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Research Article

Moderate Roasting Improves Bioactivity and Stability of Sacha Inchi (*Plukenetia volubilis* L.) Oil Without Altering Its Fatty Acid Composition

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Abstract

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Sacha Inchi oil (*Plukenetia volubilis* L.), rich in ω-3 fatty acids and vitamin E, has attracted growing global interest for its potential health benefits. However, its low oxidative stability remains a key limitation to commercial application. This study investigated the effects of moderate thermal pre-treatment (roasting) on the chemical composition, oxidative stability, and functional bioactivity of Sacha Inchi oil, comparing oils extracted from roasted and unroasted seeds. Ten seed samples were collected from six regions in Taiwan: Taichung, Changhua, Nantou, Chiayi, Tainan, and Hualien. After dehulling, the seeds were mechanically pressed without intentional heating to obtain oil. Analytical parameters included acid value (AV), peroxide value (POV), oil stability index (OSI, at 130℃), vitamin E content, fatty acid profile, phospholipid composition (via thin-layer chromatography), total phenolic content, reducing power, and anti-inflammatory activity assessed using a cell-based model. Results showed that roasting did not alter the fatty acid profile or vitamin E content, but significantly improved oxidative stability (higher OSI) and promoted the accumulation of bioactive compounds such as phospholipids (ex. phosphatidylethanolamine; PE) and total phenolics, along with stronger reducing power. Both roasted and unroasted oils exhibited anti-inflammatory activity, with roasted oil showing a slightly greater suppression of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated macrophages. In summary, controlled roasting effectively enhances the oxidative stability, bioactive composition, and physiological functionality of Sacha Inchi oil while preserving its core nutritional profile (rich in ω-3 fatty acids and vitamin E). These findings support roasting as a feasible strategy to enhance the stability and bioactivity of Sacha Inchi oil for food and nutraceutical applications.

Key words: Sacha Inchi oil, Roasting, Oxidative stability, Functional bioactivity, Anti-inflammatory activity.

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INTRODUCTION

Sacha Inchi (*Plukenetia volubilis* L.) is a perennial oilseed vine native to the eastern slopes of the Andes, particularly in Peruvian regions such as Cusco, Junín, and Pasco, where it thrives at altitudes of 1,600–2,100 m. Although its natural distribution ranges from the Lesser Antilles to northern South America, the Andean populations display the greatest morphological diversity and have a long history of traditional use by indigenous communities (Bussmann *et al*. 2013). In recent years, Sacha Inchi oil has attracted international attention due to its high content of polyunsaturated fatty acids (PUFAs), especially α-linolenic acid (ALA), as well as other bioactive compounds such as tocopherols, phytosterols, and phenolics (Mhd Rodzi & Lee 2022; Mhd Rodzi *et al*. 2025). Clinical and preclinical studies have demonstrated its potential benefits in regulating blood lipids, reducing inflammation, and supporting cardiovascular health (Mhd Rodzi *et al*. 2025). Recognizing its nutritional value, Peru has actively promoted Sacha Inchi oil as a novel food and proposed its inclusion in the Codex Standard for Named Vegetable Oils (CAC 2024).

Despite these health-promoting properties, Sacha Inchi oil is traditionally extracted via cold pressing without heat treatment, based on the prevailing belief that PUFAs are highly susceptible to thermal degradation, as noted in Peru's submission to CAC (2021). This perception has led to the avoidance of roasting or any form of pre-heating during processing. However, studies on other oilseeds, such as camellia oil, have shown that moderate roasting prior to pressing can enhance oxidative stability and promote the release of bioactive compounds without compromising oil quality (Hsieh *et al*. 2013).

Recent investigations have further demonstrated that roasting Sacha Inchi seeds under controlled conditions can significantly increase total phenolic content and antioxidant capacity (Kittibunchakul *et al*. 2023). Moreover, optimized roasting at 134–140℃ for 15–20 min has been shown to preserve fatty acid composition while improving oxidative stability and bioactivity (Bocanegra Morales & Galeano Garcia 2023).

To reassess the conventional cold-pressing paradigm, this study investigated the effects of thermal pre-treatment (roasting) on the chemical quality, oxidative stability, and functional properties of Sacha Inchi oil. Ten seed samples collected across Taiwan were roasted prior to being mechanically pressed without intentional heating. However, during pressing (~15 min per batch), mechanical friction raised the expeller chamber temperature to 70–80°C. The resulting oils were analyzed for acid value (AV), peroxide value (POV), oil stability index (OSI), vitamin E content, fatty acid composition, phospholipid profile, and bioactivity using cell-based anti-inflammatory assays. This study aims to determine whether controlled thermal processing can improve the functionality and stability of Sacha Inchi oil for food and nutraceutical applications.

MATERIALS AND METHODS

Plant material and sample preparation

Ten batches of mature *P. volubilis* L. (Sacha Inchi) seeds were collected from six major cultivation regions in Taiwan: Taichung, Changhua, Nantou, Chiayi, Tainan, and Hualien. Each batch was assigned a sample code (No. 1–10) for subsequent analysis. All seeds were harvested between April and May 2023, coinciding with the peak ripening season. Their fruits were harvested when the entire capsule turned brown, indicating full maturity. After harvest, local farmers sun-dried the fruits under ambient conditions to reduce their moisture content. Outer fruit hulls were manually removed, and defective fruits- such as moldy or shriveled ones- were discarded. Upon arrival at the laboratory, seed moisture content ranged from 8% to 14%. To ensure consistency across samples, all seeds were further equilibrated by hot-air oven drying at 50℃ until their moisture content fell below 10%.

The samples were then divided into two groups: one subjected to roasting, and the other retained as the unroasted control. Roasting was conducted in a convection oven at 140℃ for 20 min. All samples were mechanically dehulled to obtain kernels for oil extraction.

Oil extraction

Oil was extracted using a screw-type press expeller (Model SX-TB02, Orin Oil Press, Fad International Development, New Taipei, Taiwan) operated without intentional heating of seeds. Each batch (~15 min) was pressed at ambient conditions, during which mechanical friction raised the temperature of the expeller chamber to 70–80℃. The resulting crude oil was centrifuged at 13,690× *g* for 5 min to remove suspended solids, and the clear upper layer was collected for further analysis. To evaluate extraction efficiency, two indices were calculated:

Pressed oil ratio (%): the weight of crude oil obtained immediately after pressing divided by the weight of input seed material.

Clarified oil yield (%): the weight of centrifuged clear oil divided by the seed input weight.

Both values were expressed as weight percentages (w/w).

Oil quality

The AV and POV of each oil sample were determined according to standard American Oil Chemists' Society (AOCS) methods: Cd 3d-63 for AV and Cd 8b-90 for POV.

Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared via base-catalyzed transesterification. Approximately 0.015 g of oil was dissolved in 1 mL of *n*-hexane containing 3.0 mg of methyl heptadecanoate (internal standard; Sigma-Aldrich, St. Louis, MO, USA), followed by the addition of 60 μL of 2 N KOH in methanol. The mixture was vortexed vigorously for 5 min. To terminate the reaction, 0.4 mL of saturated sodium chloride solution was added, and the sample was centrifuged at 12,000× *g* for 2 min. The upper *n*-hexane layer was filtered through a 0.45 μm polyvinylidene fluoride (PVDF) filter (Millex-HV, Millipore, Darmstadt, Germany), diluted 100-fold, and subjected to GC-MS analysis (Varian CP-3800 GC coupled with MS 4000, Palo Alto, CA, USA).

Chromatographic separation was performed using a CP8822 column (Vf-23ms, 30 m × 0.25 mm i.d., 0.25 μm film thickness, Varian, Palo Alto, CA, USA), with an injector temperature of 240℃. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1} and a split ratio of 100 : 1. The oven temperature was initially held at 100℃ for 1 min, ramped to 185℃ at 15℃ min^{-1}, and maintained at 185℃ for 8 min. FAMEs were identified and quantified using Supelco standard mixtures (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany).

Vitamin E content

Vitamin E homologs were analyzed by high-performance liquid chromatography (HPLC) using an Inertsil ODS-3 column (4.6 mm × 250 mm, 4 μm; GL Sciences, Tokyo, Japan). The system consisted of a Waters 2695 gradient pump and a Waters 474 fluorescence detector (Milford, MA, USA). Oil samples were filtered through a 0.45 μm PVDF filter prior to injection.

A 20 μL injection volume was used, with the mobile phase composed of methanol and acetonitrile (40 : 60, v/v) in isocratic mode at a flow rate of 1 mL min^{-1}. The column temperature was maintained at 30℃. Fluorescence detection was performed at 298 nm (excitation) and 328 nm (emission). Calibration standards for α-, β-, γ-, and δ-tocopherol and tocotrienol were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Phospholipid preparation and two-dimensional thin-layer chromatography (2D TLC) analysis

Five mL of crude oil extract were mixed with 100 μL of deionized water (2%, v/v), vortexed briefly, and shaken on a rotary shaker

(99 rpm, Intelli-Mixer RM-2L, ELMI, Riga, Latvia) at room temperature for 1 h. The mixture was centrifuged at 5,250× *g* for 15 min, and the upper oil layer was removed. The remaining white precipitate was collected as the degummed phospholipid fraction.

To further eliminate residual oil and moisture, the precipitate was centrifuged again at 5,250× *g* for 1 min, and the supernatant was discarded. This step was repeated once. The resulting pellet was dissolved in diethyl ether and dried using a freeze-dry concentrator. The dried sample was then re-dissolved in chloroform:methanol (2 : 1, v/v) for quantification and TLC analysis.

Phospholipid analysis was performed using 2D TLC, and phospholipid visualization was conducted using a modified molybdenum blue staining method, both following the protocol described in Hsieh (2017). TLC was performed using Silica Gel 60 F_{254} plates on aluminum sheets (Merck KGaA, Darmstadt, Germany).

In the first dimension, plates were developed using Solvent System I : chloroform : methanol : water (6.5 : 2.5 : 0.4, v/v/v). After drying, the plates were rotated 90 degrees and developed in the second dimension using Solvent System II: chloroform:methanol : acetic acid : water (8 : 1.2 : 1.5 : 0.4, v/v/v/v). Phospholipid standards were prepared by dissolving 10 mg of asolectin (from soybean, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) in 1 mL of chloroform : methanol (2 : 1, v/v). Dried sample pellets were re-dissolved in 25 μL of chloroform : methanol (2 : 1, v/v) and spotted onto TLC plates. Soybean asolectin was run separately as a reference standard for qualitative comparison of phospholipids.

Total phenolic content

The total phenolic content of oil samples was determined using a modified Folin-Ciocalteu method based on Singleton & Rossi (1965), as adapted by Hsieh *et al*. (2013). Briefly, oil was first diluted with isopropanol (1 : 1, v/v). A 15 μL aliquot of the mixture was added to 240 μL distilled water, 15 μL Folin-Ciocalteu reagent (Sigma-Aldrich, Buchs, Switzerland), and 30 μL of 35% aqueous sodium carbonate. The solution was vortexed thoroughly and centrifuged at 16,200× *g* for 2 min to remove undissolved oil. A 200 μL portion of the supernatant was transferred to a 96-well microplate, and absorbance was measured at 750 nm. All samples were analyzed in triplicate. Gallic acid (0–0.5 mg mL^{-1}) was used to construct the calibration curve.

Reducing power

The reducing power of oil samples was evaluated using a modified Oyaizu (1986) method, as described by Hsieh *et al*. (2013). Briefly, 100 μL of oil sample was mixed with 100 μL of 0.2 M sodium phosphate buffer (pH 6.6) and 100 μL of 1% potassium ferricyanide (prepared in buffer), then incubated at 50℃ for 20 min. The reaction was cooled on ice and terminated by adding 100 μL of 10% trichloroacetic acid. After centrifugation at 16,200× *g* for 2 min, the supernatant was collected and mixed with 100 μL distilled water and 20 μL of 0.1% ferric chloride solution. Absorbance was measured at 700 nm using a 96-well microplate reader. All samples were analyzed in triplicate. Trolox (0.08–0.8 mM), dissolved in methanol, served as the standard.

Cell culture and anti-inflammatory assay

The murine macrophage cell line RAW264.7 (BCRC, Hsinchu, Taiwan) was cultured in Dulbecco's modified eagle medium (DMEM; HyClone, Cytiva, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Cytiva, Logan, UT, USA) at 37℃ in a humidified incubator containing 5% CO_2. Sacha Inchi oil (0.4 mL) was mixed with 1 mL of absolute ethanol and centrifuged at 16,200× *g* for 20 min to obtain the ethanol-soluble fraction, herein referred to as the "29% extract." This designation is based on the volumetric proportion of oil in the total mixture (0.4 mL oil/1.4 mL total volume ≈ 29%) and reflects the treatment concentration applied in cell assays, rather than a chemical extraction yield. This extract was further diluted

with ethanol to a final concentration of 1,000 μL L^{-1} for cell treatment (the concentration was selected based on preliminary dose-response screening, data not shown), following the method of Lee *et al*. (2023). RAW264.7 cells were seeded into 48-well plates at a density of 8×10^4 cells per well and incubated for 24 h, then co-treated with lipopolysaccharide (LPS, 1 μg mL^{-1}; Sigma-Aldrich, St. Louis, MO, USA) and 1,000 μL L^{-1} of Sacha Inchi oil extract for an additional 24 h. Hydrocortisone (HC; 50 μM; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) served as a positive control. Cell-free supernatants were collected, and nitric oxide (NO) production was quantified using a modified Griess reagent (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). All treatments were performed in triplicate. The procedure was adapted from our previous protocol for fermented sweetpotato leaves (Lee *et al*. 2025), with modifications to focus exclusively on NO analysis. Statistical analyses were performed using the *t*-test function in Microsoft Excel. Data are expressed as mean ± *SD*. Significance was indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Oil stability

The oxidative stability of Sacha Inchi oil was evaluated using the OSI, which reflects the induction period (in min) before rapid oxidation occurs under accelerated thermal conditions. The analysis was conducted at a constant temperature of 130℃ using a Rancimat apparatus (Model 873 Biodiesel Rancimat, Metrohm AG, Herisau, Switzerland). For each measurement, exactly 3.00 g of oil was accurately weighed and subjected to continuous airflow at 10 L h^{-1}. The volatile oxidation products generated during heating were captured in 60 mL of distilled water, and the increase in electrical conductivity of this water was continuously recorded. The inflection point corresponding to the sharp rise in conductivity was defined as the OSI. This method allows for comparative evaluation of oxidative resistance in oil samples under uniform stress. The experimental design was based on the protocol of Hsieh *et al*. (2013), with modifications to a single testing temperature of 130℃ to simulate mild cooking conditions and to support shelf-life prediction for Sacha Inchi oil.

RESULTS AND DISCUSSION

Oil yield and sensory characteristics

Thermal pre-treatment (roasting) consistently enhanced oil extraction efficiency across ten *P. volubilis* L. (Sacha Inchi) seed samples, as evidenced by increased pressed oil ratios (Fig. 1). For all samples (No. 1–10), roasted seeds (Y) yielded a higher pressed oil ratio (%) compared to their unroasted counterparts (N), indicating that roasting facilitated oil release during cold pressing. The increase in pressed oil ratio ranged from 3% to over 25%, with the most pronounced effect observed in sample No. 5 (from 48.2% to 72.5%). Overall, 9 out of 10 samples showed improvement, with an average increase from 45.0% (unroasted) to 54.6% (roasted), representing a 21.3% relative gain.

In contrast, the clarified oil yield showed variable responses. Only 4 out of 10 samples (No. 1, 8, 9, and 10) demonstrated a net increase in clarified yield after roasting, while 6 samples (especially No. 4, 5, 6, and 7) exhibited a marked reduction. For instance, sample No. 5 had one of the largest increases in pressed oil ratio (from 50% to 73%) but suffered a significant drop in clarified yield (from 45% to 24%). These contrasting trends between pressed and clarified yields underscore the dual impact of roasting- enhancing oil release while potentially complicating post-extraction clarification.

To summarize the impact of roasting on oil yield, Table 1 presents the average values of the pressed oil ratio and clarified oil yield across all 10 samples. While roasting consistently improved the pressed oil ratio, the clarified oil yield exhibited a less uniform response. This divergence suggests that although roasting promotes oil liberation, it may also increase the presence of suspended solids, emulsifying

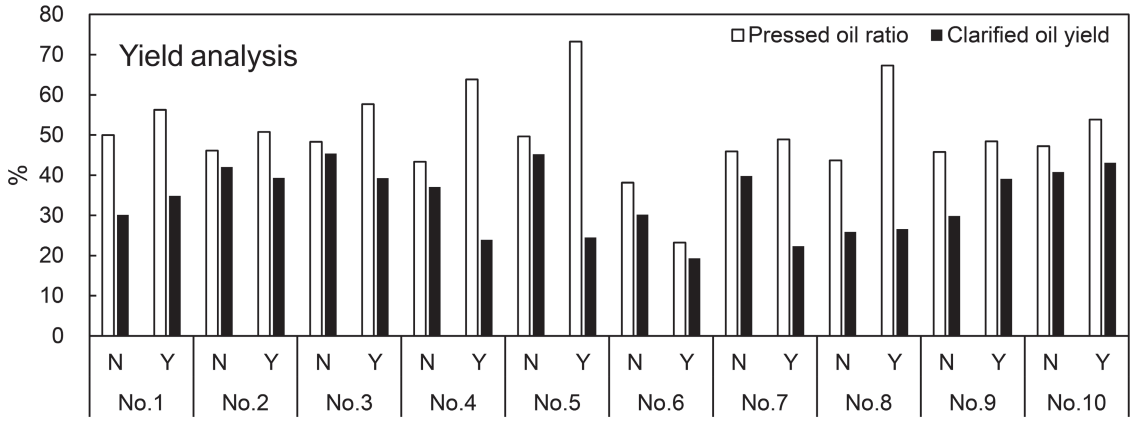


Fig. 1. Yield analysis of Sacha Inchi oil extracted from ten seed samples subjected to roasting pre-treatment (Y) or retained as unroasted controls (N). Pressed oil ratio (%) indicates the proportion of crude oil obtained by mechanical pressing relative to seed input. Clarified oil yield (%) refers to the percentage of centrifuged clear oil recovered from the pressed fraction. Roasting generally increased the pressed oil ratio across most samples, whereas its effect on clarified oil yield was more variable. Summary statistics are provided in Table 1.

Table 1. Average oil yield performance with and without roasting treatment[z].

Yield parameter	Unroasted (N, mean)	Roasted (Y, mean)	Mean difference	Observation
Pressed oil ratio (%)	45.0	54.6	9.6	Increased in 9 out of 10 samples
Clarified oil yield (%)	35.6	33.3	-2.3	Increased in 4 samples, decreased in 6

[z] Values represent mean percentages across 10 independent samples per treatment.

agents (such as phospholipids and denatured proteins), or thermally induced particulates that hinder efficient oil clarification by centrifugation. Similar observations have been reported in peanut systems, where roasting-induced emulsifying agents and heat-generated particulates interfered with oil separation efficiency (Suri *et al*. 2019; Zhang *et al*. 2023).

Interestingly, some samples, such as No. 9 and No. 10 showed improvements in both pressed and clarified yields post-roasting, suggesting that seed characteristics (e.g., initial moisture content or protein composition) may modulate the balance between oil release and sediment formation. These findings emphasize the need to optimize roasting parameters based on seed traits to balance extraction efficiency and oil clarity. Further compositional analysis of the sediment may clarify the mechanism.

In addition to yield performance, sensory evaluation revealed distinct differences in flavor profiles between unroasted and roasted oils. Unroasted Sacha Inchi oil was often described as having raw or grassy notes, which may be less appealing to consumers. In contrast, roasted oils exhibited pleasant peanut-like and nutty aromas, thereby improving flavor acceptability. These sensory enhancements further support the application of roasting as a value-adding step in cold-pressed oil production.

Oil quality: AV and POV

Oil quality was assessed using AV and POV, which represent hydrolytic and oxidative stability, respectively. AV ranged from 0.10 to 0.50 mg KOH g^{-1} in unroasted samples and from 0.10 to 0.70 mg KOH g^{-1} in roasted oils (Fig. 2A; Table 2). AV increased slightly in 7 out of 10 samples, with a maximum change of + 0.17 mg KOH g^{-1} (e.g., No. 1). Despite this increase, all values remained well below the Codex Alimentarius threshold of 2.0 mg

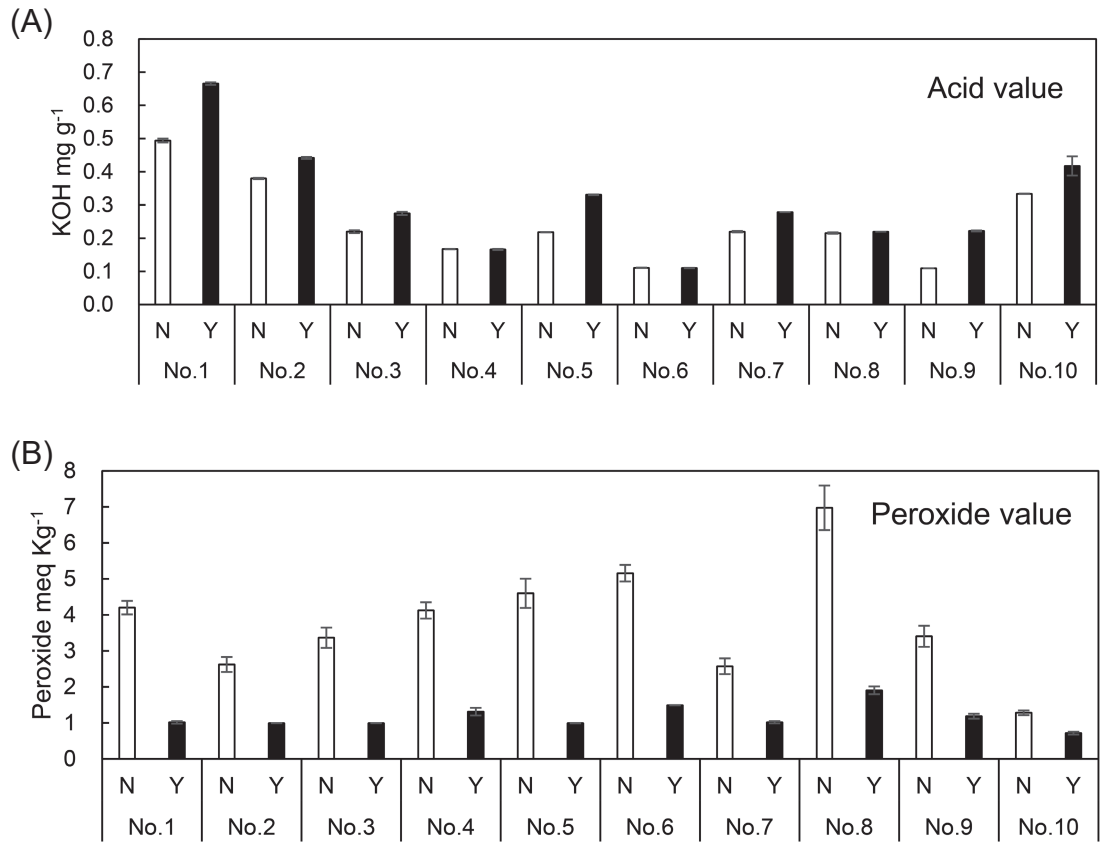


Fig. 2. Oil quality parameters of Sacha Inchi oil. (A) Acid value (AV) and (B) peroxide value (POV) of oils extracted from ten seed samples processed under unroasted (N) and roasted (Y) conditions. AV values showed slight increases after roasting but remained well within acceptable limits for edible oils. In contrast, POV values decreased markedly in all roasted samples, indicating enhanced oxidative stability associated with roasting (summary statistics provided in Table 2). Values represent mean ± *SD* of duplicate and tetraplicate measurements for AV and POV, respectively.

Table 2. AV[z] and POV[y] summary.

Quality parameter	Unroasted (N)	Roasted (Y)	Change trend
AV (mean[x], mg KOH g^{-1})	0.29	0.37	Increased in 7 samples, decreased in 3
POV (mean, meq O_2 kg^{-1})	3.65	1.16	Decreased in all samples

[z] Acid value.
[y] Peroxide value.
[x] Values represent mean measurements across 10 independent samples per treatment.

KOH g^{-1} for Sacha Inchi oil, as endorsed by the Codex Committee on Fats and Oils and submitted for final adoption at CAC47, thereby confirming that moderate roasting did not induce significant hydrolysis.

In contrast, POV showed a consistent reduction across all roasted samples (Fig. 2B). Unroasted oils exhibited POVs ranging from 1.28 to 6.98 meq O_2 kg^{-1}, while roasted counterparts ranged from 0.71 to 1.90 meq O_2 kg^{-1}. The average POV decreased from 3.65 to 1.16 meq O_2 kg^{-1}, representing a 68% reduction. This is well below the Codex recommended limit of 15 meq O_2 kg^{-1} for virgin Sacha Inchi oil, suggesting

that roasting not only avoided oxidation but actively reduced pre-existing peroxides, thereby improving oil stability.

These results indicate that controlled moderate roasting does not compromise oil quality and may enhance oxidative resilience. This finding challenges the common perception that PUFA-rich oils must be strictly cold-pressed to avoid degradation. Similar improvements have been reported in other oilseeds, such as camellia oil, where moderate roasting improved oxidative parameters without compromising lipid integrity (Hsieh *et al*. 2013).

Unaltered nutrients under roasting: Vitamin E and fatty acids

The nutritional stability of Sacha Inchi oil during roasting was evaluated by analyzing vitamin E homologs and fatty acid composition. The vitamin E profile was dominated by γ-tocopherol, followed by δ-tocopherol and α-tocotrienol (Fig. 3A). Mean concentrations of γ-T, δ-T, and α-T_3 were comparable between unroasted (779, 775, and 85 mg kg^{-1}) and roasted oils (784, 773, and 84 mg kg^{-1}), respectively. Minor fluctuations between samples- such as a slight decrease in δ-T in sample No. 3 and a slight increase in α-T_3 in No.10- fell within

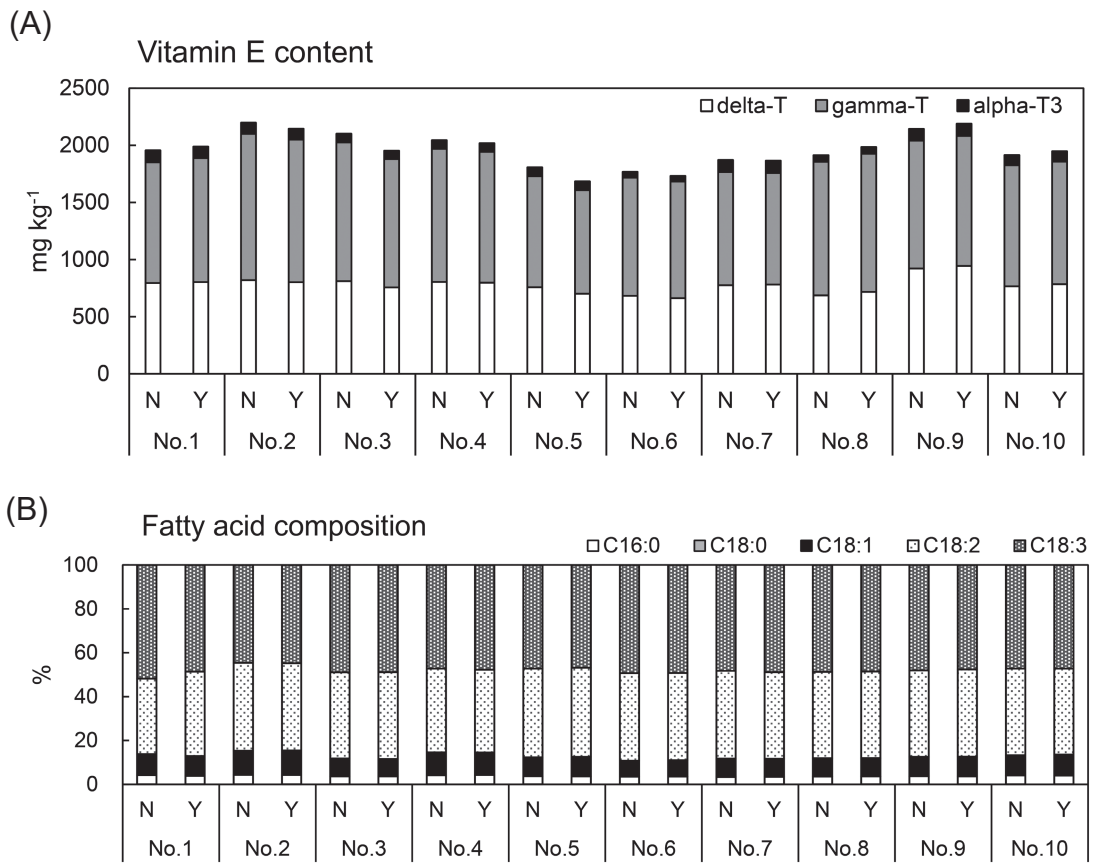


Fig. 3. Composition of functional components in Sacha Inchi oil under unroasted (N) and roasted (Y) conditions. (A) Vitamin E homologs, including δ-tocopherol (δ-T), γ-tocopherol (γ-T), and α-tocotrienol (α-T_3). (B) Fatty acid profile, including palmitic acid (C16 : 0), stearic acid (C18 : 0), oleic acid (C18 : 1), linoleic acid (C18 : 2), and α-linolenic acid (C18 : 3). No significant differences were observed between treatments, indicating compositional stability of both lipid and antioxidant components following roasting. Values are represented with duplicate measurements.

normal biological variability, and no systematic trend was observed.

Similarly, the fatty acid composition remained largely unaffected by roasting (Fig. 4B). The major components were α-linolenic acid (C18 : 3, 46–52%), linoleic acid (C18 : 2, 34–40%), and oleic acid (C18 : 1, 7–11%). Saturated fatty acids, mainly palmitic acid (C16 : 0), ranged from 3.4% to 4.3%, with negligible levels of stearic acid (C18 : 0 < 0.1%). The consistently high PUFA content (> 85%) in both treatments reinforces the nutritional value of Sacha Inchi oil as a rich source of ω-3 fatty acids, even after thermal exposure.

These results indicate that moderate roasting does not degrade vitamin E homologs or alter the oil's core lipid profile. This stability contrasts with the commonly assumed heat sensitivity of polyunsaturated oils. The findings support the feasibility of incorporating controlled roasting in Sacha Inchi oil production, balancing processing efficiency with nutritional preservation, as similarly observed in heat-treated oilseeds (Hsieh *et al*. 2013).

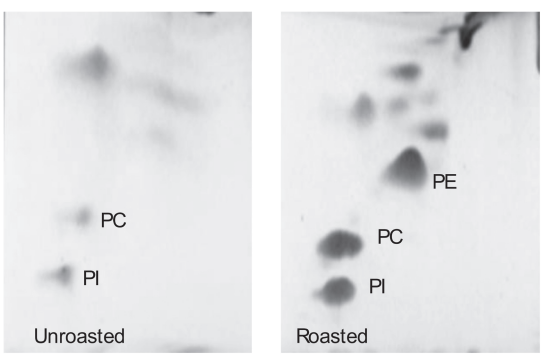


Fig. 4. Thin-layer chromatographic (TLC) analysis of phospholipid composition in Sacha Inchi oil under unroasted and roasted conditions. Representative TLC plates from a Taichung-sourced sample illustrate the separation of three major phospholipids: phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). Roasting visibly enhanced the diversity and intensity of phospholipid bands, particularly the emergence of PE. Representative TLC image shown (Taichung sample); all other batches exhibited consistent spot patterns with minor differences in intensity or background.

Roasting-induced functional enhancement

1. Enriched bioactive compounds: Phospholipids and phenolics

Roasting-induced changes in the bioactive composition of Sacha Inchi oil were examined, with a focus on phospholipids and phenolic compounds. Phospholipid profiles were assessed using TLC, based on a representative sample sourced from Taichung, Taiwan. This sample was selected for its consistent compositional traits across batches and serves as a visual example to illustrate the impact of roasting on lipid diversity (Fig. 4). Three major phospholipids were identified: phosphatidylinositol (PI), phosphatidylcholine (PC), and phos-phatidylethanolamine (PE).

In the unroasted oil, only PI and PC bands were clearly visible, while PE was faint or undetectable. In contrast, roasted oil exhibited all three phospholipids with distinct separation and visibly stronger band intensity, particularly for PE. This qualitative shift suggests that roasting promotes the release of membrane-associated phospholipids, resulting in a more diverse lipid profile.

The emergence of PE in roasted oil is especially noteworthy given its potential antioxidant activity. Although native PE exhibits limited radical-scavenging activity, recent studies have shown that thermally generated PE-derived Maillard reaction products (MRPs) can significantly enhance lipid oxidative stability (Shrestha & De Meulenaer 2014; Li *et al*. 2024). These findings suggest that roasting may not only release PE but also promote the formation of functional derivatives that contribute to the improved oxidative stability of roasted Sacha Inchi oil. While TLC does not provide precise quantification, the visual enhancement of phospholipid bands supports the conclusion that roasting modifies lipid composition in a functionally meaningful way.

In addition to changes in phospholipids, roasting significantly increased the total phenolic content of Sacha Inchi oil. Across ten seed samples, unroasted oils exhibited phenolic concentrations

ranging from 0.023 to 0.046 mg gallic acid equivalents (GAE) mL^{-1}, whereas roasted oils ranged from 0.055 to 0.124 mg GAE mL^{-1} (Fig. 5A). The most pronounced increase was observed in sample No. 8, where roasting elevated phenolic content by 170%. On average, phenolic levels increased from 0.035 ± 0.007 mg GAE mL^{-1} (unroasted) to 0.082 ± 0.018 mg GAE mL^{-1} (roasted), representing a 2.3-fold enhancement.

This consistent upward trend suggests that moderate roasting facilitates the release or transformation of bound phenolic compounds,

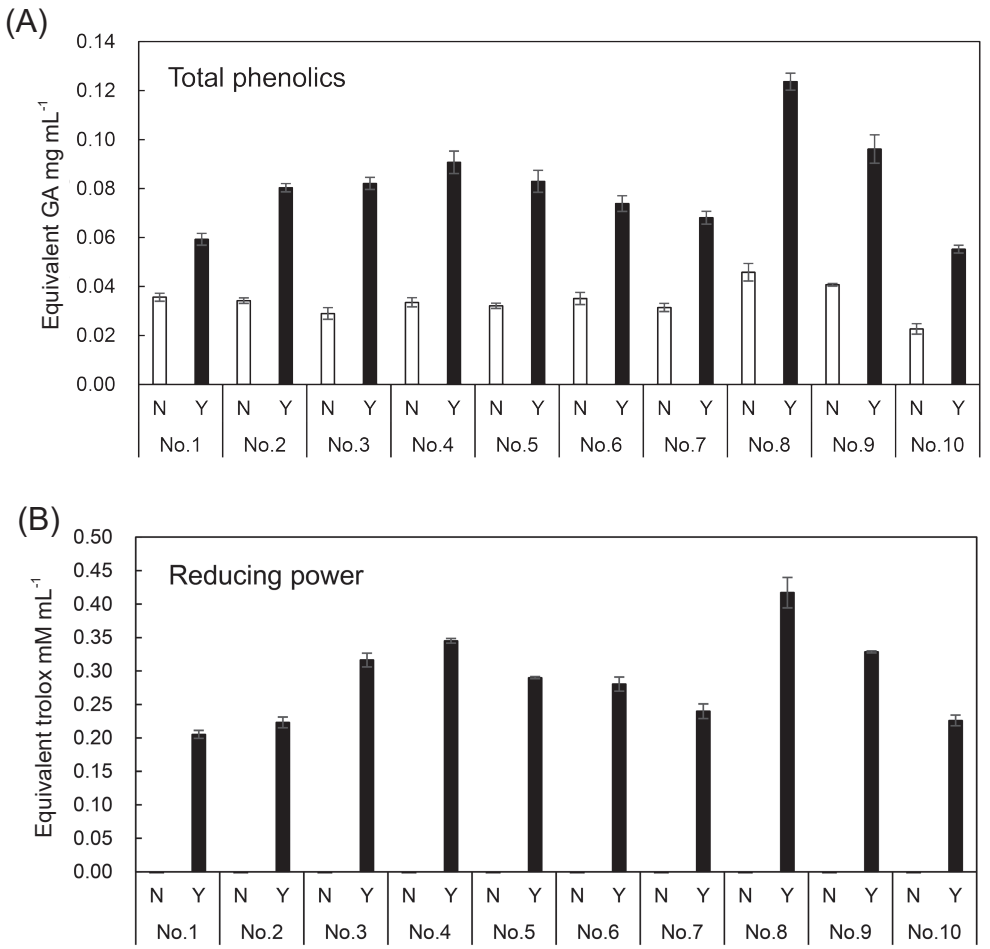
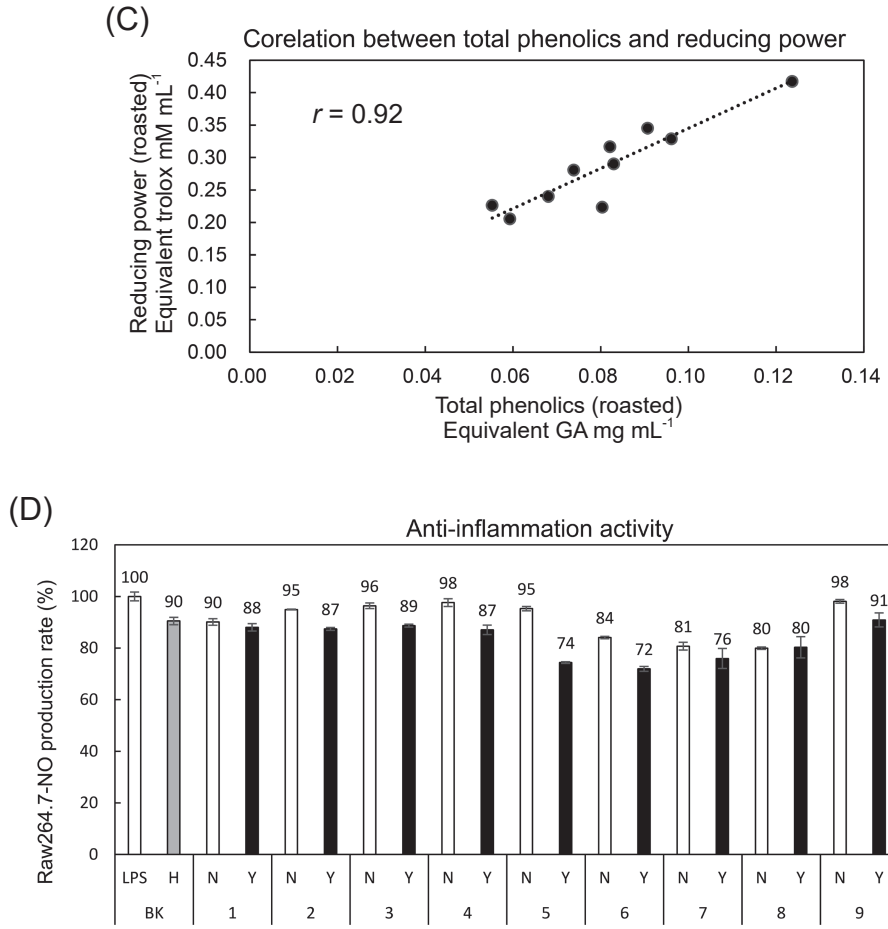


Fig. 5. Functional compound enrichment and bioactivity enhancement in roasted Sacha Inchi oil. (A) Total phenolic content of Sacha Inchi oil under unroasted and roasted conditions. Roasting consistently increased phenolic levels across all ten seed samples, with values rising from near-baseline to over 0.12 mg gallic acid equivalents (GAE) mL^{-1} in representative cases. (B) The reducing power of Sacha Inchi oil under unroasted and roasted conditions. Roasted samples exhibited measurable antioxidant activity, while unroasted oils remained near or below baseline, indicating negligible electron-donating capacity prior to thermal treatment. (C) Correlation between total phenolic content and reducing power in roasted samples. A strong positive relationship (r = 0.92) was observed, suggesting phenolic enrichment contributes directly to antioxidant functionality. (D) Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages following oil treatment. Both roasted and unroasted oils reduced NO levels relative to the LPS-only control (set at 100%), with several roasted samples (e.g., No. 5–7) exhibiting stronger inhibition than the positive control hydrocortisone (H). Statistical comparisons between unroasted and roasted treatments for each sample are summarized in Table 3. *P*-values were calculated by paired *t*-test comparing roasted (Y) and unroasted (N) treatments for each sample (n = 3). Values represent mean ± *SD* of triplicate measurements. BK: blank control.



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possibly through cell wall disruption or the formation of Maillard reaction intermediates. Recent studies have shown that Maillard-derived α-dicarbonyl compounds and Amadori rearrangement products can enhance phenolic solubility and reactivity by forming conjugates or adducts with flavonoids and hydroxycinnamic acids, thereby increasing their extractability and antioxidant potential (Han *et al*. 2024).

2. Enhanced functional activities: Antioxidant capacity and NO inhibition

To further evaluate the functional impact of roasting on Sacha Inchi oil, antioxidant and anti-

inflammatory activities were assessed through reducing power assays and NO inhibition tests. The results are summarized in Figs. 5A–D, which illustrate the enrichment of phenolic compounds, the emergence of reducing power, the correlation between these two parameters, and the suppression of NO production in macrophage cells.

Antioxidant capacity was evaluated using reducing power assays across 10 seed samples, expressed as trolox-equivalent concentrations (Equi. trolox mM mL^{-1}). As shown in Fig. 5B, unroasted oils exhibited values near or below baseline (-0.065 mM mL^{-1} to -0.009 mM mL^{-1}), indicating negligible electron-donating activity and the absence of measurable reducing capability. In contrast, roasted oils demonstrated distinctly positive values (0.205 mM mL^{-1} to 0.417 mM mL^{-1}), reflecting the activation of antioxidant functionality. Sample No. 8 showed the strongest response, transitioning from baseline to 0.417 mM. Overall, roasted samples consistently exhibited measurable reducing power, confirming that thermal treatment initiates antioxidant activity otherwise absent in native oil.

A correlation analysis between total phenolic content and reducing power in roasted samples revealed a strong positive relationship (r = 0.92), as shown in Fig. 5C. This suggests that phenolic enrichment is a key contributor to the antioxidant functionality observed after roasting. These findings support the hypothesis that moderate roasting enhances antioxidant capacity primarily through phenolic activation. Maillard reaction intermediates have also been reported to improve phenolic solubility and reactivity, further contributing to antioxidant enhancement (Han *et al*. 2024).

The anti-inflammatory potential of Sacha Inchi oil was evaluated by measuring NO production in LPS-stimulated RAW264.7 macrophages following oil treatment. As shown in Fig. 5D, all oil samples- both roasted and unroasted- exhibited inhibitory effects compared to the LPS-only control (set at 100%). Hydrocortisone (H), used as a positive control, reduced NO levels to 90.5%. Most oil-treated groups showed comparable or stronger inhibition than HC. Notably, roasted samples No. 5, 6, and 7 reduced NO production to 74.4%, 71.9%, and 75.9%, respectively- substantially lower than both HC and their unroasted counterparts.

Statistical significance was assessed using two approaches: one-sample t-tests comparing each treatment group to the LPS control, and paired t-tests comparing roasted (Y) versus unroasted (N) extracts within each sample. Significant reductions in NO production (P < 0.05) were observed in most roasted samples, particularly No. 2, 3, 5, 6, 7, 8, and 9 (Table 3). In addition, paired comparisons revealed that roasting significantly enhanced NO inhibition in 9 out of 10 samples, with P-values ranging from 0.0490 to < 0.0001. These results confirm that roasting consistently improves the anti-inflammatory activity of Sacha Inchi oil.

The enhanced inhibition may reflect thermally induced release or transformation of bioactive compounds such as phenolics and phospholipids (e.g., PE), which have been associated with immunomodulatory effects (van Dieren *et al*. 2011). Although sample No. 8 exhibited the highest phenolic content and strongest reducing power, its NO inhibition was only moderate, suggesting that the anti-inflammatory effects of Sacha Inchi oil may involve additional pathways beyond antioxidant mechanisms. Overall, samples with stronger NO inhibition tended to show elevated phenolic content and reducing power, supporting the functional relevance of roasting in enhancing both antioxidant and anti-inflammatory properties of Sacha Inchi oil.

3. Improved oxidative stability: Rancimat resistance and nutrient synergy

To simulate mild cooking scenarios and evaluate the thermal oxidative stability of Sacha Inchi oil, the OSI was measured using the Rancimat method at 130℃ (Fig. 6). OSI represents the induction period length and reflects the oil's resistance to oxidative degradation under accelerated heating conditions. This temperature

Table 3. Effects of roasted and unroasted Sacha Inchi oil extracts on nitric oxide (NO) production in LPS-stimulated RAW264.7 cells and statistical significance.

Sample No.	Treatment[z]	NO production (% of LPS)	*P*-value[y] vs. LPS (significance)	Mean difference N–Y	*P*-value N vs Y (significance)
BK[x]	LPS	100.0 ± 1.7	1.0000 (-)	-	-
	H	90.5 ± 1.4	0.0020 (**)	-	-
No. 1	N	90.1 ± 1.3	0.0017 (**)	0.0215	0.0490 (*)
	Y	88.0 ± 1.4	0.0008 (***)		
No. 2	N	94.9 ± 0.2	0.0344 (*)	0.0752	0.0010 (**)
	Y	87.4 ± 0.6	0.0030 (**)		
No. 3	N	96.4 ± 1.1	0.0445 (*)	0.0770	0.0000 (***)
	Y	88.7 ± 0.6	0.0039 (**)		
No. 4	N	97.6 ± 1.5	0.1469 (NS)	0.1059	0.0000 (***)
	Y	87.0 ± 1.8	0.0009 (***)		
No. 5	N	95.3 ± 0.8	0.0244 (*)	0.2085	0.0000 (***)
	Y	74.4 ± 0.3	0.0010 (***)		
No. 6	N	84.1 ± 0.4	0.0023 (**)	0.1213	0.0000 (***)
	Y	71.9 ± 0.9	0.0001 (***)		
No. 7	N	80.7 ± 1.5	0.0001 (***)	0.0478	0.0010 (**)
	Y	75.9 ± 3.9	0.0032 (**)		
No. 8	N	79.9 ± 0.4	0.0014 (**)	-0.0036	0.7720 (NS)
	Y	80.3 ± 4.1	0.0071 (**)		
No. 9	N	98.1 ± 0.7	0.1837 (NS)	0.0721	0.0000 (***)
	Y	90.9 ± 2.7	0.0124 (*)		
No. 10	N	99.8 ± 1.8	0.9068 (NS)	0.1414	0.0000 (***)
	Y	85.7 ± 1.2	0.0005 (***)		

[z] LPS: lipopolysaccharide-stimulated control group (set at 100% NO production); H: hydrocortisone-treated positive control; N: oil extract from unroasted Sacha Inchi seeds; Y: oil extract from roasted Sacha Inchi seeds.

[y] *P*-values (N vs Y) were calculated by paired *t*-test comparing roasted (Y) and unroasted (N) treatments for each sample ($n = 3$). Statistical analyses were performed with the *t*-test function in Microsoft Excel. Data are expressed as mean ± *SD*. Significance are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS: not significant.

[x] BK: blank control.

setting was chosen to mimic practical culinary applications such as light sautéing or warm salad preparation, offering real-world relevance.

The results showed that roasted samples consistently exhibited longer induction periods than their unroasted counterparts, demonstrating stable and reproducible oxidative resistance. OSI values for unroasted oils clustered between 8 min and 11 min, indicating the inherent susceptibility of polyunsaturated oils to oxidation in their native state. In contrast, roasted oils showed extended OSI values ranging from 21 to 53 min, confirming that moderate roasting significantly enhances heat stability.

Among all samples, No. 10 exhibited the most pronounced improvement, with OSI increasing from 11 to 53 min- a nearly 5-fold enhancement. Other notable increases were observed in sample No. 6 (from 10 to 36 min), No. 2 (from 10 to 35 min), and No. 8 (from 10 to 43 min). On average, unroasted oils had an OSI of approximately 10 min, while roasted oils reached an average of 33 min, indicating a consistent and substantial improvement in thermal oxidative stability following roasting.

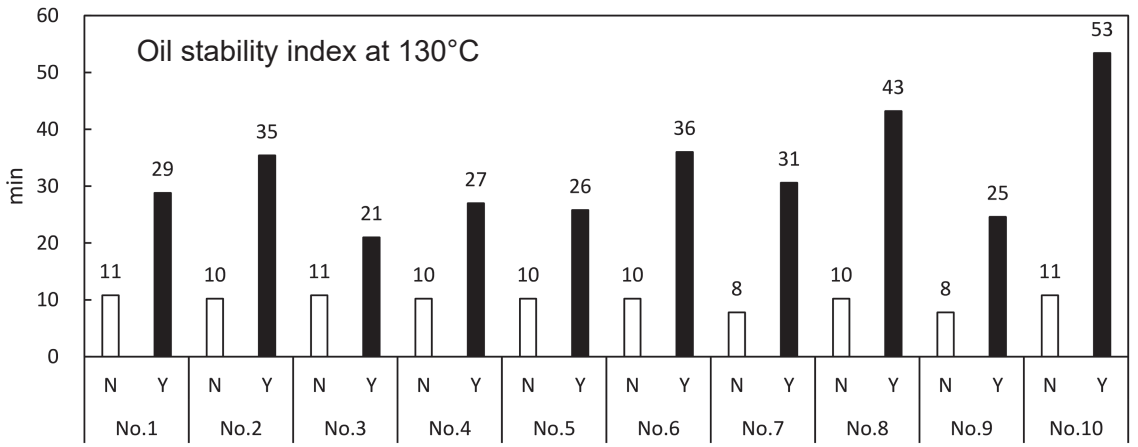

Fig. 6. Oxidative stability of Sacha Inchi oil under unroasted and roasted conditions. Oil stability index (OSI) values were measured using the Rancimat method at 130℃ to simulate light cooking conditions. Roasted samples consistently exhibited longer induction times than their unroasted counterparts, indicating improved thermal oxidative resistance. Sample No. 10 showed the most pronounced enhancement, increasing from 11 to 53 min. The average OSI increased from 10 min (unroasted) to approximately 33 min (roasted), supporting the functional benefit of controlled roasting.

This enhancement may result from multiple mechanisms, including enzyme inactivation (e.g., lipoxygenase; Zheng *et al*. 2023), moisture reduction, and the release or activation of endogenous antioxidants such as phenolics and phospholipids. Thermal stabilization has been shown to effectively suppress lipoxygenase activity and promote antioxidant release in lipid-rich matrices, thereby improving oxidative resilience. These mechanisms not only stabilize the oil matrix but also correspond with the previously observed improvements in reducing power (Hsieh *et al*. 2013, 2014). Furthermore, the earlier-documented decrease in POV reinforces this conclusion, confirming that moderate thermal treatment can effectively enhance the oxidative stability of Sacha Inchi oil.

Taken together, controlled roasting emerges as a practical strategy to enhance oil quality and extend shelf life in both food and nutraceutical applications. Beyond oxidative stability, roasting may also promote nutrient synergy by increasing the bioavailability and interaction of endogenous antioxidants such as phenolics and phospholipids, thereby strengthening the oil's functional performance. These compounds not only stabilize the lipid matrix but also help mitigate inflammatory responses, supporting the health-promoting potential of Sacha Inchi oil. The observed improvement in heat stability at 130℃ further suggests that roasted oil is suitable for mild cooking applications, such as sautéing or warm salad preparation, offering practical value while preserving bioactivity.

Integrated interpretation of roasting effects

The roasting temperature of 140℃ for 20 min was selected based on prior studies in sesame (*Sesamum indicum*), which demonstrated optimal flavor and functional compound retention under similar conditions (Huang *et al*. 2017). Preliminary trials in Sacha Inchi further confirmed that this condition preserved fatty acid integrity while enhancing phenolic content and oxidative stability. Accordingly, this thermal pre-treatment was adopted as the controlled roasting condition in this study.

Roasting under defined thermal conditions (140℃, 20 min) induced changes in the chemical composition, oxidative stability, and biofunctional properties of Sacha Inchi oil. These effects extended beyond isolated improvements, reflect-

ing a systemic enhancement of oil quality driven by physical and biochemical transformations.

From a chemical standpoint, roasting had minimal impact on AV, which remained within Codex Alimentarius specifications (AV < 2), indicating no significant hydrolytic degradation. In contrast, POV decreased substantially, suggesting that roasting either eliminated pre-existing peroxides or delayed further oxidation. The OSI at 130℃ increased by 2–5-fold in most samples, clearly demonstrating enhanced resistance to thermal oxidation.

Functionally, roasting preserved the fatty acid composition and vitamin E profile, maintaining essential lipid nutrition. Meanwhile, it significantly elevated phospholipids, total phenolic content, reducing power, and anti-inflammatory activity- indicating a functional upgrade likely attributable to heat-induced release of bound antioxidants or the formation of novel antioxidative compounds, such as Maillard-derived phenolics. Phospholipid profiling revealed the emergence of PE, a compound absent in unroasted oil, which may contribute to emulsion stability and oxidative defense.

Taken together, these findings suggest that controlled roasting induces favorable chemical and structural changes- enhancing oil extractability, increasing antioxidant potential, and improving oxidative stability- without compromising the core nutritional profile. Roasting thus offers a practical, value-adding step in Sacha Inchi oil production, particularly for food and nutraceutical applications requiring both stability and bioactivity.

Nevertheless, this study was limited by the use of a single roasting condition (140℃, 20 min) and a laboratory scale capacity (each batch used approximately 300 g of seeds, which is sufficient for chemical and functional analyses). Future studies should expand the range of roasting parameters to evaluate their effects on oil quality, biofunctional properties, and long term storage stability. Moreover, large scale production may introduce additional variability due to differences in expeller models, friction induced heating, and batch volumes; therefore, processing conditions across different expeller types should be examined to enhance generalizability and industrial relevance.

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適度烘焙處理提升印加果 (*Plukenetia volubilis* L.) 油生理活性與穩定性並不改變其脂肪酸組成

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摘要

李雅琳、洪子桓、劉威廷、林素月、何奕漢、李穎甫。2026。適度烘焙處理提升印加果 (*Plukenetia volubilis* L.) 油生理活性與穩定性並不改變其脂肪酸組成。台灣農業研究 75(2):333–349。

印加果 (*Plukenetia volubilis* L.) 油富含 ω-3 脂肪酸與維生素 E，具備潛在健康效益，近年來備受全球關注。然而，其氧化穩定性低，成為商業應用上的主要限制。本研究採用中度烘焙熱前處理，比較烘焙與未烘焙種子的榨出油脂，對於印加果油化學組成、氧化穩定性及功能活性的影響。本研究共收集臺灣 6 個地區 (臺中、彰化、南投、嘉義、臺南、花蓮) 之 10 件種子樣品，經去殼後以不加熱的物理壓榨法萃取油品。分析項目包括酸價 (acid value; AV)、過氧化價 (peroxide value; POV)、油脂氧化安定性指數 (oil stability index; OSI)，於 130℃測定)、維生素 E 含量、脂肪酸組成、磷脂組成 (以薄層層析法分析)、總酚含量、還原力，以及以細胞模式評估之抗發炎活性。結果顯示，烘焙處理未改變脂肪酸與維生素 E 組成，但顯著提升氧化安定性 (OSI 上升)，並促進磷脂 (如磷胺酯) 與總酚等生物活性成分增加並伴隨還原力增強。烘焙與未烘焙油品皆具備抗發炎活性，其中烘焙油在抑制脂多醣 (lipopolysaccharide; LPS) 刺激巨噬細胞產生一氧化氮 (nitric oxide; NO) 的反應表現略優。綜合而言，適度烘焙處理可以有效提升印加果油之氧化安定性、生物活性組成及生理功能性，同時保留其核心營養價值 (ω-3 脂肪酸與維生素 E)。本研究結果支持烘焙作為提升印加果油品質之可行加工策略，拓展其在食品與營養保健領域之應用潛力。

關鍵詞：印加果油、烘焙、氧化安定性、功能性生物活性、抗發炎活性。

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