

Impact of roasting temperature on the shelf life and antioxidant properties of cold-pressed sacha inchi oil during storage: a practical approach in Thailand

*Chotimarkorn, C. and Punvichai, T.

Integrated High-Value Oleochemical Research Center, Faculty of Innovative Agriculture, Fisheries and Food, Prince of Songkla University, Surat Thani Campus, Muang Surat Thani, 84000, Thailand

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Abstract

Cold-pressed sacha inchi oil, obtained from the seeds of the *Plukenetia volubilis* plant, is recognized for its high nutritional value and potential health benefits. However, the effects of various processing techniques on the oil's quality and shelf life warrant further investigation. This study primarily aimed to explore the impact of roasting temperature on the shelf life and antioxidant properties of cold-pressed sacha inchi oil during a 6-month storage period, with samples analyzed monthly. Oil samples were prepared from unroasted seeds and seeds roasted at 60°C and 80°C. The physicochemical properties, including acid value, iodine value, fatty acid profile, tocopherol isomers, total phenolic content, and DPPH radical scavenging activity, were assessed. The findings revealed that higher roasting temperatures enhanced oxidative stability, as indicated by lower peroxide and p-anisidine values. Oils from roasted seeds exhibited increased total phenolic content and elevated DPPH radical scavenging activity, contributing to their improved antioxidant properties. The fatty acid profile demonstrated a higher level of lipid oxidation in oil from unroasted seeds during storage, while oils from roasted seeds maintained a more stable fatty acid composition, characterized by higher contents of health-promoting unsaturated fatty acids like alpha-linolenic acid and lower levels of saturated fatty acids such as palmitic and stearic acid. Acid and iodine values remained within acceptable limits for all oil samples, signifying consistent quality throughout the storage period. In summary, this study established that roasting sacha inchi seeds at 80°C before cold pressing could significantly enhance the oil's shelf life and antioxidant properties. This finding suggested a practical approach for oil production in Thailand, with potential benefits for both consumers and the oil industry.

1. Introduction

Sacha Inchi (*Plukenetia volubilis* L.), indigenous to the Amazon rainforest region, has attracted considerable interest due to the high nutritional value and potential health benefits of its seeds. These seeds are abundant in polyunsaturated fatty acids (PUFAs), specifically omega-3 (alpha-linolenic acid) and omega-6 (linoleic acid) fatty acids, which are vital for maintaining human health (Follegatti-Romero *et al.*, 2009). Cold-pressed oils, including sacha inchi oil, have become increasingly popular as they retain most of their nutritional properties and provide better flavor and aroma compared to oils extracted using conventional methods. The cold-press extraction process involves mechanically pressing the seeds at controlled temperatures ensuring that heat-sensitive compounds and beneficial nutrients remain

intact (Ramadan, 2020). Cold-pressed sacha inchi oil has gained popularity in the functional food and nutraceutical industries owing to its exceptional nutritional profile, antioxidant properties, and potential health benefits (Wang *et al.*, 2018). Nonetheless, the oil's high PUFA content renders it prone to lipid oxidation during storage, which may compromise its quality, nutritional value, and shelf life. As a result, developing strategies to enhance the oxidative stability of sacha inchi oil is crucial for preserving its quality and ensuring consumer satisfaction. One potential approach involves roasting the seeds before oil extraction. Thermal treatments such as roasting have been demonstrated to increase the release of antioxidant compounds and modify the fatty acid composition of oils, potentially enhancing their oxidative stability (Chandrasekara and

*Corresponding author.

Email: chotimarkorn.c@gmail.com

Shahidi, 2011). In Thailand, the production and consumption of cold-pressed sacha inchi oil have grown, emphasizing the need to optimize processing conditions for superior product quality. To date, few studies have explored the influence of roasting temperature on the shelf life and antioxidant properties of cold-pressed Sacha Inchi oil during storage, particularly in the Thai context.

This study aimed to assess the impact of roasting temperature on the shelf life and antioxidant properties of cold-pressed sacha inchi oil over a 6-month storage period. Specifically, the research will compare oil obtained from unroasted seeds with oil from seeds roasted at 60°C and 80°C. Various parameters, including acid value, iodine value, peroxide value, p-anisidine value, fatty acid profile, tocopherol isomer content, total phenolic content, and DPPH radical scavenging activity, will be evaluated to determine the effect of roasting temperature on the oil's quality and oxidative stability. The findings of this study will offer valuable insights into the practical production of cold-pressed sacha inchi oil in Thailand and contribute to the development of strategies to improve its shelf life and antioxidant properties.

2. Materials and methods

2.1 Materials

The commercially sourced sacha inchi seeds (*Plukenetia volubilis*) used in this study were harvested in March 2021 from the north of Thailand and were kindly provided by community enterprises in Chiang Mai province, Thailand. The seeds were cleaned and divided into three groups of 50.0 kg each and packed in polyethylene bags. All the seeds were stored in a refrigerator until used. For roasting methods, 2.5 kg of seeds were placed in a stainless-steel tray dryer with dimensions of 50 cm × 60 cm × 2 cm and roasted at 60°C and 80°C for 20 mins using a tray dryer (Unique Tools Co. Ltd., Chachoengsao Province, Thailand). The roasted seeds were preserved in a refrigerator within polyethylene bags prior to use. For the oil extraction process, a mini screw press T3Pro model (Nature Health and Innovation Co. Ltd., Saraburi, Thailand) was utilized, featuring 186.43 watts drive motor, a screw press speed of 50 rpm, and a 6 L/h capacity. Once extracted, the oil was filtered using a 10.0 µm filter paper and subsequently stored in 1,000.0 mL polyethylene (PE) bottles at room temperature (26-32°C), and exposed to natural light. Throughout the six-month storage period, oil samples were collected and analyzed monthly.

2.2 Determination of acid value

The acid value was determined using the American

Oil Chemists' Society (AOCS) official method Cd 3d-63 (AOCS, 1990a). In this method, a weighed sample of oil (approximately 3.0 g) was dissolved in a mixture of ethanol and diethyl ether (1:1, v/v). The resulting solution was then titrated with a standardized potassium hydroxide (KOH) solution, using phenolphthalein as an indicator. The endpoint was reached when the solution turned a faint pink color that persisted for at least 15 s. The acid value was calculated considering the volume of KOH solution used in the titration and the weight of the oil sample. The acid value was expressed as mg of KOH required to neutralize the free fatty acids present in 1.0 g of oil. The acid value can be calculated using the following formula: Acid Value (AV) = $(V \times N \times 56.1) / W$, where V is the volume (mL) of the standardized potassium hydroxide (KOH) solution used in the titration, N is the normality of the potassium hydroxide solution, 56.1 is the molecular weight of KOH (mg/mmol), and W is the weight (g) of the oil sample.

2.3 Determination of iodine value

The iodine value was determined using the AOCS official method Cd 1-25 (AOCS, 1990b). In this method, a 0.3 g weighed sample of oil was dissolved in cyclohexane and reacted with an excess of Wijs solution (iodine monochloride in glacial acetic acid), followed by a 30 mins incubation in the dark. After the reaction, potassium iodide (KI) was added to form iodine, which was then titrated with a standardized sodium thiosulfate solution. The endpoint was indicated by the disappearance of the blue color after adding a starch indicator solution. The iodine value was calculated using the volumes of sodium thiosulfate solution used in the titration and the weight of the oil. The iodine value was calculated using the following formula: Iodine Value = $(12.69 \times (B - S) \times N) / W$, where B = volume (mL) of sodium thiosulfate solution used for the blank, S = volume (mL) of sodium thiosulfate solution used for the sample, N is the normality of the sodium thiosulfate solution, and W is the weight (g) of the oil sample.

2.4 Determination of fatty acids composition

The fatty acid composition was determined using the AOCS official method Ce 1j-07 (AOCS, 1989c). In this method, a weighed sample of oil was subjected to transesterification to convert the fatty acids into fatty acid methyl esters (FAMES) according to the method of AOCS official method Ce 2b-11 (AOCS, 1989b). The reaction was carried out by mixing the oil sample with a methanolic sodium hydroxide (NaOH) solution, followed by the addition of boron trifluoride (BF₃)-methanol complex. The resulting FAMES were extracted with hexane and washed with water to remove any residual impurities. The FAMES solution was then analyzed using

a Shimadzu GC 17A gas chromatograph with a DB-wax column (0.25 mm i.d. \times 30.0 m, 0.25 μ m film thickness, J&W Scientific, USA) equipped with a flame ionization detector (FID). The temperature program started at an initial temperature of 150°C, held for 1 min, and then gradually increased at a rate of 1°C per min up to a final temperature of 240°C, which was maintained for 20 mins. Helium was used as the carrier gas with a column inlet pressure of 2.0 kg/cm². The fatty acid composition was determined by comparing the retention times and peak areas of the FAMES in the sample with those of the Supelco 37 component FAME Mix reference standards from Sigma-Aldrich Chemical Co. (USA). The results were expressed as weight percentages of individual fatty acids in the oil sample.

2.5 Determination of peroxide value

The peroxide value was determined using the AOCS official method Cd 8-53 (American AOCS, 1990c). In this method, a weighed sample of oil (approximately 5.0 g) was dissolved in a mixture of acetic acid and chloroform (3:2, v/v). The resulting solution was then titrated with a standardized sodium thiosulfate (Na₂S₂O₃) solution, using potassium iodide (KI) as the catalyst and starch as an indicator. The endpoint was reached when the blue color disappeared. The peroxide value was calculated based on the volume of sodium thiosulfate solution used in the titration and the weight of the oil sample. The peroxide value was expressed as milliequivalents of active oxygen per kg of oil (meq O₂/kg oil). The peroxide value can be calculated using the following formula: Peroxide Value (PV) = (V \times N \times 1000)/W, where V is the volume (mL) of the standardized sodium thiosulfate solution used in the titration, N is the normality of the sodium thiosulfate solution, and W is the weight (g) of the oil sample.

2.6 Determination of p-anisidine value

The p-anisidine value was determined using the AOCS official method Cd 18-90 (AOCS, 1990d). In this method, a weighed sample of oil (approximately 0.2 g) was dissolved in anhydrous isooctane. A portion of the resulting solution was mixed with the p-anisidine reagent, and the absorbance of the solution was measured at 350 nm using a UV-visible spectrophotometer (Lambda EZ201 UV-VIS spectrophotometer; Perkin Elmer, USA). A reference solution, containing only the oil sample and isooctane, was prepared and measured simultaneously. The p-anisidine value was calculated based on the difference in absorbance between the sample and reference solutions, the volume of the sample solution, and the weight of the oil sample. The p-anisidine value was expressed as the increase in absorbance per 1 g of oil sample. The p-

anisidine value can be calculated using the following formula: p-Anisidine Value (AnV) = (25 \times (A_{sample} - A_{reference})) / W, where A_{sample} is the absorbance of the sample solution, A_{reference} is the absorbance of the reference solution, and W is the weight (g) of the oil sample.

2.7 Determination of tocopherol isomer contents

The tocopherol content was determined using the AOCS Official Method Ce 8-89 (AOCS, 1989a). In this method, a weighed sample of oil was saponified with a potassium hydroxide (KOH) solution in ethanol to liberate the tocopherols from the oil matrix. After cooling, the tocopherols were extracted from the saponified mixture using n-hexane. The hexane extract was then evaporated to dryness under a nitrogen stream and re-dissolved in methanol. The tocopherols in the sample were analyzed using high-performance liquid chromatography (HPLC) with an Agilent 1100 series system, equipped with a reverse-phase C18 GP column (4.6 \times 250 mm, 3 μ m, Kanto Chemical Co., Inc., Japan) and an FLD G1321A fluorescence detector. The mobile phase typically consisted of a mixture of methanol, acetonitrile, and dichloromethane (50:44:6, v/v/v), with the flow rate set at 1 mL/min. The excitation and emission wavelengths for the fluorescence detector were set at 295 nm and 330 nm, respectively. The tocopherol isomers (α , β , γ , and δ) were identified and quantified based on their retention times and peak areas in comparison to known tocopherol standards. The results were expressed as mg of each tocopherol isomer per g of oil (mg/g oil).

2.8 Determination of total phenolic compound

The total phenolic content was determined using the Folin-Ciocalteu method as described by Singleton *et al.* (1999), with slight modifications from a previous study (Chotimarkorn *et al.*, 2008). In this method, an aliquot of the extracted oil sample (approximately 5.0 g) was dissolved in 100 mL of hexane and transferred to a separating funnel. The oil sample was mixed with 40 mL of methanol and water (80:20, v/v) and shaken for 3 mins. The lower layer of methanol was removed, and the process was repeated twice before evaporating the methanol using a rotary evaporator N-N series, Eyela (Japan). The residue was dissolved in 1.0 mL of methanol. A 0.25 mL aliquot of the residual solution was mixed with 0.5 mL of Folin-Ciocalteu reagent, which was previously diluted tenfold with distilled water. The mixture was then incubated in the dark for 5 mins. After the incubation period, 0.4 mL of a 7.5% sodium carbonate (Na₂CO₃) solution was added to the mixture and allowed to stand at room temperature for 2 hrs to complete the color development. The absorbance of the

resulting blue-colored solution was measured at 765 nm using a UV-visible spectrophotometer (Lambda EZ201 UV/vis spectrophotometer; Perkin Elmer, USA) against a blank. A calibration curve was prepared using a series of gallic acid standard solutions at various concentrations (e.g., 10-200 mg/L). The absorbance of each standard solution was measured following the same procedure as for the oil sample. The total phenolic content of the oil sample was calculated by interpolating the absorbance value on the calibration curve and expressed as milligrams of gallic acid equivalents per gram of oil (mg GAE/g oil).

2.9 Determination of 2,2-diphenyl-1-picrylhydrazyl

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a widely used method to determine the antioxidant activity of oil samples, was performed according to the method described by Thaipong *et al.* (2006), with slight modifications. In this method, an aliquot of the extracted oil sample (approximately 5.0 g) was dissolved in 100 mL of hexane and transferred to a separating funnel. The oil sample was mixed with 40 mL of methanol and water (80:20, v/v) and shaken for 3 mins. The lower layer of methanol was removed, and the process was repeated twice before evaporating the methanol using a rotary evaporator N-N series, Eyela (Tokyo, Japan). The residue was dissolved in 1.0 mL of methanol. An aliquot of the methanolic extract (150 μ L) was mixed with 2.85 mL of DPPH solution. The reaction mixture was incubated in the dark at room temperature for 2 h. The absorbance of the reaction mixture was measured at a wavelength of approximately 515 nm using a UV-visible spectrophotometer (Lambda EZ201 UV/vis spectrophotometer; Perkin Elmer, USA) against a blank. During this time, antioxidants present in the oil sample reduced the DPPH radicals, leading to a decrease in the absorbance of the solution. Trolox was used to create a standard curve, and the antioxidant activity was expressed as μ g TE/g oil.

2.10 Statistical analysis

Analysis of variance (ANOVA) was performed using a completely randomized design and the least significant difference (LSD) test at a significance level of 0.05. Mean values with standard deviations (SD) were reported based on three replicates ($n = 3$).

3. Results and discussion

3.1 Acid value

Acid value is a widely used measure of the total amount of acid present in an oil, expressed as the number of mg of potassium hydroxide (KOH) required to neutralize one g of oil. This study aimed to investigate

the acid value changes during 6 months of storage at 25°C for three types of oil: oil from unroasted seeds, oil from roasted seeds at 60°C, and oil from roasted seeds at 80°C. The initial acid values did not show significant differences among the groups ($p > 0.05$), with values of 1.19 ± 0.12 , 1.34 ± 0.22 , and 1.45 ± 0.31 mg KOH/g for oil from unroasted seeds, oil from roasted seeds at 60°C, and oil from roasted seeds at 80°C, respectively (Figure 1). This result corresponds to previously reported findings, which showed that the acid value ranged from 0.5 to 4.7 mg KOH/g of sachu inchi oil (Chasquibol *et al.*, 2014). These initial values indicate that all three oils were of good quality, as the acid values were lower than the Codex maximum limits of 4.0 mg KOH/g for crude vegetable oils (Codex Alimentarius Commission, 2005). However, from the 2nd to the 6th month of storage, the acid value of the oil from unroasted seeds increased significantly compared to the oils from roasted seeds at 60°C and 80°C ($p < 0.05$). The acid values of the oil from unroasted seeds, roasted seeds at 60°C, and roasted seeds at 80°C after 6 months of storage were 5.02 ± 0.32 , 4.53 ± 0.29 , and 4.13 ± 0.28 mg KOH/g, respectively (Figure 1). After 4 months of storage, the acid value of the oil from unroasted seeds remained lower than the Codex maximum limit, with a value of 3.48 ± 0.22 mg KOH/g. However, after 5 months of storage, the acid values of the oil from roasted seeds at 60°C and 80°C were found to be 3.87 ± 0.27 mg KOH/g and 3.38 ± 0.33 mg KOH/g, respectively, which were lower than the Codex maximum limit for crude vegetable oils. These results demonstrate that the storage of oil from unroasted seeds led to a greater increase in acid value compared to the oils from roasted seeds, and the increase became significant after the 1st month of storage. These findings suggest that the oil from roasted seeds may have a longer

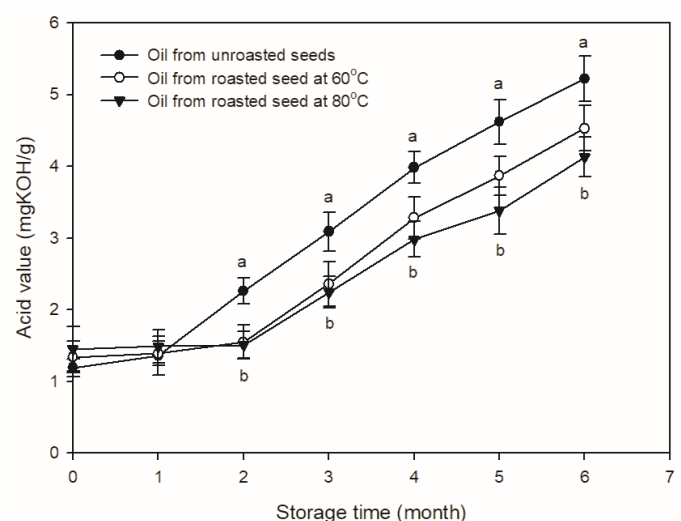


Figure 1. Acid value of oil from unroasted seeds and oil from roasted seeds at 60 and 80°C during 6 months of storage. Data is presented as mean \pm SD, $n = 3$. Data points with different superscripts are statistically significantly different within the same storage time ($p < 0.05$).

shelf life compared to the oils from unroasted seeds and that monitoring the acid value during storage is important. The higher acid value observed in oil from unroasted seeds compared to the oils from seeds roasted at 60°C and 80°C from the 2nd month of storage may be attributed to a combination of factors, including enzyme inactivation, the formation of antioxidant compounds during roasting, and changes in the seed's physical structure. These factors may result in enhanced oxidative stability and reduced acid value in oils obtained from roasted seeds compared to those from unroasted seeds.

3.2 Iodine value

The iodine value, a crucial indicator of oil unsaturation, quantifies the iodine amount absorbed by 100 g of oil. Figure 2 displays the iodine values of oil from unroasted seeds and oils from roasted seeds at 60°C and 80°C over a 6-month storage period. Roasting, conducted at 60°C and 80°C in this study, induces chemical and physical changes in seeds, impacting oil quality, composition, and iodine value (Mohamed Ahmed *et al.*, 2020). Initially, no significant differences ($p>0.05$) were observed among the groups, with values of 203.31 ± 2.38 , 198.87 ± 1.82 , and 197.77 ± 2.13 for oil from unroasted seeds, oil from roasted seeds at 60°C, and oil from roasted seeds at 80°C, respectively. The iodine values remained stable ($p>0.05$) for all three oils during the first 4 months of storage. However, from the 5th month onwards, the oil from unroasted seeds exhibited a significantly lower iodine value compared to the oils from roasted seeds ($p<0.05$). By the end of the 6-month storage period, the iodine values were 173.22 ± 3.15 , 181.66 ± 2.44 , and 182.47 ± 3.01 for oil from unroasted seeds, oil from roasted seeds at 60°C, and oil from roasted seeds at 80°C, respectively. These findings suggest that the oil from unroasted seeds had lower

oxidative stability, as evidenced by the decreased iodine value after the 5th month of storage. These findings are consistent with previous studies that have demonstrated a decrease in iodine value with increasing storage time of several oils (Abdellah and Ishag, 2012; Cobzaru *et al.*, 2016). Moreover, the formation of antioxidant compounds, such as Maillard reaction products and other heat-induced antioxidants, enhances the oxidative stability of oils from roasted seeds (Liu *et al.*, 2020). The study results validate the use of iodine value for evaluating oil unsaturation levels from unroasted and roasted seeds during storage.

3.3 Fatty acid profile

The fatty acid profile of oils is an important determinant of their nutritional value and shelf life. Table 1 shows the changes in the fatty acid profile of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during storage for 6 months. The initial percentages of palmitic acid, stearic acid, oleic acid, linoleic acid, and alpha-linolenic acid before storage ranged from 4.99 ± 0.21 - $5.04\pm 0.20\%$, 3.08 ± 0.21 - $3.14\pm 0.17\%$, 12.08 ± 0.67 - $12.20\pm 0.52\%$, 37.82 ± 0.65 - $38.42\pm 0.71\%$, and 40.05 ± 0.81 - $40.68\pm 0.69\%$, respectively. These values are consistent with previous reports by Hamaker *et al.* (1992) and Cisneros *et al.* (2014). During the first 3 months of storage, the percentages of palmitic acid, stearic acid, oleic acid, linoleic acid, and alpha-linolenic acid did not show significant differences ($p>0.05$) among the three groups of oils. However, after 4 months of storage, the palmitic acid and stearic acid content in the oil from unroasted seeds were significantly higher than in the oils from roasted seeds at 60°C and 80°C ($p<0.05$). Additionally, the alpha-linolenic acid content in the oil from unroasted seeds was significantly lower than in the oils from roasted seeds at 60°C and 80°C ($p<0.05$) after 4 to 6 months of storage. These results suggest that storage conditions may have a significant impact on the fatty acid profile of oils. The higher levels of palmitic acid and stearic acid in the oil from unroasted seeds after 4 to 6 months of storage may be due to a higher level of lipid oxidation compared to the oils from roasted seeds. Conversely, the lower level of alpha-linolenic acid in the oil from unroasted seeds may be due to its higher susceptibility to oxidation during storage. The results of this study indicate that the fatty acid profile of oils may be affected by storage conditions. The higher levels of palmitic acid and stearic acid and lower levels of alpha-linolenic acid in the oil from unroasted seeds after 4 to 6 months of storage suggest a higher level of lipid oxidation compared to the oils from roasted seeds. The decreasing content of polyunsaturated fatty acids (alpha-linolenic acid) during storage and the increasing content

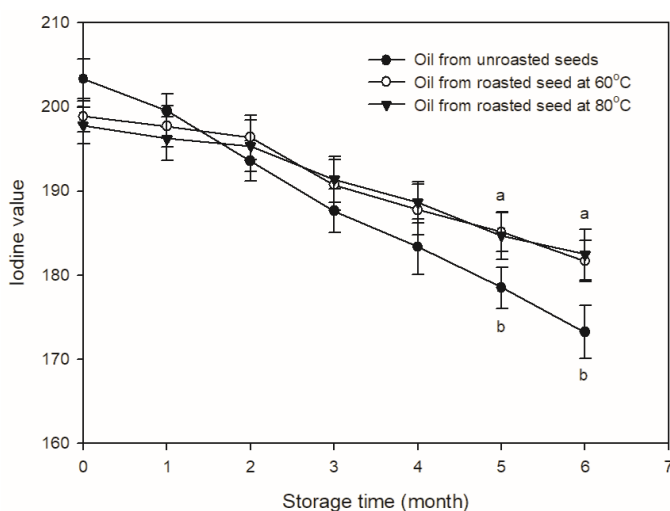


Figure 2. Iodine value of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during 6 months of storage. Data is presented as mean±SD, n = 3. Data points with different superscripts are statistically significantly different within the same storage time ($p<0.05$).

Table 1. Changes in fatty acid composition of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during 6 months storage.

Storage time (month)	Fatty acids (%)				
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	α -linolenic acid
Month 0					
Oil from unroasted seeds	4.99±0.21	3.11±0.13	12.08±0.67	37.99±0.76	40.68±0.69
Oil from roasted seeds at 60°C	5.11±0.19	3.08±0.21	12.13±0.52	37.82±0.65	40.47±0.72
Oil from roasted seeds at 80°C	5.04±0.20	3.14±0.17	12.20±0.43	38.42±0.71	40.05±0.81
Month 1					
Oil from unroasted seeds	4.91±0.19	3.15±0.14	12.11±0.56	37.86±0.66	40.82±0.73
Oil from roasted seeds at 60°C	4.98±0.17	3.12±0.19	12.17±0.41	37.79±0.56	40.57±0.66
Oil from roasted seeds at 80°C	5.02±0.19	3.19±0.24	12.16±0.32	38.40±0.65	40.08±0.74
Month 2					
Oil from unroasted seeds	5.02±0.24	3.48±0.21	12.34±0.48	37.89±0.64	40.03±0.69
Oil from roasted seeds at 60°C	5.24±0.21	3.24±0.23	12.33±0.25	37.85±0.49	39.93±0.53
Oil from roasted seeds at 80°C	5.11±0.21	3.26±0.25	12.21±0.36	38.29±0.57	39.97±0.79
Month 3					
Oil from unroasted seeds	5.56±0.16	3.99±0.22	12.65±0.39	37.94±0.46	38.52±0.57
Oil from roasted seeds at 60°C	5.42±0.19	3.77±0.16	12.39±0.32	37.87±0.59	39.46±0.47
Oil from roasted seeds at 80°C	5.23±0.22	3.67±0.19	12.31±0.28	38.18±0.48	39.77±0.82
Month 4					
Oil from unroasted seeds	5.99±0.19 ^a	4.59±0.35 ^a	12.97±0.42	38.02±0.57	37.25±0.49 ^b
Oil from roasted seeds at 60°C	5.56±0.22 ^b	3.51±0.24 ^b	12.49±0.27	37.91±0.41	39.11±0.37 ^a
Oil from roasted seeds at 80°C	5.34±0.24 ^b	3.48±0.22 ^b	12.35±0.34	38.13±0.53	39.53±0.69 ^a
Month 5					
Oil from unroasted seeds	6.48±0.25 ^a	4.87±0.32 ^a	13.06±0.55	38.11±0.62	36.38±0.58 ^b
Oil from roasted seeds at 60°C	5.74±0.32 ^b	3.64±0.18 ^b	12.46±0.42	38.01±0.62	38.59±0.44 ^a
Oil from roasted seeds at 80°C	5.48±0.17 ^b	3.52±0.27 ^b	12.41±0.24	38.04±0.49	39.82±0.61 ^a
Month 6					
Oil from unroasted seeds	6.79±0.24 ^a	5.18±0.34 ^a	13.22±0.28 ^a	38.24±0.81	35.46±0.77 ^b
Oil from roasted seeds at 60°C	5.98±0.23 ^b	3.75±0.24 ^b	12.86±0.31 ^a	38.03±0.75	37.80±0.49 ^a
Oil from roasted seeds at 80°C	5.64±0.28 ^b	3.66±0.21 ^b	12.44±0.23 ^b	37.99±0.72	38.75±0.72 ^a

Values are presented as mean±SD, n = 3. Values with different superscripts are statistically significantly different within the same storage time (p<0.05).

of saturated fatty acids (palmitic and stearic acid) may be due to the susceptibility of unsaturated fatty acids to oxidation (Li *et al.*, 2014). These findings suggest that the oils from roasted seeds may have a longer shelf life compared to the oil from unroasted seeds due to their fatty acid profile. The roasting process can help maintain the quality and shelf life of oils. This is evidenced by the higher content of health-promoting unsaturated fatty acids like alpha-linolenic acid and lower levels of saturated fatty acids like palmitic and stearic acid in the oil from roasted seeds compared to the oil from unroasted seeds.

3.4 Peroxide value

The peroxide value is an important parameter for assessing the oxidative stability of oils during storage.

The changes in the peroxide value of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during storage for 6 months are shown in Figure 3. The initial peroxide values of the oils ranged from 1.62±0.09 to 2.13±0.17 meqO₂/kg oil, which is less than the maximum limit of 15.0 meqO₂/kg oil set by the Codex Alimentarius standard for cold press oil (Codex Alimentarius Commission, 2005). The Codex Alimentarius standard provides guidelines to ensure the quality and safety of cold-pressed oils. During the first month of storage, the peroxide values did not show significant differences (p>0.05) among the three groups of oils, indicating the induction period of lipid oxidation. However, from 2 to 4 months of storage, the peroxide value of the oil from unroasted seeds was significantly higher than the oils from roasted seeds at 60°C and 80°C (p<0.05). The peroxide value of the oil from unroasted

seeds reached its highest level at 9.92 ± 0.24 meqO₂/kg oil after 4 months of storage before slightly decreasing to 9.02 ± 0.33 meqO₂/kg oil at the end of the 6-month storage period. This suggests that at 4 months of storage, the formation of lipid hydroperoxides in the oil from unroasted seeds was lower than the decomposition of these hydroperoxides to produce secondary lipid peroxidation products (Andersen and Skibsted, 2002). Furthermore, at the end of the 6-month storage period, the peroxide values of the oil from roasted seeds at 60°C and 80°C were 9.56 ± 0.28 and 8.57 ± 0.32 meqO₂/kg oil, respectively. These results suggest that the oil from unroasted seeds may have a shorter shelf life compared to the oils from roasted seeds due to its higher susceptibility to lipid oxidation during storage. The lower peroxide values observed in the oils from roasted seeds indicate a higher level of oxidative stability compared to the oil from unroasted seeds. The storage conditions can significantly affect the peroxide value of oils, with the oil from unroasted seeds displaying a higher susceptibility to lipid oxidation and a shorter shelf life than the oils from roasted seeds.

oils from roasted seeds at 60°C and 80°C ($p > 0.05$). However, starting from the 3rd month of storage, the p-anisidine value of the oil from unroasted seeds increased more rapidly than that of the oils from roasted seeds at 60°C and 80°C ($p < 0.05$). The formation of secondary lipid peroxidation products was supported by a previous report, which showed that when the rate of lipid hydroperoxide decomposition is higher than the rate of their formation, secondary lipid peroxidation products such as aldehydes increase rapidly (Chotimarkorn *et al.*, 2008). At the end of the 6-month storage period, the p-anisidine values were 9.23 ± 0.33 , 4.99 ± 0.36 , and 3.99 ± 0.19 for the oil from unroasted seeds, oil from roasted seeds at 60°C, and oil from roasted seeds at 80°C, respectively. These results suggest that the oil from unroasted seeds may have undergone greater lipid oxidation during storage, resulting in the formation of higher levels of secondary lipid peroxidation products compared to the oils from roasted seeds. The lower p-anisidine values in the oils from roasted seeds at 60°C and 80°C indicate a higher level of oxidative stability, which may be due to the protective effect of roasting on the oil. Roasting can lead to the degradation of oxidation-prone compounds and the formation of antioxidant compounds through reactions such as the Maillard reaction (Liu *et al.*, 2020), which can effectively inhibit lipid oxidation in roasted seed oils.

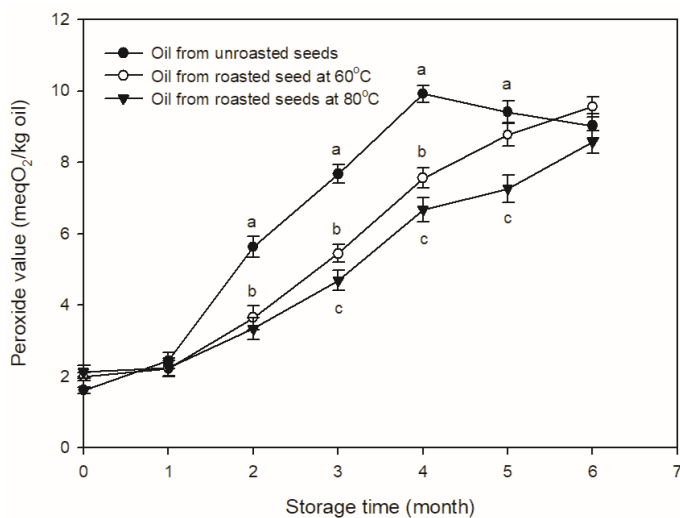


Figure 3. Peroxide value of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during 6 months of storage. Data is presented as mean \pm SD, n = 3. Data points with different superscripts are statistically significantly different within the same storage time ($p < 0.05$).

3.5 p-anisidine value

The p-anisidine value is a measure of secondary lipid peroxidation products in oils that can affect their flavor and odor. Figure 4 illustrates the changes in p-anisidine value for oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during a 6-month storage period. The initial p-anisidine values of the oils ranged from 1.52 ± 0.21 to 1.69 ± 0.23 , consistent with the reported range of values for sacha inchi seed oils (Cisneros *et al.*, 2014). During the first 2 months of storage, no significant differences in p-anisidine value were observed between the oil from unroasted seeds and the

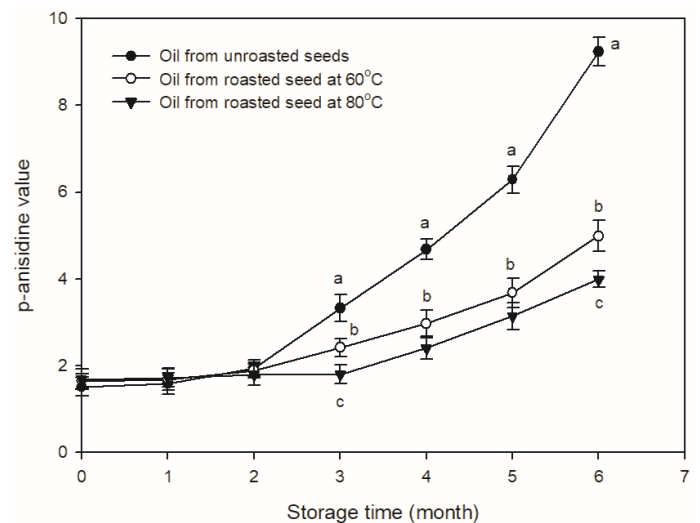


Figure 4. p-anisidine value of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during 6 months of storage. Data is presented as mean \pm SD, n = 3. Data points with different superscripts are statistically significantly different within the same storage time ($p < 0.05$).

3.6 Tocopherol isomer contents

The study aimed to investigate the changes in tocopherol isomer content of oil extracted from unroasted seeds and oil from roasted seeds at 60°C and 80°C during a 6-month storage period. Figure 5 shows that the main tocopherols found in the oils were alpha, gamma, and delta-tocopherol, consistent with previous

studies and reports (Cisneros *et al.*, 2014; Wang *et al.*, 2018). The tocopherol isomer contents in oil are important because they have different antioxidant effects (Madhavi *et al.*, 1996). The initial content of alpha, gamma, and delta-tocopherol in the oils ranged from 4.34 ± 0.32 - 4.67 ± 0.19 , 150.98 ± 2.46 - 152.66 ± 2.34 , and 87.06 ± 1.13 - 88.45 ± 1.27 mg/100 g oil, respectively. The results showed that there was no significant difference in the alpha-tocopherol content among the three groups of oil until 2 months of storage. During the 6-month storage period, the content of tocopherol isomers tended to decrease with time. This result is consistent with previous reports in several studies that observed decreasing tocopherol isomer contents during oil storage (Shendi *et al.*, 2019b; Shendi *et al.*, 2020). Interestingly, between 3 and 6 months of storage, the alpha-tocopherol content in oil from unroasted seeds was significantly lower than that in oil from roasted seeds at 60°C and 80°C. One possible reason behind this observation could be that the roasting process degrades oxidation-prone compounds or forms new antioxidant compounds that help preserve tocopherol content in the roasted seeds during storage. This effect could contribute to the higher alpha and gamma-tocopherol content in oil from roasted seeds, as compared to oil from unroasted seeds. At the end of the 6-month storage period, the alpha-tocopherol content in oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C was 1.19 ± 0.08 , 1.55 ± 0.11 , and 1.81 ± 0.13 mg/100 g oil, respectively (Figure 5A). The gamma-tocopherol isomer was found to be the main isomer in all three groups of oil, with an initial content ranging from 150.98 ± 2.46 to 152.66 ± 2.34 mg/100 g oil. The gamma-tocopherol content of oil from unroasted seeds and oil from roasted seeds did not differ significantly during the first 2 months of storage. However, from 3 to 6 months of storage, a significant decrease in gamma-tocopherol content was observed. At the end of 6 months of storage, the gamma-tocopherol content in oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C was 30.89 ± 1.41 , 39.75 ± 1.23 , and 52.28 ± 1.16 mg/100 g oil, respectively (Figure 5B). The delta tocopherol content in the three groups of oil remained stable during the first 3 months of storage, with no significant difference observed among the groups. Although the delta-tocopherol content decreased slightly until the end of the 6-month storage period, no significant difference was found among the three groups. At the end of 6 months of storage, the delta-tocopherol content in oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C was 66.74 ± 2.14 , 69.52 ± 2.41 , and 70.52 ± 1.36 mg/100 g oil, respectively (Figure 5C). Throughout the entire storage period, roasting seeds at 60°C and 80°C appeared to have a positive effect on preserving the alpha and gamma-tocopherol content

compared to oil from unroasted seeds, as the content remained higher. These findings suggest that roasting seeds before oil extraction could help maintain higher tocopherol contents during storage, potentially enhancing the antioxidant properties of the oil.

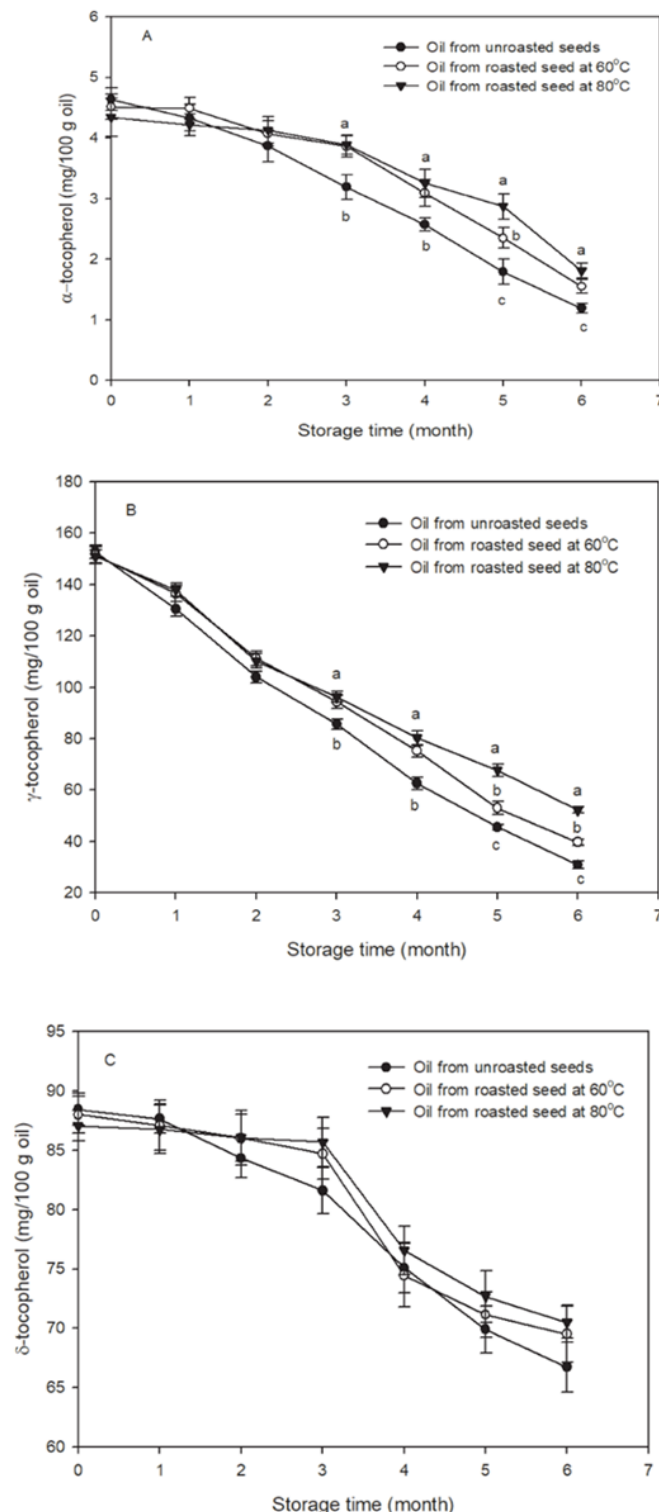


Figure 5. a (5A), g (5B) and d (5C)- tocopherol isomer contents of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during 6 months of storage. Data is presented as mean \pm SD, n = 3. Data points with different superscripts are statistically significantly different within the same storage time (p<0.05).

3.7 Total phenolic compounds

Phenolic compounds, known for their potent antioxidant properties, contribute to the oxidative stability of oils, extending their shelf life by delaying lipid oxidation and rancidity. Figure 6 illustrates the total phenolic content of oil extracted from unroasted seeds and roasted seeds at 60°C and 80°C. Initially, the total phenolic contents in oil from unroasted seeds, and oil from roasted seeds at 60°C and 80°C were 3.26 ± 0.13 , 6.59 ± 0.14 , and 9.88 ± 0.45 mg GAE/100 g oil, respectively. The increased total phenolic content in oil from roasted seeds can be attributed to several factors. As a thermal treatment, roasting may cause the breakdown of cell walls and cellular structures in seeds, leading to the release of bound phenolic compounds. This enhanced release increases the extractability of phenolic compounds into the oil, resulting in a higher total phenolic content in oils from roasted seeds. Consistent with these findings, thermal processing has been shown to increase total phenolic contents in several studies (Yu *et al.*, 2005; Locatelli *et al.*, 2010; Chandrasekara and Shahidi, 2011). Throughout the 6-month storage period, the total phenolic content of oil from roasted seeds at 80°C was significantly higher than that of oil from unroasted seeds and roasted seeds at 60°C ($p < 0.05$). Throughout the 6-month storage period, the total phenolic contents in all three groups of oil decreased with time. A similar decrease in total phenolic compounds was observed in olive oil during a 12-month storage period (Shendi *et al.*, 2019a). At the end of the 6-month storage period, the total phenolic content of oil from unroasted seeds, and oil from roasted seeds at 60°C and 80°C, were 1.08 ± 0.01 , 3.68 ± 0.17 , and 6.98 ± 0.24 mg GAE/100 g oil, respectively. The decline in total phenolic content across all groups during the 6-month

storage period suggests that storage conditions may influence the phenolic content of oils. The significantly higher total phenolic content in oil from roasted seeds at 80°C, compared to oil from unroasted seeds and roasted seeds at 60°C, may be attributed to the effect of roasting temperature on enhancing total phenolic content in oil. This could lead to a greater retention of phenolic compounds, which are renowned for their antioxidant properties and potential health benefits.

3.8 DPPH scavenging activity

The DPPH radical scavenging activity of the oil was investigated during storage since it serves as an indicator of the oil's antioxidant capacity. A high DPPH scavenging activity implies a strong ability to neutralize free radicals, which can help delay lipid oxidation and prolong the oil's shelf life. Initially, the DPPH levels in oil from unroasted seeds, oil from roasted seeds at 60°C, and oil from roasted seeds at 80°C were 55.23 ± 0.38 , 68.91 ± 0.37 , and 79.45 ± 0.45 µg Trolox equiv/g oil, respectively (Figure 7). Throughout the 6-month storage period, the DPPH level in oil from roasted seeds at 80°C remained significantly higher than in oil from unroasted seeds and oil from roasted seeds at 60°C ($p < 0.05$). During this time, DPPH levels in all three groups of oil slightly decreased as storage time increased. At the end of the 6-month storage period, the DPPH levels in oil from unroasted seeds, oil from roasted seeds at 60°C, and oil from roasted seeds at 80°C were 33.46 ± 0.18 , 47.99 ± 0.32 , and 59.87 ± 0.36 µg Trolox equiv/g oil, respectively. The notably higher DPPH levels in oil from roasted seeds at 80°C, compared to oil from unroasted seeds and oil from roasted seeds at 60°C, may be ascribed to the protective effect of roasting on the oil's antioxidant capacity. Monitoring DPPH activity in oil

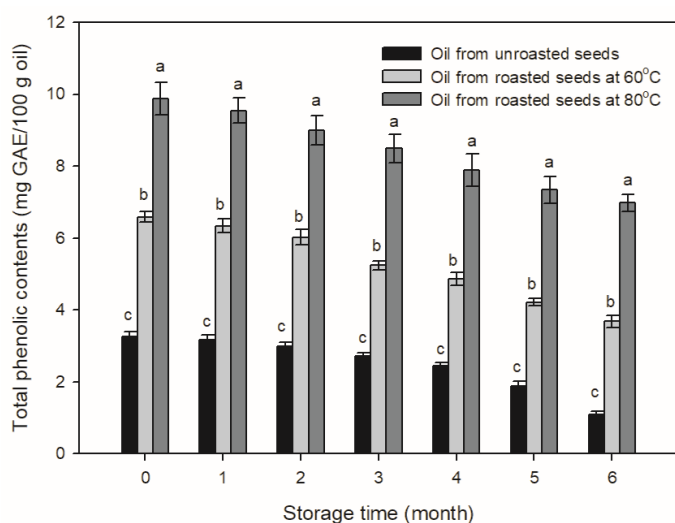


Figure 6. Total phenolic content of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during 6 months of storage. Data is presented as mean \pm SD, n = 3. Bars with different superscripts are statistically significantly different within the same storage time ($p < 0.05$).

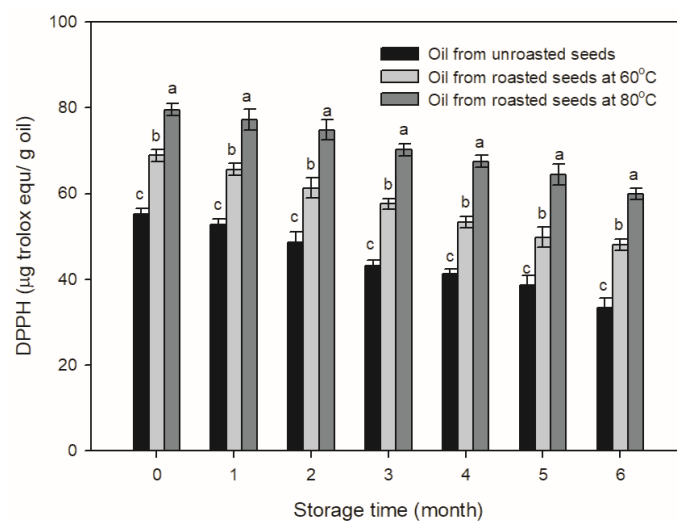


Figure 7. DPPH free radical scavenging of oil from unroasted seeds and oil from roasted seeds at 60°C and 80°C during 6 months of storage. Data is presented as mean \pm SD, n = 3. Bars with different superscripts are statistically significantly different within the same storage time ($p < 0.05$).

during storage can assist manufacturers and consumers in better understanding the optimal storage conditions and duration needed to preserve the oil's antioxidant potential and overall quality. By the end of the storage period, oil from roasted seeds at 80°C exhibited the highest DPPH level among the samples. This can be attributed to its higher content of total phenolic compounds compared to oil from unroasted seeds and oil from roasted seeds at 60°C. Phenolic compounds are renowned for their potent antioxidant properties, which can effectively neutralize free radicals and help safeguard the oil from oxidative deterioration. As a result, the higher concentration of total phenolic compounds in oil from roasted seeds at 80°C is likely responsible for its enhanced DPPH radical scavenging activity. This finding suggests that roasting seeds at a higher temperature (80°C) may contribute to preserving the oil's antioxidant capacity, resulting in an improved shelf life and overall quality.

4. Conclusion

In conclusion, this study examined the influence of roasting temperature on the shelf life and antioxidant properties of cold-pressed sacha inchi oil over a 6-month storage period. The findings revealed that roasting seeds at a higher temperature (80°C) positively impacted the oil's quality and oxidative stability, as indicated by lower peroxide and p-anisidine values, and elevated levels of total phenolic content and DPPH scavenging activity, in comparison to oil from unroasted seeds and oil from seeds roasted at 60°C. Moreover, the fatty acid profile analysis showed that oils from roasted seeds retained higher amounts of alpha-linolenic acid and lower quantities of saturated fatty acids throughout storage, suggesting reduced susceptibility to lipid oxidation. The enhancement in antioxidant properties and oxidative stability of oil obtained from seeds roasted at 80°C can be attributed to the greater phenolic content and DPPH scavenging activity, both of which are known to contribute to the oil's overall antioxidant capacity. This study, conducted in Thailand, underscores the significance of optimizing roasting conditions to boost the shelf life and nutritional value of cold-pressed sacha inchi oil. Additionally, the conclusion highlights the potential benefits of these findings for both consumers and the oil industry, offering valuable insights into the production of premium-quality and stable Sacha Inchi oil, ultimately promoting its potential health advantages and market appeal.

Conflict of interest

The authors declare no conflict of interest.

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