extracting Sacha Inchi oil, each with distinct advantages and limitations. While hydraulic pressing is considered a gentle process that preserves bioactive compounds and fatty acids, it often results in lower yields. Conversely, Soxhlet extraction, which uses organic solvents and heat, may increase oil yield but could also degrade sensitive compounds such as tocopherols and polyphenols due to thermal exposure [11]. These findings necessitate a detailed comparative analysis of both methods to determine their respective impacts on the oil's nutritional and antioxidant properties.

Scientific literature has extensively explored the composition and benefits of Sacha Inchi oil. For instance, studies have confirmed the presence of high levels of alpha-linolenic acid, which contributes to cardiovascular health, as well as other essential nutrients like protein and fiber in the press cake, a by-product of oil extraction [12,13]. Furthermore, antioxidant properties linked to phenolic compounds and tocopherols present in Sacha Inchi seeds highlight the oil's potential to mitigate oxidative stress, making it valuable for both food and cosmetic applications [6]. However, discrepancies in findings exist, often due to differences in extraction techniques and seed origin, emphasizing the need for standardization in processing methods to achieve consistent quality and efficacy.

Research also indicates that the choice of extraction method not only affects the yield but also the

quality of Sacha Inchi oil in terms of its physicochemical properties, such as density, refractive index, and peroxide value, which are critical indicators of oil purity and stability [14]. While hydraulic pressing minimizes exposure to high temperatures and solvents, thus preserving sensitive compounds, Soxhlet extraction may enhance oil yield but can compromise quality through increased oxidative degradation [15]. This study explores these dynamics by comparing these extraction methods and evaluating their effects on Sacha Inchi oil's physicochemical properties, fatty acid profile, and antioxidant potential.

Existing literature demonstrates that Sacha Inchi oil has been successfully integrated into various functional food products and cosmetics due to its high omega-3 and omega-6 fatty acid content [16]. However, while numerous studies provide insights into the general composition of the oil, few have conducted a comprehensive analysis encompassing not only the fatty acid profile but also the proximate composition, amino acid profile, and antioxidant activity specific to different extraction methods. Furthermore, studies rarely explore the press cake, a valuable by-product, in detail. Understanding its composition is crucial for maximizing the utilization of Sacha Inchi seeds and ensuring that the entire processing chain is optimized for both economic and nutritional benefits.

This study addresses these gaps by providing a detailed analysis of Sacha Inchi seed oil and its by-products. We compare the hydraulic press and Soxhlet extraction methods to determine their respective impacts on the physicochemical properties, proximate composition, fatty acid profile, and antioxidant activity of the oil, as well as the amino acid profile of the press cake. This study is novel in its holistic approach, as it not only evaluates the oil's quality based on standard physicochemical parameters but also considers the nutritional and functional potential of the press cake, which is often overlooked in existing research. Our findings aim to offer practical insights for optimizing extraction techniques to enhance the quality and value of Sacha Inchi oil for use in the food and health industries.

By investigating these factors, this study contributes to the broader understanding of Sacha Inchi oil processing and utilization. Sacha Inchi oil has numerous potential applications for the future,

particularly in the fields of functional foods, nutraceuticals, and cosmetics. Its rich omega-3 and omega-6 content make it an excellent dietary supplement for cardiovascular health, brain function, and inflammation control. Additionally, its balanced fatty acid ratio allows for better formulation in plant-based omega-3 supplements, offering a vegetarian alternative to fish oil. In the cosmetics industry, Sacha Inchi oil's antioxidant properties and essential fatty acid content make it valuable for skincare formulations, promoting hydration, elasticity, and anti-aging benefits. Furthermore, ongoing research into its bioactive compounds suggests its potential use in pharmaceuticals targeting metabolic disorders and inflammatory conditions. With increasing demand for plant-based and sustainable health products, Sacha Inchi oil is poised to become a key ingredient in various industries in the coming years.

The results are expected to inform future strategies for optimizing extraction methods, improving product stability, and increasing the overall yield of valuable compounds. Furthermore, this research emphasizes the need for a comprehensive approach in assessing oil quality, considering not just the main product (the oil) but also the by-products that can enhance the economic and nutritional value of Sacha Inchi seeds.

## Materials and methods

### Plant materials and chemicals

Sacha inchi seeds were obtained from a commercial plantation in Tabongo Timur, Gorontalo, Indonesia. Seeds were harvested at optimal maturity and stored under controlled conditions until further processing to preserve their quality. The plant material was determined in Department of Biology, Universitas Negeri Gorontalo, with a voucher specimen number of YKS001.

All analytical-grade chemicals, including n-hexane ( $\geq 99\%$ ), acetic acid, methanol, chloroform, and potassium iodide, were purchased from Merck (Darmstadt, Germany). Reagents for antioxidant assays, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), were prepared following standard protocols.

### Seed oil extraction method

Prior to oil extraction, Sacha Inchi (SI) seeds were cleaned, dried, and de-hulled to remove extraneous

materials. The seeds were then oven-dried at 78 °C for 1 h to reduce moisture content, following established protocols from previous studies [4]. For Soxhlet extraction, the dried seeds were ground into a fine powder using a mechanical grinder (Fritsch Pulverisette 14, Germany) to achieve a uniform particle size, ensuring consistent oil extraction efficiency. However, for hydraulic pressing, the dried SI seeds were used directly without grinding. The prepared samples were subsequently divided into 2 portions for hydraulic pressing and Soxhlet extraction.

The hydraulic press and Soxhlet extraction methods were chosen due to their differing impacts on cost efficiency, bioactive compound preservation, and oil yield. Hydraulic pressing is a solvent-free, environmentally friendly method that better preserves bioactive compounds like sterols, polyphenols, and tocopherols, ensuring higher nutritional quality, lower peroxide values, and improved oxidative stability [6,17]. It also produces a press cake rich in protein and antioxidants, making it valuable for functional foods [3]. In contrast, while soxhlet extraction produced higher oil yields, it can cause degradation of PUFA content due to prolonged heat and solvent exposure, affecting sensory quality and shelf life [5,7].

Prior to oil extraction, Sacha inchi seeds were cleaned, dried, and de-hulled. The seeds were then oven-dried at 78 °C for 1 h to reduce moisture content, based on methods outlined by previous studies [8]. The dried seeds were ground into a fine powder using a mechanical grinder (Fritsch Pulverisette 14, Germany) to achieve uniform particle size, which is crucial for consistent oil extraction. The prepared samples were divided into 2 portions for hydraulic pressing and Soxhlet extraction.

Hydraulic pressing was conducted using a DO3S hydraulic press. Approximately 200 g of the ground Sacha inchi seeds were placed into the pressing chamber and subjected to a pressure of 150 MPa at room temperature. The extraction duration was set at 30 min (SIOP). After pressing, the crude oil was allowed to settle for 24 h to separate the solid residues (SIOPC). The oil was then filtered through Whatman No. 1 filter paper to obtain a clear sample for subsequent analysis. This method was selected based on its efficiency in preserving the bioactive compounds, as hydraulic pressing minimizes thermal and solvent exposure [10].

The Soxhlet extraction was carried out using 200 mL of n-hexane as a solvent. Five grams of the ground Sacha inchi seed powder were placed in a cellulose thimble and inserted into the Soxhlet apparatus. The extraction was conducted at a temperature of 90 °C for 6 h, as previously described in the literature [9]. The extracted oil (SIOS) was then concentrated using a rotary evaporator (Buchi R-300) at 40 °C under reduced pressure to remove residual solvent. The oil was further filtered to remove any impurities before storage. This solvent-based method was chosen to compare its efficiency and effects on oil quality relative to the hydraulic pressing technique.

### Physicochemical analysis

The extracted oils (SIOP and SIOS) were analyzed for density and refractive index. Density measurements were performed using a pycnometer, while the refractive index was determined using an Abbe refractometer, following AOAC guidelines [14]. Additionally, moisture content was determined by the oven-drying method at 105 °C until a constant weight was achieved, as outlined in the SNI 01-3555 standards [15]. The peroxide value, an indicator of oxidation level, was assessed by titrating the oil sample with sodium thiosulfate solution. The acid value, which measures the free fatty acid content, was determined through titration with potassium hydroxide, while the saponification value, which quantifies the amount of potassium hydroxide required for saponification, was also calculated.

### Fatty acid composition analysis

The fatty acid composition of the extracted oils (SIOP and SIOS) was analyzed using gas chromatography-mass spectrometry (GC-MS). Fatty acids were first converted to fatty acid methyl esters (FAMES) using methanolic KOH, following the method described by [18]. Identification of fatty acids was based on retention times and comparison with known standards, and quantification was performed using peak area integration [3]. The analysis was performed using a Shimadzu GC-MS QP2010 Plus equipped with a DB-23 column (60 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed to start at 150 °C and increase to 250 °C at a rate of 5 °C per min.

### Proximate analysis of SIOPC and SIOSC flour

Proximate analysis was performed on Sacha Inchi Oil Press Cake (SIOPC) and Sacha Inchi Oil Soxhlet Cake (SIOSC) based on AOAC 2016 guidelines [19]. The proximate analysis included moisture content analysis (oven-drying method at 105 °C), ash content analysis (dry ashing method), crude protein content analysis (Kjeldhal method), and crude fiber analysis (strong acid-base method).

### Protein hydrolysate extraction

The SI by-products (SIOPC and SIOSC) were each mixed with distilled water in a ratio of 1:10. The pH was adjusted to 11.0 using 1 N NaOH, and the samples were stirred at 100 rpm while being heated at 50 °C for 1 h. The suspended material was filtered through filter paper, and the resulting supernatant was adjusted to pH 4.5 using 1 N HCl before centrifuging at 2600×g for 15 min. The protein content of the hydrolysates was analyzed using the Kjeldahl method. The obtained SI protein hydrolysate samples were dried overnight in a hot air oven at 40 °C.

The hydrolysate with the highest protein content (SIOPC) was selected for amino acid composition analysis using high-performance liquid chromatography (HPLC). The HPLC analysis was performed using an Agilent 1260 Infinity II system equipped with a ZORBAX Eclipse Plus C18 column (4.6×250 mm, 5 µm). The mobile phase consisted of acetonitrile and 0.1 % formic acid in water, with a flow rate of 1 mL/min, and detection was carried out at 260 nm.

### Antioxidant activity

The antioxidant activity of SIOP, SIOS, SIOPC, and SIOSC was evaluated using the DPPH radical scavenging method, adapted from the protocol by [8].

Oil samples were prepared at different concentrations (1000, 500, 250, 100, and 50 ppm) in methanol. One milliliter of each sample was mixed with 1 mL of DPPH solution (0.1 mM) and incubated in the dark for 30 min. Absorbance was measured at 517 nm using a UV-1780 double beam spectrophotometer (Shimadzu). The percentage inhibition was calculated, and the IC<sub>50</sub> value, indicating the concentration required to inhibit 50 % of the DPPH radicals, was determined through linear regression analysis.

### Statistical analysis

In this study, statistical analyses were conducted to compare the mean values of physicochemical properties and proximate analysis between SIOPC and SIOSC. The analysis was performed using the R programming language. Independent t-tests were then employed to evaluate the significance of the differences in means between the 2 samples for each parameter of the proximate analysis. Furthermore, ANOVA analysis followed by post-hoc tests was conducted to evaluate the significant differences in antioxidant activities among SIOP, SIOS, SIOPC, and SIOPS.

### Results and discussion

#### *The physicochemical characteristics of sacha inchi seed oil*

The physicochemical properties of Sacha Inchi oil (SIO) extracted using the hydraulic press (SIOP) and Soxhlet extraction (SIOS) methods (**Figure 1**) are crucial in determining its stability, quality, and potential applications in various industries. The density, refractive index, moisture content, peroxide value, saponification value, and acid value of SIO extracted using both methods are presented in **Table 1**.

**Table 1** Physicochemical characterization of sacha inchi seed oil.

Physicochemical Parameters	Extraction Method		Technical Standard Peru	Australian Oilseeds
	SIOP	SIOS	NTP 151.400	Federation
Density (g/cm <sup>3</sup> )	0.9350 ± 0.009	0.9340 ± 0.009	0.926 - 0.931	-
Refractive index	1.4771 ± 0.081	1.4774 ± 0.129	1.478 - 1.481	-
Moisture values (%)	0.0900 ± 0.003	0.1600 ± 0.008	-	Max 0.2
Peroxide values (mEq O <sub>2</sub> /kg)	2.3100 ± 0.022	3.6500 ± 0.053	-	Max 10

Physicochemical Parameters	Extraction Method		Technical Standard Peru NTP 151.400	Austalian Oilseeds Federation
	SIOP	SIOS		
Saponification values (mg KOH/g)	186.2800 ± 2.570	192.1400 ± 2.052	183 - 199	-
Acid Values (mg KOH/g)	1.0250 ± 0.032	1.3050 ± 0.064	-	Max 2

Density is an essential physicochemical characteristic that influences oil quality, oxidation stability, and industrial application [16,20,21]. The density values for SIOP and SIOS were not significantly different ( $p$ -value = 0.91) suggesting minor variations, likely influenced by fatty acid composition and extraction method. These values are within the standard range for vegetable oils, as reported in previous studies [16,21]. Generally, the density values of SIOP and SIOS (**Table 1**) fall within the standard range, indicating that the oil composition is consistent with common vegetable oils. However, according to the Peruvian Technical Standard [22], these values exceed the established threshold, which may indicate oil degradation, oxidation, or contamination with other substances [23]. The variation in the density of sacha inchi oil can be attributed to several factors, primarily the fatty acid composition and the extraction method used. Sacha inchi seed oil is rich in polyunsaturated fatty acids, especially  $\alpha$ -linolenic acid and linoleic acid, which influence its physical properties, including density. Variations may occur based on the specific fatty acid profile, as oils with higher saturated fatty acid concentrations tend to have higher densities, while those rich in unsaturated fatty acids, like sacha inchi oil, typically exhibit lower densities [24].

In terms of refractive index, there is no significant difference between the SIOP and SIOS ( $p$ -value = 0.1). The refractive index for both samples align with that of common vegetable oils and indicates slight variations in oil purity and degree of unsaturation [14,25]. The refractive index measures how much light is bent when passing through. These refractive index of sacha inchi oil fall below the Peruvian Technical Standard [12]. The dominant content of unsaturated fatty acids, such as  $\alpha$ -linolenic and linoleic acids, can influence these variations. A change in the refractive index may indicate changes in the chemical composition, oil degradation, or mixing with other oils. According to Chasquibol *et al.* [21], the values are ranging between 1.480 and 1.481, while Vicente *et al.* [14] reported lower refractive index values of 1.475. Additionally, studies from Colombia and China reported refractive index values of 1.481 and 1.475, respectively [25,26]. These variations may be associated with factors such as differences in extraction techniques, the geographic origin of the seeds, and specific conditions during oil extraction [27]. A higher refractive index often indicates purer oil or oil with a different fatty acid profile, which can affect the overall quality of the oil [28]



**Figure 1** SIOP (1) and SIOS (2).

The moisture content of sacha inchi seed oil is an important factor that affects its quality, stability, and suitability for various applications [24]. This study shows that the hydraulic press method yielded sacha inchi oil with significantly lower moisture content compared to that of the soxhlet extraction method ( $p$ -value = 0.001). The moisture values for SIOP (0.09 %) and SIOS (0.16 %) fall within the acceptable range set by the Australian Oilseeds Federation standard ( $\leq 0.2$  %) [29]. The lower moisture content in SIOP suggests that hydraulic pressing minimizes residual moisture, making the oil more stable and resistant to hydrolytic degradation. In contrast, the higher moisture content in SIOS may be attributed to solvent retention or prolonged exposure to heat, impacting oil purity and oxidative stability. The difference in moisture content between the 2 extraction methods may be linked to the nature of the processes involved. The hydraulic press method is generally considered a gentler extraction technique that minimizes exposure to heat and solvents, which may help reduce moisture content. In contrast, the soxhlet extraction method, which involves prolonged heating and solvent use, may introduce more moisture into the final product. The moisture content of sacha inchi seed oil is influenced by genetic traits, environmental conditions during cultivation, and extraction methods. Different seed varieties exhibit varying moisture levels due to genetic differences. Environmental factors such as soil moisture, temperature, and humidity at seed maturation significantly impact moisture content. Seeds harvested in wet conditions tend to have higher moisture levels, which can affect the oil extraction process and quality. High moisture levels can cause emulsification issues during extraction, resulting in less clear oil and reduced shelf life and stability [30]. The moisture content in sacha inchi seed oil directly affects the stability and shelf life of the extracted oil. Elevated moisture levels can enhance hydrolysis and promote microbial growth, leading to spoilage [31,32]. Additionally, higher moisture content can accelerate oxidative degradation, thereby shortening the shelf life and diminishing the quality of the oil [33].

The peroxide value is an indicator of the initial oxidation level in the oil. Lower values indicate better oil quality in terms of oxidative stability [34]. The peroxide values in this study vary for sacha inchi seed

oil, highlighting the influence of the extraction method and storage conditions on oil quality [24]. In this study (**Table 1**), the peroxide value of SIOP was significantly lower than that of SIOS ( $p$ -value =  $4.3 \times 10^{-5}$ ). Nevertheless, these values were well below the Australian Oil seeds Federation standard, which allows a maximum peroxide value of 10 mEq O<sub>2</sub>/kg [29], indicating that both extraction methods produce oil with low oxidation levels. The peroxide values obtained in this study are comparable to those reported in previous studies. Chasquibol *et al.* [21] recorded a peroxide value of 2.90 mEq O<sub>2</sub>/kg, while Paucar-Menacho *et al.* [16], reported a lower value of 2.00 mEq O<sub>2</sub>/kg from Myanmar, and Almeida *et al.* [35] reported a value of 1.85 mEq O<sub>2</sub>/kg from China. These variations may be associated with factors such as seed quality, extraction methods, and storage conditions.

The extraction method plays a significant role in determining the peroxide value of sacha inchi seed oil. The hydraulic press method, which operates at lower temperatures and does not involve solvents, is highly effective in maintaining oil quality and reducing oxidative degradation. On the other hand, the soxhlet extraction method, despite yielding a higher peroxide value, produces oil that still meets acceptable quality standards. This indicates that both methods can be used to produce high-quality sacha inchi seed oil, but the hydraulic press method may offer advantages in terms of oxidative stability. Peroxide values can change over time due to factors such as exposure to light, heat, and oxygen. Research by Nghiem *et al.* [36], has shown that sacha inchi oil can experience fluctuations in peroxide values during storage, highlighting the need for proper storage conditions to maintain oil quality. For example, oil stored in dark and cool environments tends to maintain quality longer than oil exposed to light and heat [35].

The saponification value of sacha inchi seed oil is a crucial indicator of its fatty acid composition and potential applications in various industries [31]. This study shows that the saponification values for SIOP and SIOS, were not significantly different ( $p$ -value = 0.06). These values fall within the range set by the Peruvian Technical Standard [14]. This saponification value reflects the amount of potassium hydroxide (KOH) needed to saponify a specific amount of oil, which is

directly related to the oil's fatty acid profile. The slightly higher saponification value in SIOS suggests a higher proportion of short-chain fatty acids, which could influence oil stability and reactivity in formulations. Stability and shelf life are influenced by saponification value, as oils with higher values are more prone to oxidation, leading to rancidity and off-flavors [37]. The results obtained in this study are consistent with previous findings, with Paucar-Menacho *et al.* [16], reporting a saponification value of 190.5 mg KOH/g and Kyaw *et al.* [31], reporting a value of 190.85.

Acid value measures the free fatty acid content in oil, reflecting its purity and resistance to rancidity [38,39]. SIOP exhibited a lower acid value compared to SIOS ( $p$ -value = 0.005), indicating superior oil quality and lower susceptibility to hydrolysis. Lower acid values are preferred for culinary and cosmetic applications, ensuring better sensory attributes and shelf stability [14]. The higher acid value in SIOS suggests greater exposure to oxidative degradation, potentially due to prolonged heat and solvent interactions during Soxhlet extraction.

The findings of this study demonstrate that the hydraulic press method (SIOP) produces oil with superior physicochemical properties compared to Soxhlet extraction (SIOS). The lower peroxide values,

lower acid values, and higher retention of essential fatty acids in SIOP confirm that mechanical pressing better preserves bioactive compounds. These results are in agreement with previous studies on solvent-free extraction methods, emphasizing their advantages in maintaining oil quality and oxidative stability [6,17]. Additionally, SIOP's higher oxidative stability suggests that it may be more suitable for long-term storage and industrial applications, including functional foods, cosmetics, and pharmaceuticals. The variations in density and refractive index observed in **Table 1** also have practical implications for emulsification properties and lipid-based formulations, making Sacha Inchi oil a valuable ingredient for food processing and nutraceutical applications [40,41].

#### *Fatty acid composition of sacha inchi seed oil*

The identification of  $\alpha$ -linolenic acid, linoleic acid, and oleic acid compounds in sacha inchi seed oil using Gas Chromatography-Mass Spectrometry (GC-MS) involves the separation and analysis of fatty acid molecules present in the oil. This process begins with the methylation of fatty acids to form volatile methyl esters, which are then injected into the GC-MS [42]. The fatty acid composition of Sacha Inchi oil extracted using both methods is shown in **Table 2**.

**Table 2** Essential fatty acid composition of sacha inchi seed oil.

Compound	Percentage (%)		Peruvian Technical Standard NTP 151.400 (%)
	SIOP	SIOS	
$\alpha$ -linolenic acid	46.77	44.87	44.7
Linoleic acid	41.41	39.59	32.1
Oleic acid	0.95	0.89	8.9

The linolenic acid content in SIOP and SIOS closely aligns with the Peruvian Technical Standard, suggesting that both methods are effective in preserving this essential fatty acid. The slight variation might be attributed to the differing efficiencies of the extraction methods. These results highlight the high linolenic acid content of sacha inchi oil, which is beneficial due to its anti-inflammatory properties [43–45] and potential cardiovascular benefits [46–48]. In contrast, the linoleic acid content showed a more noticeable difference between the 2 extraction methods and the standard. SIOP yielded a significantly higher linoleic acid content

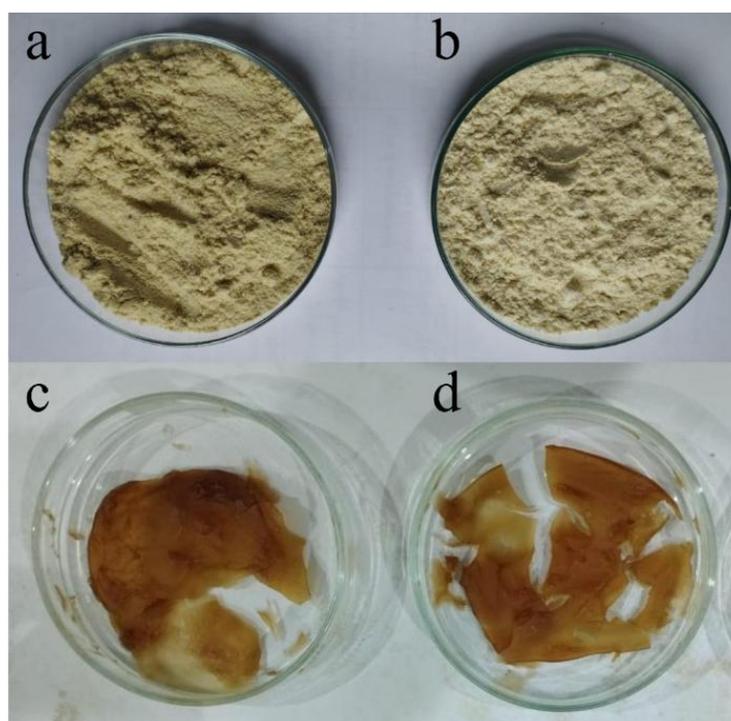
compared to SIOS and the Peruvian Technical Standard. This disparity indicates that the hydraulic press method might be more efficient in extracting linoleic acid from the oil. Linoleic acid, being an omega-6 fatty acid, plays a crucial role in maintaining skin health [49] and supporting overall immune function [50,51], thus making this difference quite impactful.

Interestingly, the oleic acid content in both SIOP and SIOS was significantly lower than the standard. Oleic acid, an omega-9 fatty acid, is known for its benefits in reducing inflammation [52,53]. Overall, these findings contribute valuable information to the

understanding of sacha inchi oil's nutritional qualities and potential health benefits. These results are particularly relevant in the context of health implications, as Katrencikova *et al.* [54] and Paduchova *et al.* [55] highlight the importance of an optimal omega-3 to omega-6 ratio in dietary intake. Sacha Inchi oil, with an omega-3 to omega-6 ratio of approximately 1.39:1 [6], is notably superior to many commonly used vegetable oils, such as sunflower oil (20:1) and corn oil (30:1 - 50:1). This balanced ratio is associated with anti-inflammatory benefits, improved cardiovascular health, and reduced risks of metabolic disorders, as supported by previous literatures [56,57].

#### *Proximate analysis of sacha inchi cakes*

The proximate analysis of SIOPC (**Figure 2(a)**) and SIOSC (**Figure 2(b)**) is presented in **Table 3**. Statistical analysis revealed that the SIOPC flour has lower mineral compared to SIOSC ( $p$ -value = 0.0037). This may be due to the pressing method, which extracts oil and possibly leaves behind most of the inorganic (mineral) components [54]. The higher ash content in SIOSC suggests that the Soxhlet method may extract more components from the sample, including inorganic materials such as minerals, leading to greater residue (ash) remaining after combustion. Ash content reflects the mineral composition of the press cake, and the observation aligns with the findings of Lavenburg *et al.* [58], who suggested that pressing methods primarily extract oil while leaving behind most inorganic components



**Figure 2** SIOPC (a), SIOSC (b), SIOPC protein hydrolysate (c), and SIOSC protein hydrolysate (d).

SIOPC produced a higher crude fiber content than the soxhlet method, but the difference is not significant ( $p$ -value = 0.16). This difference may be attributed to variations in processing methods, affecting how much fiber remains after processing. In the press method, the material is mechanically extracted, likely protecting the crude fiber from the solvent, thus leaving more fiber after combustion. In terms of protein content, the SIOPC flour sample contain lower protein content than the

SIOSC ( $p$ -value =  $1.3 \times 10^{-7}$ ). On the other hand, the protein hydrolysate of SIOPC (**Figure 2(c)**) was higher than SIOSC (**Figure 2(d)**) ( $p$ -value = 0.0088). The high protein content in the protein hydrolysate of SIOPC makes the press cake a valuable by-product for both food and nutraceutical applications. This is consistent with Thuanthong *et al.* [59] and Teran *et al.* [60], who reported the use of Sacha Inchi protein in functional food products such as protein powders, snack bars, and

dietary supplements. Additionally, previous studies by Sanchez *et al.* [15], reported that Sacha Inchi press-cake samples contain 56.1 % total crude protein, supporting the observed trend.

The lipid content in SIOPC and SIOSC provides insights into the efficiency of oil extraction methods. Lower residual lipid content in SIOSC indicates that

Soxhlet extraction removes a greater proportion of oil compared to hydraulic pressing. However, this intensive extraction process may also alter the composition of essential nutrients and bioactive compounds in the press cake. The fat content between the 2 methods did not differ significantly ( $p$ -value = 0.57), suggesting that both techniques are effective in extracting oil.

**Table 3** Proximate analysis comparison of SIOPC and SIOSC.

Parameters	Values		$p$ -values
	SIOPC	SIOSC	
Ash content	4.56 ± 0.07	5.25 ± 0.13	3.7×10 <sup>-3</sup>
Fat content	6.59 ± 0.15	6.47 ± 0.32	0.57
Fiber content	0.82 ± 0.11	0.70 ± 0.05	0.16
Moisture content	12.43 ± 0.41	14.99 ± 0.08	6×10 <sup>-3</sup>
Protein content	49.48 ± 0.13	60.57 ± 0.16	1.3×10 <sup>-7</sup>
Protein hydrolysate content	82.48 ± 0.60	79.96 ± 0.28	8.8×10 <sup>-3</sup>

The moisture content of both SIOPC and SIOSC plays a critical role in determining their shelf life and stability. The moisture content of SIOPC was lower than that of SIOSC ( $p$ -value = 0.006). Low moisture content is beneficial for product stability, while high moisture content may lead to quality degradation. Overall, the proximate analysis highlights the significant differences in the composition of SIOPC and SIOSC, reinforcing the advantages of hydraulic pressing in preserving protein, fiber, and mineral content while minimizing excessive processing that may compromise nutrient retention. These insights contribute to the growing interest in utilizing Sacha Inchi press cake as a

functional ingredient in food and nutraceutical applications.

#### ***Antioxidant activity of sachu inchi seed oil and cakes***

The antioxidant activity of sachu inchi seed oil using the DPPH method is presented in **Table 4**. Sachu inchi seed oil is rich in various bioactive compounds, including flavonoids, triterpenoids, and steroids, which contribute to its antioxidant activity. These compounds have been shown to exhibit significant antioxidant properties, capable of inhibiting oxidative damage and reducing free radical formation [14].

**Table 4** Antioxidant activity of SIOP, SIOS, SIOPC, and SIOSC.

Sample	IC <sub>50</sub> value (mg/mL)
SIOP	104.05 ± 4.48 <sup>a</sup>
SIOS	108.53 ± 8.42 <sup>a</sup>
SIOPC	0.212 ± 0.01 <sup>b</sup>
SIOSC	0.314 ± 0.02 <sup>b</sup>

The antioxidant activity in sachu inchi seed oil, obtained using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and measured through IC<sub>50</sub> values, is crucial in assessing the oil's ability to neutralize free radicals and provide health benefits. The

IC<sub>50</sub> value indicates the concentration of oil required to inhibit 50 % of radical activity, with lower values indicating stronger antioxidant activity [61]. The study found that sachu inchi seed oil extracted using hydraulic press (SIOP) and soxhlet methods (SIOS) resulted in

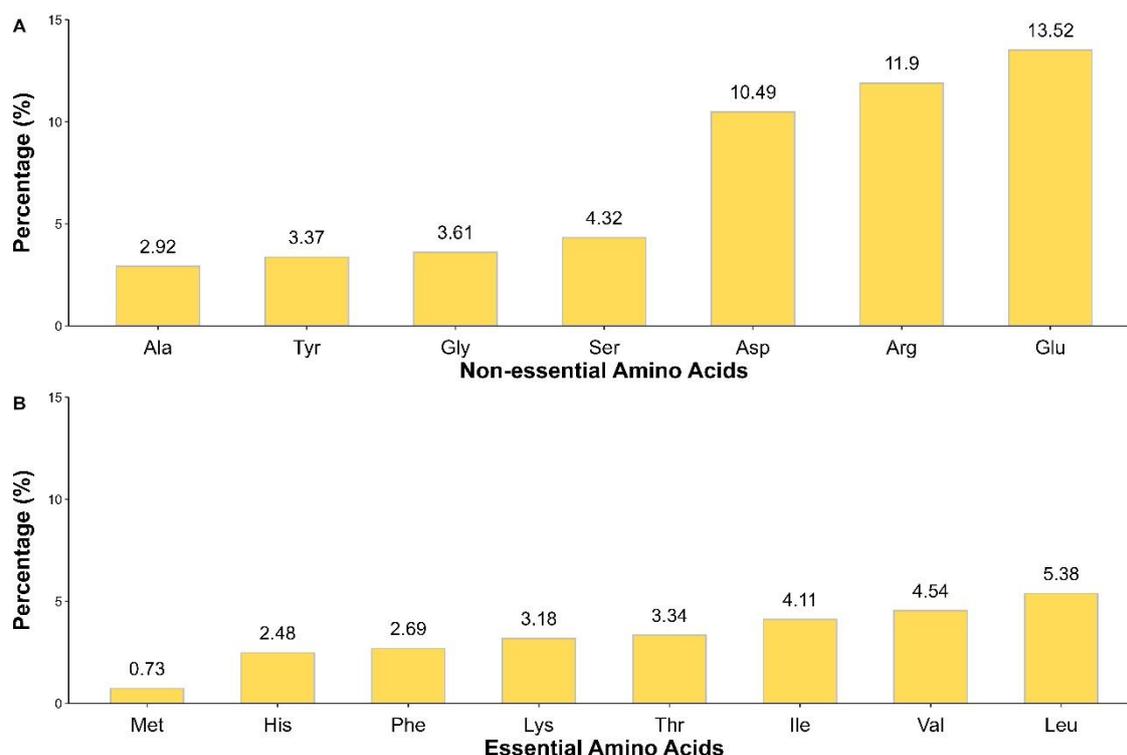
IC<sub>50</sub> values of 104.05 ± 4.48 and 108.53 ± 8.42 mg/mL, respectively. Interestingly, both the SIOPC and SIOSC extract exhibited significantly higher antioxidant activity compared to SIO and SIOS with IC<sub>50</sub> of 0.212 ± 0.01 and 0.314 ± 0.02 mg/mL.

The SIOPC's antioxidant activity is rarely studied. However, previous reports indicated a 32 % value and 730 µmol FeSO<sub>4</sub>/L for SIOPC's antioxidant capacity, evaluated through DPPH and ferric reducing antioxidant power (FRAP) assays. This activity is attributed to phenolic compounds, ranging between 51 and 312 mg gallic acid equivalents (GAE)/100 g. Sacha inchi seed oil extracted by hydraulic press showed significant antioxidant activity at this concentration, suggesting that this method preserves bioactive compounds and their effectiveness in retaining natural antioxidants [62].

Conversely, oil extracted via soxhlet showed slightly higher antioxidant activity, which may be due to solvent usage and repeated heating that could potentially damage heat-sensitive antioxidant compounds. Antioxidant activity standards for vegetable oils are often compared with IC<sub>50</sub> values of oils with very high antioxidant activity, such as olive or grape seed oil, which typically have IC<sub>50</sub> values below 0.5 mg/mL [63].

#### *Amino acid analysis of sacha inchi press-cake*

The amino acid composition of SIOPC was analyzed using HPLC. HPLC is an advanced development of column chromatography. It measures retention time based on the time it takes from sample injection until the maximum peak height of the compound is observed [21].



**Figure 3** Non-essential (A) and essential (B) amino acids content in protein hydrolysate of SIOPC.

The HPLC analysis of SIOPS revealed significant findings regarding its amino acid composition and nutritional value. The analysis identified 7 non-essential amino acids, with glutamic acid (13.52 %) and arginine (11.9 %) being the most abundant (**Figure 3(A)**). These amino acids are critical for protein synthesis and various metabolic pathways, where glutamic acid serves a pivotal role in cellular metabolism [64–66] and arginine

is recognized for its contributions to immune function and cardiovascular health [67, 68]. In addition to non-essential amino acids, the presence of essential amino acids underscores the completeness of SIOPS as a protein source (**Figure 3(B)**). Essential amino acids, such as leucine (5.38 %), valine (4.54 %), and isoleucine (4.11 %), are vital for muscle protein synthesis, tissue repair, and overall growth [69, 70]. The relatively high

percentages of these amino acids in sachu inchi oil make it a valuable dietary component, particularly for individuals seeking plant-based protein options. Moreover, the presence of lysine (3.18 %) is particularly noteworthy, as this amino acid is often limited in plant proteins [71,72], making sachu inchi an excellent supplement for a balanced vegetarian or vegan diet.

A previous study conducted by Vanegas-Azuero & Gutiérrez, reported that SIOPC is rich in essential amino acids (EAAs) such as lysine, histidine, and leucine, and contains significant levels of isoleucine, valine, tryptophan, and phenylalanine, with lower concentrations of threonine and methionine. This composition of essential amino acids meets the FAO/WHO/ONU recommendations, except for lysine and leucine [73]. SIOPC can be regarded as a balanced protein source, comparable to soy protein in terms of total EAA content. Regarding non-essential amino acids (NEAAs), SIOPC contains high levels of tyrosine, glutamic acid, aspartic acid, and cysteine.

While this study provides significant insights into Sachu Inchi oil extraction, some limitations must be acknowledged. First, the study focuses only on hydraulic press and Soxhlet extraction methods, excluding advanced techniques such as supercritical CO<sub>2</sub> extraction, which might yield different results. Second, while we ensured consistency in sample preparation, variations in seed quality and environmental factors could impact reproducibility. Additionally, the stability of the extracted oils over extended storage periods was not assessed, which could influence long-term industrial applications. One of the key disadvantages of this study is the lack of an economic feasibility analysis comparing the cost-effectiveness of hydraulic pressing and Soxhlet extraction for large-scale production. While SIOP demonstrated superior oil quality, its lower yield compared to SIOS may limit its commercial viability. We suggest future research exploring optimization strategies to enhance oil recovery without compromising bioactive retention.

## Conclusions

This study confirms that hydraulic press extraction is a superior method for obtaining high-quality Sachu Inchi (*Plukenetia volubilis* L.) oil compared to Soxhlet extraction. SIOP demonstrated lower peroxide values

( $2.31 \pm 0.022$  mEq O<sub>2</sub>/kg), higher retention of polyunsaturated fatty acids (46.77 %  $\alpha$ -linolenic acid), and greater antioxidant activity (IC<sub>50</sub>:  $104.05 \pm 4.48$  mg/mL). In contrast, SIOS exhibited slightly lower polyunsaturated fatty acid content (44.87 %  $\alpha$ -linolenic acid) and reduced oxidative stability due to solvent exposure. GC-MS analysis confirmed higher essential fatty acid retention in SIOP, including 41.41 % linoleic acid and 0.95 % oleic acid. The balanced omega-3 to omega-6 ratio (1.39:1) makes Sachu Inchi oil a healthier alternative to conventional vegetable oils. Additionally, hydraulic pressing eliminates chemical solvents, offering a sustainable extraction method. The proximate analysis of the press cake (SIOPC) showed higher protein and fiber content than Soxhlet-extracted press cake (SIOSC), making it a valuable functional food ingredient. HPLC analysis identified 15 amino acids, including 9 essential ones such as leucine, lysine, and valine. Hydraulic pressing, being solvent-free, is a more sustainable method as it eliminates chemical waste and reduces environmental impact. However, the lower yield of SIOP compared to SIOS raises feasibility concerns for large-scale production. This suggests that while SIOP is preferable for high-quality oil, further optimization is needed to enhance its yield for industrial viability. Additionally, long-term storage stability studies and cost-benefit analyses will be valuable for assessing commercial scalability.

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