

Comparative Study of Thermal Pre-treatment on the Extraction, Antioxidant, Fatty Acid Profile, and Physicochemical Properties of Inca Inchi Seed Oil

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ABSTRACT

Inca Inchi oil, an edible oil with high amounts of polyunsaturated fatty acids such as omega 3 and omega 6 fatty acids, has a wide range of applications in therapeutic, food, and pharmaceutical industries. Increasing its oil yield during oil extraction is important due to its high value. However, conventional techniques such as screw press extraction pose a limitation in terms of oil yield. Thus, in this study, the seeds were pre-treated in a microwave and hot air oven prior to oil extraction. The effects of this pre-treatment on the oil yield, fatty acid profile, antioxidant profile, and physicochemical properties were compared. Microwave treatment (4 min) was found to have the highest oil yield (43.39%) compared to control (37.76%). The proximate analysis revealed that the protein content in the oil meal was high (51–60%) compared to oil seed (24.2%), indicating that it has potential application to be developed into plant-based protein foods. The fatty acid profile indicates that the oil had high omega 3 (49%) and omega 6 (37%) fatty acids. The free fatty acids and peroxide values of the pre-treated oil samples were less than 1% and 10 meq O₂/kg oil, respectively, compared to the control (1%), while the iodine value was high due to double bonds. The 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid study shows that the oil has good radical scavenging activity (70 and 90%), which shows the oil's potential in functional food applications.

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INTRODUCTION

Inca Inchi (*Plukenetia volubilis* Linneo), an oleaginous, perennial plant from the Euphorbiaceae family, is found naturally in the tropical rainforest of the Amazon region in South America. They are most abundant in Peru and grow at an altitude level below 900 m (Gillespie, 1993). The plants have been cultivated for centuries by the Peruvians (Guillén et al., 2003). The seed is commonly known as “forest peanut”, or “sacha inchi” (Bussmann et al., 2009). The indigenous plant is also cultivated in Southeast Asia due to its high economic value (Medina-Mendoza et al., 2021).

Inca Inchi seeds are made up of seed layers (33–35%), which include the husk, shell, and oleaginous seed kernel (65–67%), which is commercially turned into oil (Chirinos et al., 2016). The oil content in Inca Inchi seeds is high (35–60%) and comparable to sunflower seeds (48%) and peanuts (45%) (Gutiérrez et al., 2011; Hamaker et al., 1992). Commercially, the oil is recognized for its health attributes and distinct sensory characteristics (taste and flavor) (Garmendia et al., 2011).

The major fraction of the oils is made up of polyunsaturated fatty acids (PUFAs) (77.5–84.4%). The remaining lipid fraction is composed of monounsaturated fatty acids (MUFAs) (8.4–13%) and saturated fatty acids (SFAs) (6.8–9.1%) (Chirinos et al., 2013; Follegatti-Romero et al., 2009; Gutiérrez et al., 2011; Kodahl et al., 2022; Maurer et al., 2012). A few vegetable oils with an equivalent high percentage of PUFA are linseed (*Linum usitatissimum* L.) (74%)

and chia (*Salvia hispanica* L.) (80%) (Ciftci et al., 2012). The PUFAs are composed of two essential fatty acids, α -linolenic acid, an omega 3 (ω -3) (35–50%) and linoleic acid, an omega 6 (ω -6) (33–41%), respectively (Chirinos et al., 2013; Cisneros et al., 2014). These essential fatty acids are required for biological processes. However, they cannot be synthesized in the human body due to the lack of Δ -12 and Δ -15 desaturases and hence must be obtained through diet (Sinclair et al., 2002).

The primary mechanism by which α -linolenic acid is transformed to eicosapentaenoic acid (EPA) and ultimately docosahexaenoic acid (DHA) in the human body is by β -oxidation, which uses the same enzyme cascade that transforms linoleic acid to docosapentaenoic acid (DPA) (Glick & Fischer, 2013). The composition of the fatty acids varies depending on the factors such as climate, time of harvest, quality of seed, and storage condition (Torres Sánchez et al., 2021). The PUFAs aid in managing the cardiometabolic syndrome, specifically the prevention of coronary heart disease, hypertension, and exhibits hypocholesterolemic effect when used as a dietary supplement (Follegatti-Romero et al., 2009). Furthermore, topical products containing Inca Inchi oil have promising anti-inflammatory, anti-aging, antibacterial, and moisturizing properties (Gonzalez-Aspajo et al., 2015). Additionally, phytosterols, phenolic compounds, tocopherols, and carotenoids also improve health (Lagarda et al., 2006; Moreau et al., 2002). With the growing recognition of Inca Inchi

in international markets in recent years, dietary products such as gourmet oil, protein powder, and encapsulated oil are available. Other seeds, such as roasted, salted, or candied, are also available (Kodahl & Sørensen, 2021).

The oil from the Inca Inchi seeds is generally obtained through various methods such as solvent extraction, supercritical carbon dioxide (CO₂) extraction, and screw press extraction (Follegatti-Romero et al., 2009; Sayyar et al., 2009). Among these, screw press extraction is preferred due to the absence of solvent in the oil product, simple equipment, convenience of use, inexpensive investment, and low running costs (Siregar et al., 2016). The screw-pressing method is gaining popularity as an alternative to solvent extraction.

Additionally, screw pressing also helps retain the beneficial components, such as antioxidants, hence improving the quality of the oil (Lutterodt et al., 2011; Maier et al., 2009). In addition to the oil extraction process, pre-treatment is important in producing high oil yield with great reproducibility (Mwaurah et al., 2020). The applied pre-treatment will ease and speed up the process of releasing oil as the chemical bonds of the seed have been broken down (Mwaurah, 2020). It is important to ensure that the cellular structure of the plant seed has been loosened up before the extraction process.

This study aimed to extract the Inca Inchi oil using thermal pre-treatment methods such as microwaves and hot air ovens. Although oil extraction has been

done using thermal pre-treatment methods on various oil seeds, very limited work has been done on Inca Inchi oil and its effect on the properties of the oil. Hence, in this work, the oil seeds were thermally pre-treated using a microwave with different times of exposure (1, 2, 4, and 6 min) and a hot air oven with three different temperatures (60, 80, and 120°C) to evaluate the effects on oil yield, fatty acid profile, antioxidant activity, and physicochemical properties. Subsequently, the results from the study will be used to produce various geriatric functional foods.

MATERIALS AND METHODS

Materials

Inca Inchi seeds were purchased from Myanmar (Wusang Group Sdn. Bhd., Malaysia). The chemicals and solvents used in the study were high-performance liquid chromatography (HPLC) or analytical grades and purchased from Fisher Scientific Sdn. Bhd. (Malaysia). Standards such as gallic acid and quercetin were purchased from Sigma-Aldrich (USA). Folins-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) salt were purchased from Merck (Germany).

Inca Inchi Seeds Preparation

Inca Inchi seeds from the supplier were cleaned to remove the foreign particles. Then, the seeds were dried in an oven (Binder, Germany) at 50–60°C for a day.

After drying, the seeds were cooled and packaged in re-sealable storage bags and stored in a dehumidifier room until further processing.

Pre-treatment Techniques

Pre-treatment of oilseeds is essential in increasing the quality and yield of oil. The major pre-treatment step goes to preheating to reduce the moisture content and weaken the cell structure of the oilseed. Applying heat prior to extraction enables the oil to flow more easily. Besides this, crushing plays an important role by destroying the cell wall of the oil-bearing material, which further aids the process of releasing the oil (Saurabh et al., 2011). Conventionally, hot air oven is commonly applied as pre-treatment before being replaced with modern techniques such as microwave radiation, ultrasonication, supercritical fluid, and enzyme treatment due to their excellent benefits (Danlami et al., 2014).

In this study, the seeds were pre-treated using a microwave and hot air oven prior to oil extraction to improve the oil yield. A control was performed without any pre-treatment.

Microwave Pre-treatment. The seeds were placed in the Pyrex Petri dish and inside the oven (Panasonic 800 W model, Japan). The sample rotates inside the oven during the process. This configuration allows the samples to pass through the electromagnetic field pattern, allowing unified energy absorption within the seeds. The samples were pre-treated for 1, 2, 4, and 6 min. The

selection of time was based on a preliminary study in which the exposure to microwave pre-treatment beyond 6 min had burnt the Inca Inchi seeds (Azadmard-Damirchi et al., 2010). After pre-treatment, the seeds were loaded into an oil expeller for extraction.

Hot Air Oven Pre-treatment. Thermal treatment using hot air ovens inactivates antinutrients and improves the antioxidant activity of seed samples (Bueno-Borges et al., 2018). Dry roasting improves the general aroma of the seeds. In this method, 200 g of Inca Inchi seeds were roasted in an oven at 60 (T60), 80 (T80), and 120°C (T120) for 15 min, respectively. After pre-treatment, the seeds were loaded into an oil expeller for extraction.

Oil Extraction

Oil extraction from Inca Inchi seeds was done using lab scale oil expeller (model: Household Oil Presser, Best Day, China). Two hundred (200) g of Inca Inchi seeds were manually dehulled for the study. Prior to the extraction of oil, the oil presser was pre-heated for a few minutes. After pre-heating, 200 g of dehulled seeds were loaded slowly into the hopper to obtain a steady flow of oil extraction. The crude oil obtained was kept in the chiller for the particles to sediment. The sedimented particles were removed by filtration to obtain the actual oil yield. The oil meal from the extraction was vacuum sealed and stored at room temperature to determine their proximate composition. The oil extraction process has been summarized in Figure 1.

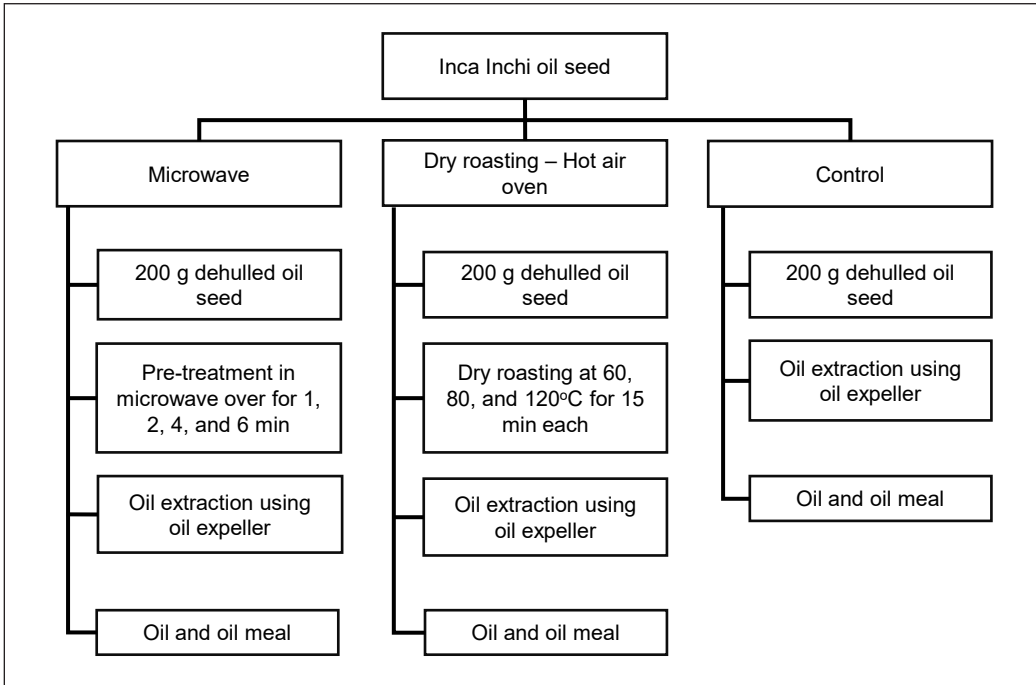


Figure 1. Summarized methods for the extraction of Inca Inchi oil

Proximate Analysis

The proximate analysis of the Inca Inchi oil seed and oil meal was analyzed in terms of moisture, ash, crude protein, crude fat, and crude fiber using the standard method of the Association of Official Analytical Chemists (AOAC) (Horwitz & Latimer, 2005). The protein content in the sample was analyzed by the micro Kjeldahl method by determining the total nitrogen in the sample (factor 6.25 was used to convert it to total protein). The moisture content in the sample was determined by drying the sample in a hot air oven at 105°C till constant weight was obtained. The ash content in the sample was analyzed by incinerating the sample in a muffle furnace at 550°C, while Soxhlet was used to analyze the crude fat. The crude fiber was analyzed by the acid-alkali digestion

method. The difference in the above analysis calculated the carbohydrate content in the sample. All analyses were carried out in triplicate.

Thermal Profile Study

The melting profile of the oil samples was studied using differential scanning calorimetry (DSC). PerkinElmer DSC 8000 (USA) was used to determine the melting thermograms of the oil samples. Nitrogen gas was used to purge the system during the analysis. An empty and hermetically sealed aluminum pan was used as a reference. Seven (7.0) mg of sample was accurately weighed and hermetically sealed in an aluminum pan. The following temperature program was applied to the sample. The sample was initially heated to 80°C and held

for 5 min. The sample was then heated from 50 to 80°C at 5 min/s and held at 80°C for 10 min. Duplicate analysis was performed for each sample (Y.-Y. Lee et al., 2015).

Fatty Acid Composition

The fatty acid methyl esters (FAMES) of the oil samples were derived by transesterification, based on O'Fallon et al. (2007). The analyses were done on Agilent Technologies (USA) gas chromatograph 7890A equipped with flame ionization detector (FID), a split/split less injector, and a capillary column (25 m × 0.32 mm × 0.25 µm, type BPX70, SGE™ Analytical Science, Australia). The injector and detector temperatures were set at 250 and 280°C. The temperature program for the analysis was as follows: Holding at 100°C for 0.5 min, increasing up to 180°C at the rate of 10°C/min, increasing from 180 to 220°C at the rate of 1.5°C/min, increasing from 220 to 250°C at the rate of 30°C/min and holding at 250°C for 5 min. Nitrogen was used as carrier gas. Triplicate analysis was done on each sample. Peaks were integrated using Agilent Chem Station software (version: B.04.01). The area under each fatty acid peak relative to the total area of all fatty acid peaks was used to quantify the fatty acids. Results are reported as a percentage of the total fatty acids.

Physicochemical Properties

The physicochemical properties of the oil studied were free fatty acid (FFA), peroxide value (PV), and iodine value (IV). The properties were analyzed using

American Oil Chemist Society (AOCS) standards (Firestone, 2009). Free fatty acid (the method by Ca 5a-40), iodine value by cyclohexane-acetic acid method (the method by Cd 1d-92), and peroxide value using acetic acid-isooctane method (the method by Cd 8b-90), respectively. All the analyses were carried out in triplicate. FFA in oils was expressed as % oleic acid, while PV of the oil samples was expressed as meq (milli equivalent) O₂/kg of oil, and IV of the oil samples was expressed as grams of iodine absorbed by 100 g of sample (g I₂/100 g).

Antioxidant Study

Extraction of Bioactive Compounds.

The oil's bioactive compounds, such as phenolics and flavonoids, were extracted using a solvent. A known amount of the oil was first dissolved in an equal proportion of *n*-hexane (Fisher Scientific Sdn. Bhd., Malaysia) and was vortexed vigorously. Then an equal volume of aqueous methanol (methanol: water, 80:20 v/v) (Fisher Scientific Sdn. Bhd., Malaysia) was added to the solution and vortexed vigorously. The methanolic phase was carefully collected to estimate the oil's total phenolic content and flavonoids. The analysis was conducted in triplicate (Muangrat et al., 2018).

Total Phenolic Content. The total phenolics in the oil extracts were analyzed using Folin's phenol reagent with slight modification. Twenty (20) µl of the sample extracts were diluted to 500 µl with distilled water. The solution was vigorously vortexed. One hundred fifty (150) µl of Folin's phenol

reagent (Merck, Germany) was added to the solution. The solution was incubated at room temperature for 10 min. Five hundred (500) μ l of 7.5% (w/v) sodium carbonate solution (Fisher Scientific Sdn. Bhd., Malaysia) was added to this solution. The solution was incubated at room temperature in the dark for 60 min, and the absorbance was taken at 650 nm using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA). Gallic acid (Sigma-Aldrich, USA) was used as the standard. The results were expressed as milligrams of gallic acid equivalents per gram of the oil sample (mg GAE/g oil sample) (Baba & Malik, 2015).

Estimation of Total Flavonoids. The total flavonoids in the oil extracts were analyzed using the method proposed by Djeridane et al. (2006) with slight modifications. This study mixed 0.5 ml of the oil extracts with 0.5 ml of 2% aluminum chloride methanolic solution (Fisher Scientific Sdn. Bhd., Malaysia). The samples were vigorously agitated and incubated at room temperature in the dark for 15 min. The absorbance was measured at 430 nm using a spectrophotometer (Cary UV-Vis, Agilent Technologies, USA). Methanol (Fisher Scientific Sdn. Bhd., Malaysia) was used as blank, and quercetin (Sigma-Aldrich, USA) was used as standard. The flavonoid content was expressed in mg quercetin equivalent (QE) /g oil extract (OE).

Radical Scavenging Activity. The radical scavenging activity of the oil sample was assessed by DPPH and ABTS scavenging

assays. The method proposed by L. Liu et al. (2009) was followed with slight modifications to assess the oil's overall scavenging capacity by DPPH. The sample (20 μ l) was taken in an Eppendorf tube and diluted to 1 ml using 95% ethanol (Fisher Scientific Sdn. Bhd., Malaysia). The sample was vigorously agitated. To this solution, 3 ml of 0.004% DPPH solution (4 mg of DPPH in ethanol, Merck, Germany) was added. The solution was vigorously agitated for a minute and incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA). Ethanol (Fisher Scientific Sdn. Bhd., Malaysia) was used as a blank. The study was conducted in the dark as the DPPH chemical is light-sensitive. The % scavenging activity was calculated using Equation 1.

$$\text{Scavenging activity (\%)} = [A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}] \times 100$$

(Equation 1)

where, A_{control} = Absorbance of control and A_{sample} = Absorbance of sample

The ABTS method is based on reducing the ABTS radical cation (Cheong et al., 2018; Marfil et al., 2011). In this assay, a stock solution consisting of 7 mM ABTS (Merck, Germany) and 2.45 mM potassium persulfate (Sigma-Aldrich, USA) were combined in the ratio of 1:1 and incubated at room temperature in the dark for 16 hr to create ABTS radical cation stock solution. The resulting solution was diluted with ethanol to adjust the absorbance to $0.700 \pm$

0.020 at 734 nm prior to sample analysis. To determine the radical scavenging activity of the sample, 100 µl of the oil sample was added to 2.0 ml of diluted ABTS solution and vortexed vigorously. The solution was incubated at room temperature in the dark for 3 min, and the absorbance was measured at 734 nm using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA). Ethanol (Fisher Scientific Sdn. Bhd., Malaysia) was used as a blank. The percentage of scavenging activity was calculated using Equation 1.

Statistical Analysis

The experimental analysis data were presented as mean ± standard error (SE) for at least three analyses. Data were analyzed by one-way analysis of variance (ANOVA) to determine the significant difference, and Tukey pairwise comparisons were used to compare the significant difference among the thermal pre-treatment conditions. The statistical analysis was performed using Minitab version 17 (USA).

RESULTS AND DISCUSSION

Oil Extraction

The study results show that microwave and hot air oven thermal pre-treatment can improve oil yield. Among the thermal pre-treatment methods, microwave treatment for 4 min gave the highest oil yield of 43.39% compared to control (37.76%). The results of the study are shown in Table 1. The oil yield in the hot air oven and pre-treated method was between 41.44–42.68%, and in the microwave, the method was between 40.31–43.39%, indicating that pre-treating the oil seeds before oil extraction is necessary to increase the oil yield. Untreated Inca Inchi seeds had low oil yield as the intact cell wall significantly hindered oil extraction. Preliminary lab studies on microwave exposure duration revealed that Inca Inchi seeds thermally pre-treated longer than 4 min were slightly burnt and had reduced the oil yield. However, the Inca Inchi seeds were not affected in the hot air oven, so the oil yield increased with the roasting temperature.

Table 1
Effect of pre-treatment process on the yield of oil

Treatment	Sample weight (g)	Oil obtained (g)	Oil meal (g)	% yield of oil	% increase in oil yield
M1	200	80.63±0.09 ^d	90.90±0.63 ^a	40.31±0.05 ^d	6.7
M2	200	83.61±0.54 ^c	78.08±0.59 ^f	41.30±0.27 ^c	9.37
M4	200	86.77±0.59 ^a	81.49±0.58 ^e	43.39±0.29 ^a	14.9
M6	200	84.27±0.08 ^{bc}	77.11±0.47 ^f	42.14±0.04 ^{bc}	11.59
T60	200	74.88±0.54 ^c	82.80±0.57 ^d	41.44±0.27 ^c	9.74
T80	200	83.73±0.76 ^{bc}	87.92±0.07 ^b	41.87±0.38 ^{bc}	10.86
T120	200	85.10±0.43 ^b	85.39±0.17 ^c	42.68±0.22 ^b	13.0
Control	200	75.52±0.61 ^c	85.22±0.11 ^c	37.76±0.31 ^c	

Note. M1 = Microwave 1 min; M2= Microwave 2 min; M4 = Microwave 4 min; M6 = Microwave 6 min; T60 = Oven roasting 60°C; T80 = Oven roasting at 80°C; T120 = Oven roasting at 120°C; Different small letters indicate significant differences ($p < 0.05$)

The % increase in oil yield for microwave pre-treatment was between 6.7–14.9% for microwave and 9.74–13.0% for oven roasting. During heating, the microstructure of the seed changes due to water evaporation. It creates internal pressure and breaks the cell wall and the membrane, forming pores within. Generally, the oil droplets in the seed are in the form of an emulsion with protein. During roasting, the protein gets denatured, coagulates which causes the emulsion to break, and the oil droplets combine to form a bigger droplet. Moreover, the hot temperature reduces the surface tension and increases the fluidity. Thereby, the oil is easily released from the seed. Thus, the oil yield is better after thermal pre-treatment (Shahidi, 2005). The oil yield is better with microwaves as compared to oven roasting due to the efficient transfer of heat in the microwave. In the microwave, the energy is transmitted directly to the sample via molecular interaction with the electromagnetic field (Rękas et al., 2017).

Uquiche et al. (2008) conducted a study on Chilean hazel nuts seeds to determine the effect of pre-treatment on oil extraction using two different microwave power (400 and 600 W) for 3, 3.5, and 4 min. The highest oil yield of 45.3% was recorded in seeds exposed at 400 W for 4 min, followed by 43.5% at 600 W for 4 min, while control seeds recorded only 6.1%. Likewise, studies by Fathi-Achachlouei et al. (2019) on milk thistle seeds using microwave power (800 W) for 2 and 4 min revealed that the highest oil yield of

35.41% was obtained when the seeds were exposed to 4 min as compared to 32.33% when exposed to 2 min.

Studies on cold press extraction of oil from whole and dehulled seeds of Inca Inchi showed that the highest oil yield extraction (40.15%) was obtained in de-hulled seeds, as compared to whole seeds, which had only 27.20%, indicating that it is necessary to dehull the seeds prior to oil extraction (Gutiérrez et al., 2019).

Proximate Analysis

The proximate analysis of the Inca Inchi oil meal (with and without pre-treatment) and the oil seed were studied. The results are shown in Table 2.

Among the pre-treatments, the maximum and minimum moisture content obtained were in M1 (5.14%) and M6 (1.26%). The moisture content in the Inca Inchi oil seed was 3.58%, and a similar value (3.3%) was reported by (L. F. Gutiérrez et al., 2011). Moisture content is an important determinant of the shelf life of seeds. These values are within the safe range for storage and processing (0-13%) of Inca Inchi seeds without microbial deterioration of the triglyceride. Microbial growth can be prevented with low water activity (Alemu et al., 2022). The protein content of the Inca Inchi oil seed in this study was slightly lower (24.2%) than those reported by other studies (29.2 and 27%) (Bueno-Borges et al., 2018; Hamaker et al., 1992).

The protein content of the Inca Inchi oil seeds is similar to other oil seeds such as rape seeds (22%), sunflower seeds

Table 2
Proximate analysis of the Inca Inchi oil meal (with and without pre-treatment) and oil seed

Treatment	Moisture content (%)	Ash content (%)	Crude fat (%)	Crude protein (%)	Crude fiber (%)	Carbohydrate (%)
Microwave-oil meal						
M1	5.14±0.11 ^a	3.01±0.88 ^a	6.09±0.07 ^d	60.18±1.00 ^{ab}	11.47±0.20 ^c	14.11±1.96 ^d
M2	3.75±0.10 ^b	3.04±0.35 ^a	5.70±0.09 ^d	58.41±1.20 ^{abc}	12.07±0.40 ^b	17.03±1.11 ^d
M4	2.19±0.05 ^c	3.06±0.46 ^a	3.88±0.04 ^f	59.37±1.30 ^{abc}	10.87±0.15 ^d	20.63±1.85 ^{bc}
M6	1.26±0.08 ^f	3.08±0.02 ^a	4.21±0.14 ^{ef}	57.01±0.80 ^c	11.40±0.10 ^c	23.04±0.80 ^b
Hot air oven-oil meal						
T60	3.62±0.02 ^b	3.01±0.54 ^a	7.54±0.31 ^c	57.65±1.50 ^{bc}	12.70±0.10 ^a	15.48±0.71 ^d
T80	3.19±0.08 ^c	3.01±0.84 ^a	7.46±0.06 ^c	51.74±1.30 ^d	12.13±0.10 ^b	22.47±1.93 ^b
T120	2.75±0.03 ^d	3.00±0.49 ^a	4.70±0.06 ^c	60.97±0.90 ^a	11.13±0.30 ^{cd}	17.45±0.86 ^{cd}
Oilseed	3.58±0.01 ^b	2.49±0.14 ^a	40.95±0.44 ^a	24.20±0.70 ^f	12.47±0.20 ^{ab}	16.31±0.87 ^d
Control-oil meal	5.19±0.20 ^a	3.04±0.30 ^a	8.26±0.04 ^b	41.15±0.20 ^c	11.33±0.20 ^{cd}	31.03±0.18 ^a

Note. M1 = Microwave 1 min; M2 = Microwave 2 min; M4 = Microwave 4 min; M6 = Microwave 6 min; T60 = Oven roasting 60°C; T80 = Oven roasting at 80°C; T120 = Oven roasting at 120°C; Different small letters indicate significant differences ($p < 0.05$)

(18.72%), ground nut seeds (26.5%), and melon seeds (25.4%) (McKevith, 2005; Muhammad Anjum et al., 2012; Onyeike & Acheru, 2002). A plant product with more than 12% protein is regarded as an excellent source of proteins and is referred to as a protein-rich food (Pearson & Carr, 1976).

The crude fat content of the Inca Inchi oil seed in this study was 41%, and the value was within the range reported in earlier studies (35–60%) (Follegatti-Romero et al., 2009; Guillén et al., 2003). This variation in the oil content could be due to differences in geographical conditions and seed maturity. The fat content in the oil meal was between 3.88–8.26%. M4 had the lowest value, and oil meal (control) had the highest value.

Thermal Profile Study

The melting profile of Inca Inchi oil was studied using DSC. Figure 2 shows the

DSC melting thermogram of the Inca Inchi oil obtained under different extraction conditions. At the given temperature program, when the oil was heated to 80°C, the melting curve showed a single prominent peak at about -20°C with a transition starting at -12°C and ending at -25°C. A small shoulder peak was visible at 25°C. This type of shoulder can be seen during the melting of more than one group of triglycerides with melting points that are too close to be distinguished by DSC. The melting of a triglyceride is determined by the extent of branching in the carbon chain, the degree of unsaturation of its fatty acids, and their stereo-specific arrangement along the glycerol molecules (Ferrari et al., 2007). Vegetable oils, in general, have a combination of triglycerides, and hence the melting occurs over a wide range of temperatures rather than at a single

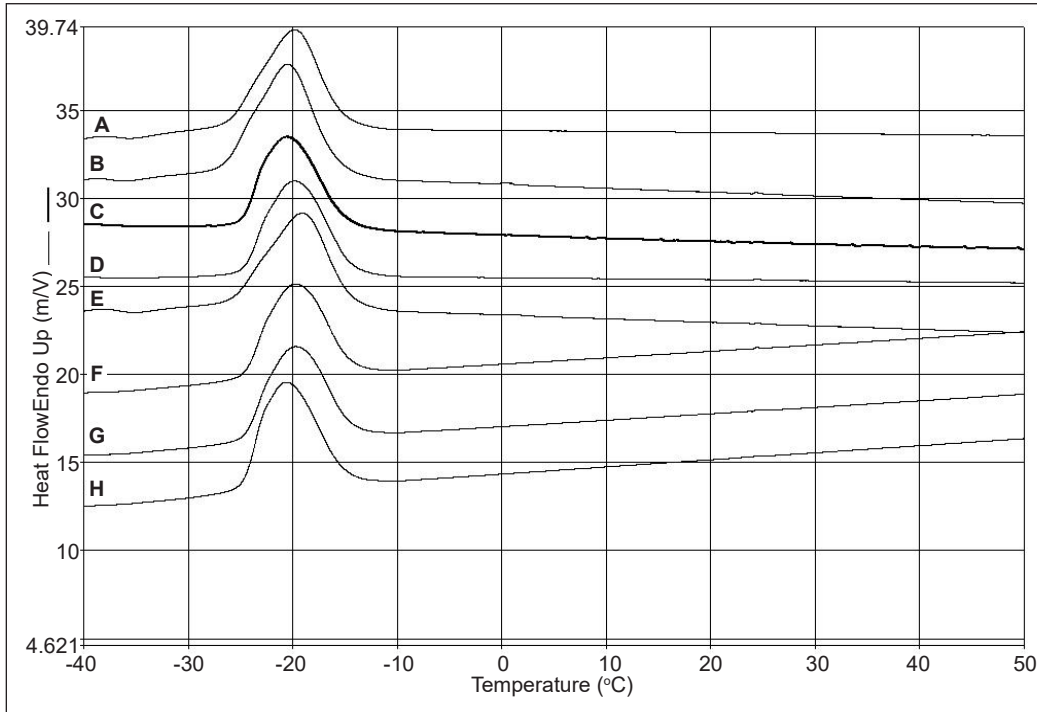


Figure 2. DSC melting curves of Inca Inchi oil samples with and without pre-treatment
 Note. A = Control; B = M1 (microwave 1 min); C = M2 (microwave 2 min); D = M4 (microwave 4 min); E = M6 (microwave 6 min); F = T60 (oven roasting at 60°C); G = T80 (oven roasting at 80°C); H = T120 (oven roasting at 120°C)

temperature (Tan & Che Man, 2002). Thus, due to the combination of triacylglycerols in most vegetable oils, the shoulder-like feature in a DSC curve is generally common in these oils. The melting curve of Inca Inchi oil also confirms that the oils rich in unsaturated fatty acids often melt at a lower temperature. The melting profile of Inca Inchi oil obtained through solvent extraction was at -18.5°C (Gutiérrez et al., 2011).

Fatty Acid Composition

Inca Inchi oil is an excellent source of the beneficial linolenic and linoleic acids that aid in preventing coronary heart disease, rheumatoid arthritis, diabetes, hypertension,

and microbial infection (Alayón et al., 2018, 2019; da Silva Soares et al., 2019). Table 3 shows the fatty acid composition in Inca Inchi oil obtained through various pre-treatments. From the gas chromatography (GC) study, the major fatty acids in the oil were linolenic acid and linoleic acid, which were about 48 and 37%, respectively. The MUFA found in the oil was oleic acid, which was about 8%. The saturated fatty acids present in the oil were palmitic acid and stearic acid, which were about 4 and 2%, respectively. The oleic acid present in the oil helps to reduce the risk of coronary heart disease (Capurso et al., 2014; Lopez-Huertas, 2010). The PUFAs and MUFA

Table 3
Fatty acid composition of Inca Inchi oil with and without pre-treatment (%)

Treatment	ω -3	ω -6	ω -9	Palmitic acid	Stearic acid
M1	48.8±0.0 ^a	37.5±0.0 ^a	7.7±0.1 ^a	3.9±0.0	2.1±0.0 ^a
M2	48.7±0.0 ^a	37.6±0.0 ^a	7.7±0.0 ^a	4.0±0.0 ^a	2.0±0.0 ^a
M4	49.1±0.0 ^a	37.2±0.1 ^a	7.7±0.0 ^a	3.8±0.1 ^a	2.2±0.1 ^a
M6	48.5±0.0 ^a	37.6±0.0 ^a	7.8±0.1 ^a	4.0±0.0 ^a	2.1±0.0 ^a
T60	48.4±0.1 ^a	37.7±0.0 ^a	7.8±0.0 ^a	4.0±0.1 ^a	2.1±0.1 ^a
T80	48.7±0.0 ^a	37.6±0.0 ^a	7.7±0.0 ^a	3.8±0.0 ^a	2.2±0.0 ^a
T120	48.4±0.1	37.8±0.1 ^a	7.8±0.0 ^a	3.9±0.1 ^a	2.1±0.0 ^a
Control	48.3±0.0 ^a	37.3±0.0 ^a	7.7±0.0 ^a	4.3±0.1 ^a	2.4±0.0 ^a

Note. M1 = Microwave 1 min; M2= Microwave 2 min; M4 = Microwave 4 min; M6 = Microwave 6 min; T60 = Oven roasting 60°C; T80 = Oven roasting at 80°C; T120 = Oven roasting at 120°C; Different small letters indicate significant differences ($p < 0.05$)

values in the Inca Inchi oil range between 77.5–84.4% and 8.0–13.2%, respectively.

However, the total SFA in the Inca Inchi oil is lower than other oil seeds such as sunflower, flaxseed, and canola (Follegatti-Romero et al., 2009; Maurer et al., 2012). Studies on the fatty acid composition of cold press extraction of oil from Inca Inchi seeds using whole seeds and dehulled seeds reveal that the fatty acid composition was not affected in both the samples, and there was no significant difference ($p > 0.05$) between the samples (Gutiérrez et al., 2019). Likewise, Gutiérrez et al. (2017) reported that γ -irradiation treatments of Inca Inchi shell at doses 1, 5, and 8 kGy did not affect the fatty acid composition of the oil. The omega 3 and omega 6 fatty acids were not affected at the high dose of 8 kGy, thereby maintaining the nutritional value of the oil.

Physicochemical Properties

The physicochemical properties of the Inca Inchi seed oil with and without pre-treatment are shown in Table 4. Peroxide

value and free fatty acid are the most crucial parameters for assessing the oil quality. The hydrolysis of oils and fats results in the production of free fatty acids. Oils and fats are exposed to various conditions such as processing, storage heating, and frying, and due to this, the level of FFA varies with time, temperature, and moisture content. FFA are more susceptible to oxidation, which becomes rancid as they are less stable than neutral oil. Therefore, FFA is a crucial component associated with oils and fats' quality and commercial viability (Mahesar et al., 2014). To be acceptable for oral consumption, the free fatty acid in the oil must not be higher than 5% (Esuoso & Odetokun, 1995). Results show that the % free fatty acid for Inca Inchi oil for pre-treated oil samples was lower (0.3–0.4%) than for the control (1.1%). A similar value (1.2%) was reported by Gutiérrez et al. (2011). The low free fatty acid in the pre-treated samples was due to moisture loss during roasting. The moisture content of the oil is one of the important factors that cause

Table 4
Physicochemical properties of Inca Inchi oil with and without pre-treatment

Treatment	Free fatty acid (%)	Peroxide value (meq O ₂ /kg oil)	Iodine value (g I ₂ /100 g oil)
Control	1.1±0.04 ^a	8.32±0.07 ^a	194.35±0.96 ^a
M1	0.4±0.03 ^b	3.82±0.02 ^b	192.42±0.26 ^a
M2	0.4±0.02 ^b	3.67±0.04 ^{cd}	192.56±0.96 ^a
M4	0.3±0.01 ^b	3.54±0.03 ^{de}	193.90±1.00 ^a
M6	0.3±0.03 ^b	3.50±0.29 ^e	191.30±1.12 ^a
T60	0.3±0.01 ^b	3.78±0.02 ^{bc}	192.66±0.34 ^a
T80	0.3±0.02 ^c	3.69±0.03 ^{cd}	192.55±0.80 ^a
T120	0.3±0.02 ^b	3.58±0.03 ^{de}	191.48±1.58 ^a

Note. M1 = Microwave 1 min; M2= Microwave 2 min; M4 = Microwave 4 min; M6 = Microwave 6 min; T60 = Oven roasting 60°C; T80 = Oven roasting at 80°C; T120 = Oven roasting at 120°C; Different small letters indicate significant differences ($p < 0.05$)

rancidity (Roger et al., 2010). Hence, Inca Inchi oil is an edible oil.

PV determines the amount of peroxides (primary oxidation products) in the oil. Peroxides are important intermediate products of oxidative reactions because they decompose to produce free radicals when exposed to transition metal irradiation and high temperatures. The organoleptic properties of the oil are also correlated with PV, which denotes the oil's freshness (Uquiche et al., 2008). PV greater than 10 meq O₂/kg oil is unacceptable (Shahidi, 2005). Results of the study exhibit that the PV for the oil samples was less than 10 meq O₂/kg. The PV of control is 8.32 meq O₂/kg, which is closer (7.36 meq O₂/kg) to commercial Inca Inchi oil (Vicente et al., 2015). Oils with peroxide levels of more than 10 meq O₂/kg are less stable and have a shorter shelf life. Lower the peroxide values better the resistance towards oxidation and lipolytic hydrolysis (Akanni et al., 2005). Studies by Fathi-

Achachlouei et al. (2019) on the effect of microwave pre-treatment of milk thistle seeds reveal that the PV was low (2.09 meq O₂/kg) against control (5.11 meq O₂/kg).

However, studies by Mazaheri et al. (2019) on *Nigella sativa* seeds reveal that the PV increased with microwave and oven roasting compared to unroasted seeds. A similar result was seen in cottonseed oil due to the presence of reactive radicals that might be formed by exposure to microwaves (Frag et al., 1992). IV quantifies the average degree of unsaturation in oils and fats. The lower the IV, the lesser the number of unsaturated bonds. Thus, the oil is stable to rancidity (Haile et al., 2019). In this study, the IV was between 191–194 g I₂/100 g oil, and there was no significant difference ($p > 0.05$) between the extraction conditions. A similar value (193.1 g I₂/100 g) on non-treated Inca Inchi oil was reported by (Gutiérrez et al., 2011). The slight reduction in the iodine value in the pre-treated samples could be due to the breakage of long-chain

fatty acids, oxidation, or polymerization (Anjum et al., 2006). Generally, the iodine value decreases with roasting.

A study was done to determine the effect of IV on sunflowers seeds using microwave power (500 W) at 5, 10, and 15 min prior to solvent extraction. The study exhibits the highest IV of 140 g I₂/100 g of oil for control against 113.5 g I₂/100 g at 15 min. Likewise, studies by Fathi-Achachlouei et al. (2019) on milk thistle seeds using microwave power (800 W) for 2 and 4 min showed that pre-treatment by microwave had a significant effect on the IV. Studies on oven roasting (6, 9, 10, and 12 min) at 210°C on red pepper seed oils did not affect the iodine value (Jung et al., 1999). A similar result was reported by (Arab et al., 2022) on oven-roasted white sesame seeds.

Antioxidant Study

An antioxidant is a substance that can delay or block the oxidation of lipids or other molecules by reducing the beginning or propagation of oxidative chain reactions, thereby preventing or repairing damage caused by oxygen to the body's cells. Various functions of antioxidants are free radical scavenging, reducing activity, quenching singlet oxygen, and potential complexing of pro-oxidant metals (Tachakittirungrod et al., 2007). The presence of naturally existing or newly formed antioxidant compounds during the seed pre-treatment process is represented by the antioxidant activity of seed oil. Enhancing the oil's antioxidant properties through pre-treatment is important in food, cosmetic and pharmaceutical applications.

Antioxidant compounds such as flavonoids, tannins, and phenolics are present in various parts of the plant, such as seed and oil leaves (Jeong et al., 2004). Bioactive compounds such as flavonoids and phenolic acids have health-promoting properties, especially for oxidative-stress-related diseases such as cancer, diabetes, neurodegenerative, and cardiovascular (Gutfinger, 1981; Li et al., 2014).

The antioxidant activities of the Inca Inchi oil were studied in terms of DPPH, ABTS, flavonoids, and total phenolic content. The results are presented in Table 5.

Total Phenolic Content and Flavonoid Content.

Phenolic compounds are important plant constituents with antioxidant activity due to their redox properties. The phenolic content is analyzed using Folin's reagent. Folin's reagent is a mixture of phosphomolybdic acid and phosphotungstic acid. Under alkaline conditions (due to the presence of sodium carbonate) during the oxidation process, these acids are reduced to blue oxides of molybdene and tungstene, respectively. The intensity of the blue color represents the concentration of phenolic compounds present, which is measured using a spectrophotometer (Conforti et al., 2006). In this study, the phenolic content was (6.3-6.4 mg GAE/g oil), and the value reported was similar to that reported (6.2 mg GAE/g of oil extract) by (Q. Liu et al., 2014). In another study, the seeds were subjected to three different roasting conditions (lightly roasted, medium roasted, and highly roasted), and the phenolic content

Table 5
Antioxidant activities of Inca Inchi oil with and without pre-treatment

Treatment	Total phenolic content (mg GAE/g oil extract)	Total flavonoids (mg QE/g oil extract)	DPPH (%) scavenging activity	% increase in activity	ABTS (%) scavenging activity	% increase in activity
M1	6.4±0.0 ^a	0.6±0.01 ^a	71.23±0.1 ^b	3.2	90.49±0.48 ^a	11.71
M2	6.4±0.0 ^a	0.6±0.01 ^a	72.45±0.0 ^{ab}	4.96	90.75±0.23 ^a	12.03
M4	6.4±0.1 ^a	0.7±0.0 ^a	74.43±0.1 ^a	7.83	91.55±0.48 ^a	13.02
M6	6.3±0.0 ^a	0.6±0.1 ^a	73.44±0.0 ^{ab}	6.40	90.44±0.18 ^a	11.65
T60	6.4±0.0 ^a	0.6±0.0 ^a	73.44±0.0 ^{ab}	6.40	90.44±0.17 ^a	11.65
T80	6.3±0.01 ^a	0.6±0.0 ^a	73.46±0.1 ^{ab}	6.43	90.46±0.12 ^a	11.67
T120	6.3±0.0 ^a	0.7±0.0 ^a	73.43±0.0 ^b	6.38	90.54±0.11 ^a	11.77
Control	6.4±0.0 ^a	0.6±0.01 ^a	69.02±0.2 ^c		81.00±1.00 ^b	

Note. M1 = Microwave 1 min; M2= Microwave 2 min; M4 = Microwave 4 min; M6 = Microwave 6 min; T60 = Oven roasting 60°C; T80 = Oven roasting at 80°C; T120 = Oven roasting at 120°C; Different small letters indicate significant differences ($p < 0.05$)

was compared to unroasted seeds. The study shows that the lowest phenolic content was in unroasted seeds (2.32 mg GAE/g of oil extract). However, it was noted that with an increase in roasting condition, there was an increase in phenolic content (3.72, 9.40, and 12.32 mg GAE/g of oil), respectively (Cisneros et al., 2014).

However, this concentration is lower than that reported by other commercial oils such as flax seed oil (39.16 mg GAE/g of oil extract), perillartine (20.38 mg GAE/g of oil extract), rice bran oil (19.59 mg GAE/g of oil extract), grape seed oil (15.56 mg GAE/g of oil extract), Inca Inchi oil (13.29 mg GAE/g of oil extract), respectively (Xuan et al., 2018). Commercial Inca Inchi oil in Japan had phenolic content of 13.29 mg GAE/g of oil extract). However, the value is still lower than the above commercial oils. These differences in the values of total phenolic content can be attributed to the processing condition or the environmental

conditions where the oil seed was cultivated.

From the study, the flavonoid content in the oil extract was low. The flavonoid content in the oil extract is between 0.6–0.7 mg QE/g oil extract, and there was no significant difference ($p > 0.05$) among the processing condition. Incidentally, the flavonoid content reported by commercial Inca Inchi oil is 0.34 mg QE/g oil extract (Xuan et al., 2018). Thus, the oil had low phenolic and flavonoid content in our study, irrespective of the processing condition.

Radical Scavenging Activity. The radical scavenging activity of Inca Inchi seed oil with and without pre-treatment was investigated using DPPH and ABTS. DPPH indicates an antioxidant's ability to donate electrons throughout an assay since it is a persistent free radical (purple color) that transforms into a non-radical form (yellow color) by removing a single electron. Low absorbance at 530 nm measures the

reduction in the color transition from purple to yellow (Brand-Williams et al., 1995). In this study, the radical scavenging activity was 71–74% for the pre-treated oil, and the control had a scavenging activity of 69%. The percentage increase in DPPH scavenging activity was between 3.2 to 7.83. The higher the antioxidant activity, the higher the stability towards oxidation. Moreover, the slight reduction within the microwave pre-treatment conditions could be because antioxidants deteriorate at higher temperatures.

The ABTS method also studied the free radical scavenging activity of the oil. The percentage increase in scavenging activity was between 11 to 13. The radical scavenging activity for Inca Inchi oil was higher in the ABTS method when compared to the DPPH method.

Most plant compounds appear to have higher antioxidant activity against ABTS radicals than against DPPH radicals. It is due to the ABTS assay's increased sensitivity in detecting antioxidant activity, which causes the kinetic reaction to be faster, resulting in higher antioxidant activity (Floegel et al., 2011; K. J. Lee et al., 2015). The scavenging activity positively correlates to pre-thermal conditions (Cisneros et al., 2014).

In a nutshell, the variation between the DPPH and ABTS studies can be attributed to differences in the mechanism of action for each method. Since an extract is made up of several molecules with varied biological properties, its synergism could result in strong antioxidant activity; hence antioxidant activities cannot be attributed to

only one or two components (Viuda-Martos et al., 2010).

CONCLUSION

Common thermal pre-treatment methods such as oven roasting, microwave, infrared radiation, boiling, and steaming impact oil yield and, to a considerable extent, the concentration and types of extracted minor lipid components in oil. In this work, microwave, and oven roasting of the Inca Inchi oil seed prior to oil extraction improved oil yield (40.31–43.39%). Among the pre-treatment conditions, M4 (microwave 4 min) had the highest oil yield (43.39%), and the pre-treated oil samples had good radical scavenging activity (about 70 and 90%) as studied through DPPH and ABTS assays. The thermal pre-treatment condition did not affect the fatty acid composition. The quality of the oil in terms of free fatty acid and peroxide values was lower in pre-treated oil, which indicates that pre-treating helps to improve the oil quality.

Further studies can be done to determine the effect of microwave and oven roasting on the anti-nutritional factors, as the Inca Inchi seeds are edible. Thus, to summarize, microwave and oven roasting are suitable methods to produce Inca Inchi oil since there are no undesirable changes in the fatty acid composition or the quality of the oil.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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