Indonesian Journal of Global Health Research
Volume 8 Number 5, October 2026
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

PHYSICOCHEMICAL CHARACTERISTICS AND ANTIOXIDANT ACTIVITY OF SACHA INCHI (Plukenetia volubilis L.) SEED OIL: EFFECT OF MICROWAVE ROASTING PRE-TREATMENT

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ABSTRACT
Sacha inchi seed oil (Plukenetia volubilis L.) has considerable potential as a functional oil source due to its high content of unsaturated fatty acids and bioactive compounds. The processing method, especially the roasting pre-treatment, can affect the quality and stability of the resulting oil, both in terms of physicochemical characteristics and antioxidant activity. This study aims to evaluate the effect of microwave roasting pre-treatment on the physicochemical characteristics and antioxidant activity of Sacha inchi seed oil extracted using the Soxhlet method. Roasting was carried out at Med-Low 30P, Medium 50P, and Med-High 70P power levels. The oil was extracted using the Soxhlet method. Physicochemical properties, including acid value, peroxide value, iodine value, and saponification value, were analyzed using AOCS standard methods, while antioxidant activity was evaluated spectrophotometrically using DPPH, ABTS, and β-carotene bleaching assays methods. The results showed that increasing roasting power increased oil yield, with the highest yield (62%) obtained at Med-High 70P treatment. Variation in roasting power did not have a significant effect on acid value (1.301–1.418 mg KOH/g oil) and iodine value (101.940–103.883 g I_2/100 g oil), but had a significant effect on peroxide value (5.32–5.82 meq O_2/kg) and saponification value (171.591–180.422 mg KOH/g oil). The highest antioxidant activity was obtained at Medium 50P treatment for the β-carotene bleaching assay (501.58 mg/L) and DPPH (1214.92 mg/L) methods, and at Med-Low 30P treatment for the ABTS method (673.70 mg/L). Microwave roasting pre-treatment affects the increase in yield as well as several physicochemical parameters and antioxidant activity of sacha inchi seed oil.

Keywords: IC50; physicochemical characteristics; roasting pre-treatment; sacha inchi (Plukenetia volubilis L.)

How to cite (in APA style)
Saputri, U. M., Irnawati, I., Jabbar, A., Sahidin, S., Nasrudin, N., Sartinah, A., & Kusumadewi, A. P. (2026). Physicochemical Characteristics and Antioxidant Activity of Sacha Inchi (Plukenetia volubilis L.) Seed Oil: Effect of Microwave Roasting Pre-Treatment. *Indonesian Journal of Global Health Research*, *8*(5), 791–800. https://doi.org/10.37287/ijghr.v8i5.2108.

INTRODUCTION
Sacha inchi (Plukenetia volubilis L.) is a plant native to Peru originating from the Amazon basin and has traditionally been utilized as both a food source and medicinal plant. It is widely cultivated in Latin America and several Asian countries, including Indonesia. In Indonesia, this plant was first found in Java and is known as kacang inka or kacang gunung and used as vegetables (Kong et al., 2023; Tianara et al., 2024). Sacha inchi seed oil is a nutrient-rich oil containing various essential minerals and high levels of unsaturated fatty acids, particularly alpha-linolenic acid (Omega-3), linoleic acid (Omega-6), and oleic acid (Omega-9) (Borges et al., 2018; Chinh et al., 2020; Fong et al., 2023; Kim & Joo, 2019; Maya et al., 2023). The bioactive compounds present in Sacha inchi oil offer numerous health benefits, including reducing the risk of coronary heart disease, diabetes, hypertension, hyperactivity disorders, and inflammatory skin diseases, while also exhibiting antioxidant activity.(Kittibunchakul et al., 2023; Kyaw et al., 2019; Maya et al., 2023) Chinh et al., 2020; Herrera-calderon et al., 2019.

To obtain high-quality oil, extraction processes and raw material treatment conditions are crucial aspects that must be carefully considered. Previous studies have shown that preparation methods significantly affect the physicochemical characteristics and composition of sacha inchi seed oil (Borges et al., 2018; Kim & Joo, 2019; Kittibunchakul et al., 2023; Muangrat et al., 2018). However, particular attention should be given to sacha inchi seed oil, as it is recognized as one of the oils with a high omega-3 fatty acid content, which is highly susceptible to oxidation. Consequently, high temperatures during the roasting process may reduce nutritional quality and alter the fatty acid profile and mineral content of the oil (Kim & Joo, 2019; Kittibunchakul et al., 2023). In addition, the roasting process may accelerate lipid oxidation in the seeds, potentially affecting the shelf life of the product (Borges et al., 2018).

The development of microwave roasting methods for nuts and seeds offers an innovative approach capable of accelerating the heating process, thereby improving efficiency while maintaining the composition and quality of the resulting oil. Appropriate roasting conditions can enhance extraction efficiency while preserving the active compounds and overall oil quality (Chirinos et al., 2015; Muangrat et al., 2018). Studies conducted by Christopher et al. (2023), Fong et al. (2023), dan Pisco & Lujerio (2016), reported higher oil yields obtained through microwave roasting without reducing biological activity, while also decreasing acid and peroxide values, resulting in improved oil stability. Considering the potential of sacha inchi as a high-value oil source and the limited related studies conducted in Indonesia, this study was carried out to investigate the effect of microwave roasting pre-treatment on the yield, physicochemical characteristics, and antioxidant activity of locally cultivated sacha inchi seed oil.

METHOD

Microwave Pre-treatment

A total of 100 g of cleaned sacha inchi seeds were roasted separately using a microwave oven (Sharp R728(S)IN) at Med-Low 30P, Medium 50P, and Med-High 70P power levels for 4 minutes. The roasted seeds were subsequently ground using a blender. The ground samples were then stored in airtight containers and kept under refrigerated conditions until extraction (Christopher et al., 2023; Fong et al., 2023; Pisco & Lujerio, 2016).

Extraction of Sacha Inchi Seed Oil

Sacha inchi seed oil extraction was carried out based on the method by Muangrat et al. (2018), with slight modifications. Approximately 50 g of ground sample was extracted using a Soxhlet apparatus with 500 mL of n-hexane as the solvent. The extraction process was conducted for 3 h, after which the solvent was removed using a rotary evaporator (Infitek®) at 50°C. The extracted oil was subsequently weighed, and the oil yield was calculated. The oil samples were then stored in airtight containers at 4°C until further analysis. The oil yield was calculated using the following equation:

$$\%\text{Oil yield} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100\%$$

Physicochemical Characterization

Physicochemical analyses included acid value, peroxide value, iodine value, and saponification value, following AOAC (1990). standard methods. All analyses were performed in triplicate.

Antioxidant Activity Assay

Antioxidant activity was evaluated using DPPH, ABTS, and β-carotene bleaching assays. Antioxidant activity was expressed as the IC_{50} value obtained from the linear relationship between sample concentration and percentage inhibition. A lower IC_{50} value indicates stronger antioxidant activity.

DPPH Radical Scavenging Assay

The DPPH antioxidant activity assay was performed according to a modified procedure adapted from Chan et al. (2024), Maya et al. (2023), dan Muangrat et al. (2018). Oil samples were prepared at various concentrations ranging from 500–2000 ppm using chloroform as the solvent. A total of 2 mL of 0.4 mM DPPH solution was added to each sample concentration. The mixture was homogenized and incubated in the dark for 30 min at room temperature. Absorbance was measured at 515 nm using a UV–Vis spectrophotometer (Jenway 6800®). Trolox and BHT solutions were used as standards. Antioxidant activity was expressed as the percentage of DPPH radical inhibition calculated using the following equation:

$$\%\text{DPPH inhibition} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of sample}}{\text{Absorbance of DPPH}} \times 100$$

ABTS Radical Scavenging Assay

The ABTS assay was conducted based on a modified method described by Chan et al. (2024) dan Muangrat et al. (2018). The ABTS radical solution was prepared by mixing 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$ solution (1:1, v/v), followed by incubation at room temperature for 16 h. Prior to use, the solution was diluted until the absorbance reached 0.700 ± 0.020 at 734 nm. Oil samples were prepared at concentrations ranging from 500–2000 ppm using chloroform as the solvent. The sample solution was mixed with ABTS solution to a final volume of 5 mL, shaken for 30 s, and allowed to stand for 6 min. Absorbance was measured at 734 nm. Trolox and BHT solutions were used as standards. The percentage of ABTS radical inhibition was calculated using the following equation:

$$\%\text{ inhibition ABTS} = \frac{\text{Absorbance of ABTS} - \text{Absorbance of sample}}{\text{Absorbance of ABTS}} \times 100$$

β-Carotene Bleaching Assay

The β-carotene bleaching assay was performed according to the modified methods of Hamdi et al. (2017) dan Xuan et al. (2018). A total of 125 mg of β-carotene was dissolved in 5 mL chloroform, followed by the addition of 125 µL linoleic acid and 1 g Tween-20. The mixture was homogenized, and the chloroform was evaporated to dryness. Subsequently, the mixture was gradually diluted with water for injection to a final volume of 500 mL to obtain a β-carotene emulsion. Oil sample solutions were prepared at concentrations ranging from 500–2000 ppm. The sample solution was mixed with the β-carotene emulsion to a final volume of 5 mL, vortexed for 30 s, and incubated at 50°C for 120 min. Absorbance was measured at 450 nm at 0 and 120 min. BHT and Trolox were used as standards. The β-carotene bleaching rate was calculated using the following equation:

$$\text{Belaching rate} = ln\left(\frac{Absorbance\ t_0}{Absorbance\ t_{120}}\right)$$

$$\%\text{ Inhibition} = \left(\frac{A0 - A1}{A0}\right) \text{ x } 100\ \%$$

Where:

A_0 : Belaching rate of the control

A_1 : Belaching rate of the sample

Data Analysis

Physicochemical characteristics and antioxidant activity data were analyzed using one-way ANOVA at a 95% confidence level (α = 0.05) with Minitab 17 software.

RESULT

Microwave roasting at different power levels resulted in variations in the color and yield of sacha inchi seed oil, as presented in Figure 1.

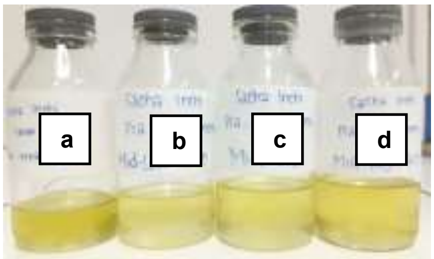

Figure 1. Sacha inchi seed oil: (a) without pre-treatment, (b) Med-Low 30P pre-treatment, (c) Medium 50P pre-treatment, and (d) Med-High 70P pre-treatment.

The resulting sacha inchi seed oil exhibited color variations ranging from pale yellow to deep yellow with different levels of intensity. Oil subjected to the Med-Low 30P pre-treatment appeared the palest and nearly transparent, whereas the Medium 50P treatment produced a more intense light-yellow color. Meanwhile, the Med-High 70P treatment resulted in a darker yellow color, similar to that of the oil without pre-treatment. In line with these color changes, increasing roasting power also contributed to higher oil yields (Table 1).

Table 1.
Oil yield of sacha inchi seeds

Sample	Sample weight (g)	Oil weight (g)	%Yield
Without pre-treatment	50	19	38
Pre-treatment Med-Low 30P		25	50
Pre-treatment Medium 50P		29	58
Pre-treatment Med-High 70P		31	62

The results showed that increasing roasting power led to an increase in oil yield. The highest oil yield was obtained under the Med-High 70P treatment (62%), whereas the lowest yield was observed in oil extracted from seeds without roasting pre-treatment (38%). Seed pre-treatment, particularly roasting, is known to influence the physicochemical characteristics of the resulting oil, as presented in Table 2.

Table 2.
Physicochemical characteristics of sacha inchi seed oil

Sample	Acid Value (mg KOH/g oil)	Peroxide Value (meq O_2/kg oil)	Iodin Value (g I_2/100 g oil)	Saponification Value (mg KOH/g oil)
Without pre-treatment	1,405 ± 0,100 ^{a}	5,32 ± 0,0 ^{b}	101,940 ± 1,03 ^{a}	180,422 ± 0,74 ^{a}
Pre-treatment Med-Low 30P	1,313 ± 0,017 ^{a}	5,40 ± 0,10 ^{a,b}	102,386 ± 0,15 ^{a}	175,752 ± 1,32 ^{b}
Pre-treatment Medium 50P	1,301 ± 0,030 ^{a}	5,62 ± 0,13 ^{a,b}	103,244 ± 0,62 ^{a}	174,443 ± 1,39 ^{b,c}
Pre-treatment Med-High 70P	1,418 ± 0,021 ^{a}	5,82 ± 0,28^{a}	103,883 ± 1,51 ^{a}	171,591 ± 1,04 ^{c}

Values are expressed as mean ± SD (n = 3).
Different letters within the same column indicate significant differences ($p < 0.05$).
Identical letters within the same column indicate no significant differences ($p > 0.05$).

The ANOVA results showed that variations in roasting power did not have a statistically significant effect on the acid value and iodine value ($p > 0.05$). However, roasting power significantly affected the peroxide value and saponification value as the roasting power increased ($p < 0.05$). The antioxidant activity of sacha inchi seed oil in this study was evaluated using DPPH, ABTS, and β-carotene bleaching assays, as presented in table 3.

Table 3.
Antioxidant activity of sacha inchi seed oil

Sample	IC_{50} of Antioxidant activity (mg/L)		
	DPPH	ABTS	*β-caroten belaching assay*
Without pre-treatment	1284,86 ± 4,15^{a}	842,71 ± 2,08^{a}	611,95 ± 1,17^{a}
Pre-treatment Med-Low 30P	1258,17 ± 2,27^{b}	673,70 ± 2,56^{b}	536,99 ± 1,18^{b}
Pre-treatment Medium 50P	1214,92 ± 1,02^{c}	746,87 ± 3,15^{c}	501,58 ± 2,12^{c}
Pre-treatment Med-High 70P	1238,94 ± 1,10^{d}	976,37 ± 4,79^{d}	699,24 ± 4,95^{d}
BHT	160,12 ± 0,70^{e}	7,85 ± 0,015^{e}	0,10 ± 0,002^{e}
Trolox	22,09 ± 0,39^{f}	3,84 ± 0,006^{e}	0,22 ± 0,002^{e}

Values are expressed as mean ± SD (n = 3).

Different letters within the same column indicate significant differences ($p < 0.05$). Based on the ANOVA results, roasting pre-treatment had a significant effect ($p < 0.05$) on the antioxidant activity of sacha inchi seed oil in all assays, including DPPH, ABTS, and β-carotene bleaching assays. All oil treatments, both without pre-treatment and with roasting pre-treatment, showed significant differences from one another. Oils subjected to Medium 50P pre-treatment (DPPH and β-carotene bleaching assays) and Med-Low 30P pre-treatment (ABTS assay) exhibited statistically lower IC_{50} values compared to oils without pre-treatment and those treated at Med-High 70P. These findings indicate that roasting at Medium 50P was the most effective condition for enhancing the antioxidant activity of sacha inchi seed oil.

DISCUSSION

Effect of Microwave Power Variation on the Yield of Sacha Inchi Seed Oi

The results of this study demonstrated that variations in microwave roasting power produced different effects on the color and yield of sacha inchi seed oil. Increasing roasting power tended to alter the color intensity of the resulting oil. This phenomenon was likely associated with the presence of natural pigments in sacha inchi seeds, such as carotenoids, which may be extracted during the oil extraction process. These pigments can impart yellowish or greenish hues to the oil (Valencia et al., 2024). Low to moderate heating intensities may partially degrade natural pigments without causing extensive browning reactions, resulting in a lighter-colored oil (Ramos-escudero et al., 2019). In contrast, higher roasting intensities produced a darker oil color due to decreased brightness associated with chemical reactions occurring during roasting, including Maillard reactions, caramelization, Strecker degradation, and lipid oxidation, which generate yellow to brown-colored compounds (Chan et al., 2024).

Increasing roasting power also resulted in higher oil yields, indicating that thermal treatment contributed to improved extraction efficiency. Microwave radiation interacts directly with polar materials and induces water evaporation within the cellular structure, thereby increasing internal pressure, which leads to membrane rupture and enhances oil extraction efficiency (Bakhshabadi et al., 2025). Consequently, the cellular structure of oil-bearing seeds becomes damaged or ruptured. These microstructural changes enhance tissue porosity, allowing the solvent to penetrate the cells more effectively and accelerate the mass transfer of oil into the solvent, ultimately improving extraction efficiency (Kaseke et al., 2020). In addition, uniform microwave heating may inactivate hydrolytic enzymes, thereby preventing triacylglycerol hydrolysis and preserving oil quality (Gaber et al., 2020). The increase in microwave power was directly proportional to the oil yield due to the greater thermal energy absorbed by the seeds. During microwave heating, heat is generated directly within the material through the oscillation of polar molecules induced by microwave radiation. The generated heat is then distributed throughout the material, resulting in more uniform heating across the entire seed matrix rather than only at the surface (Ramadhaningtyas et al., 2023). Furthermore, higher temperatures reduce surface tension and increase oil fluidity, thereby facilitating oil release from the seed tissue and improving extraction yield (Christopher et al., 2023).

Physicochemical Characteristics of Sacha Inchi Seed Oil

Physicochemical characteristics are important parameters for evaluating the quality and stability of vegetable oils, including sacha inchi seed oil, as they reflect the level of oxidation, degree of unsaturation, and potential deterioration during processing (Maya et al., 2023; Muangrat et al., 2018; Soimee et al., 2020). In this study, these parameters were analyzed to assess the effect of roasting power variation on oil quality. Roasting pre-treatment was found to influence the physicochemical characteristics of the oil, as presented in Table 2. The acid value obtained in this study ranged from 1.313–1.418 mg KOH/g oil, which remained below the maximum allowable limit (<2.0 mg KOH/g) established by Codex (2024), indicating good oil quality. Increasing roasting power tended to reduce the acid value, which was likely due to the thermal inactivation of lipase enzymes, thereby reducing the formation of free fatty acids (Amakhmakh et al., 2024;

Tolouie et al., 2018). However, a slight increase was observed at higher power levels, possibly as a result of accelerated hydrolysis during intensive heating.

The peroxide value, which serves as an indicator of primary oxidation, ranged from 5.32–5.82 meq O_2/kg oil. The values tended to increase with increasing roasting power, indicating lipid oxidation due to the formation of hydroperoxides during heating (Karrar et al., 2021; Muangrat et al., 2018). Nevertheless, all values remained below the maximum permissible limit (<10 meq O_2/kg), suggesting that the oil was still considered stable (Konuskan et al., 2019). This increase is consistent with previous reports indicating that high temperatures may accelerate free radical formation that attacks unsaturated fatty acid bonds (Bakhshabadi et al., 2017; Borges et al., 2018). The iodine value, which reflects the degree of fatty acid unsaturation, increased from 101.940 to 103.883 g I_2/100 g oil as roasting power increased. These findings are consistent with previous studies on sacha inchi seed oil (Christopher et al., 2023; Muangrat et al., 2018). Meanwhile, the saponification value showed a decreasing trend with increasing roasting power. This decrease indicates the predominance of long-chain fatty acids as well as possible compositional changes caused by thermal treatment. Similar results have been reported in other seed oils, where microwave roasting reduced the saponification value (Mohammed et al., 2017; Özcan et al., 2018). Overall, microwave roasting affected the physicochemical characteristics of sacha inchi seed oil, particularly oxidation-related parameters and fatty acid structure, without significantly reducing oil quality.

Antioxidant Activity of Sacha Inchi Seed Oil

Roasting treatment under controlled conditions is known to reduce antinutritional compounds and enhance antioxidant activity, while the oxidative changes that occur remain within acceptable oil quality limits (Borges et al., 2018; Christopher et al., 2023; Kittibunchakul et al., 2023; Muangrat et al., 2018). The antioxidant activity of sacha inchi seed oil in this study was evaluated using DPPH, ABTS, and β-carotene bleaching assays, as presented in Table 3. In the DPPH assay, sacha inchi seed oil without roasting pre-treatment exhibited the highest IC_{50} value (1284.86 mg/L), indicating the weakest antioxidant activity compared to the other oil samples. Roasting gradually reduced the IC_{50} values under Med-Low 30P (1258.17 mg/L) and Medium 50P (1214.92 mg/L) treatments, followed by an increase at Med-High 70P (1238.94 mg/L). This pattern suggests that low to moderate roasting intensities enhanced antioxidant activity, whereas excessive roasting reduced antioxidant activity due to the possible degradation of bioactive compounds. These findings are consistent with previous reports demonstrating increased antioxidant activity under moderate heating conditions and reduced activity under excessive heating (Chan et al., 2024; Muangrat et al., 2018; Oubannin et al., 2024).

The lowest IC_{50} value in the ABTS assay was obtained under the Med-Low 30P treatment (673.70 mg/L), indicating the highest antioxidant activity, whereas the value increased at Med-High 70P (976.37 mg/L). In general, the antioxidant activity obtained using the ABTS assay was higher than that observed in the DPPH assay, suggesting that the bioactive compounds present in the oil were more effective in reducing $ABTS^{\bullet+}$ radicals. This may be attributed to the higher sensitivity and broader solubility range of the ABTS method toward various antioxidant compounds (Lee et al., 2015). These findings are in agreement with previous studies reporting optimum antioxidant activity under mild roasting conditions (Muangrat et al., 2018; Chan et al., 2024). In the β-carotene bleaching assay, oil without roasting pre-treatment showed the highest IC_{50} value (611.95 mg/L). Roasting at Med-Low 30P and Medium 50P reduced the IC_{50} values, with the lowest value observed at Medium 50P (501.58 mg/L), indicating an enhanced ability to inhibit lipid oxidation. In contrast, the IC_{50} value increased under the Med-High 70P treatment (699.24 mg/L), indicating reduced effectiveness due to the thermal degradation of antioxidant compounds.

Differences in antioxidant activity among the assays may be attributed to variations in the reaction mechanisms between antioxidant compounds and free radicals in each method. Heat treatment may modify the mechanism of action of antioxidant compounds and induce synergistic or antagonistic interactions among compounds, thereby affecting the ability of the antioxidant system to scavenge free radicals (Kulik, 2025). The increase in antioxidant activity observed under roasting conditions was likely associated with the release of bioactive compounds from the cellular matrix, whereas the decline at higher roasting intensities may be attributed to the thermal degradation of heat-sensitive compounds (Alkaltham et al., 2020).

CONCLUSION

Overall, the results of this study demonstrated that microwave roasting pre-treatment increased the yield of sacha inchi seed oil, with the highest yield obtained under the Med-High 70P treatment. Variations in roasting power did not significantly affect the acid value and iodine value; however, they significantly influenced the peroxide value and saponification value. Antioxidant activity increased following roasting, with optimal conditions observed at Medium 50P for the DPPH and β-carotene bleaching assays and at Med-Low 30P for the ABTS assay, whereas the Med-High 70P treatment resulted in a decline in antioxidant activity.

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