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Research Article

Impact of Roasting on Fatty Acids, Tocopherols, Phytosterols, and Phenolic Compounds Present in *Plukenetia huayllabambana* Seed

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The effect of roasting of *Plukenetia huayllabambana* seeds on the fatty acids, tocopherols, phytosterols, and phenolic compounds was evaluated. Additionally, the oxidative stability of the seed during roasting was evaluated through free fatty acids, peroxide, and *p*-anisidine values in the seed oil. Roasting conditions corresponded to 100, 120, 140, and 160°C for 10, 20, and 30 min, respectively. Results indicate that roasting temperatures higher than 120°C significantly affect the content of the studied components. The values of acidity, peroxide, and *p*-anisidine in the sacha inchi oil from roasted seeds increased during roasting. The treatment of 100°C for 10 min successfully maintained the evaluated bioactive compounds in the seed and quality of the oil, while guaranteeing a higher extraction yield. Our results indicate that *P. huayllabambana* seed should be roasted at temperatures not higher than 100°C for 10 min to obtain snacks with high levels of bioactive compounds and with high oxidative stability.

1. Introduction

Five species belonging to the genus *Plukenetia* have been identified in Peru. These species correspond to *P. volubilis* (commonly known as sacha inchi, SI), *P. brachybotrya*, *P. polyadenia*, *P. loretensis*, and recently *Plukenetia huayllabambana,* all proceeding from the Amazonian Region. *P. huayllabambana* has only been found in the upper Amazon Region, at altitudes above 1200 m [1, 2], displaying some similar characteristics to *P. volubilis* and differing in its small number of stamens, stylar column length, and very large seeds, with pronounced ridges. Not only morphological but also molecular and physicochemical differences have been reported between *P. volubilis* and *P. huayllabambana* [2–4].

Sacha inchi seed has extensively been studied as a rich source of oil (35–60%) and proteins (~27%). Sacha inchi oil is characterized by its high content of polyunsaturated fatty acids (PUFA), mainly α-linolenic and linoleic acids (~82% of the total oil content), and also by presenting high levels of bioactive compounds such as tocopherols, phytosterols, and phenolic compounds [5–7]. The amino acid profile of sacha inchi protein showed a relatively high level of cysteine, tyrosine, threonine, and tryptophan [8]. *P. huayllabambana*, similar to *P. volubilis*, is considered as an important source of oil and good nutritional quality protein [4, 9]. *P. huayllabambana* seeds showed higher oil content (44.1–54.3%) than *P. volubilis* seeds (35.4–49.0%) [3, 4], showing also higher content of α-linolenic acid (51.3%) and lower content of linolenic acid (26.6%) when compared to *P. volubilis* (with 45.6 and 32.6%, resp.). However, the total PUFA in both species was very similar. Important contents of γ-tocopherol and δ-tocopherol as well as phytosterols have been found in both species [3, 4]. Due to these characteristics, *P. huayllabambana* oil is commercialized in the local market as sacha inchi oil.

Roasting is an important pretreatment in different oleaginous seeds destined to be consumed as snacks or previously to oil extraction from the seeds. This treatment can cause either desirable or undesirable changes in the physicochemical and nutritional characteristics of the seed and extracted oil [10]. Roasting modifies the cellular structure facilitating the extraction of antioxidants. It has been previously reported that roasted seeds yielded oil with higher content of polyphenols [11] and tocopherols [12].

The evaluation of the roasting conditions to obtain snacks from *P. huayllabambana* and the effect of the roasting conditions on the composition of the bioactive compounds present in the seed have not been studied yet now. Empirically, it is known *in situ* that sacha inchi seeds are roasted previous consumption to eliminate off-flavors and possibly antinutritional factors [13]. Thus, the main aims of this work were (i) to determine the contents of fatty acids, tocopherols, phytosterols, and phenolic compounds of *P. huayllabambana* seed at different roasting conditions (100–160°C for 10–30 min) and (ii) to evaluate the seed oxidative stability by measuring the free fatty acids, peroxide, and *p*-anisidine values in its oil. These results aim to promote the consumption of this new species as snacks, in local and/or international markets, enhancing its integral consumption and offering alternative sources of income for the Amazonian native population.

2. Materials and Methods

2.1. Sample Material and Roasting Conditions. *P. huayllabambana* seeds were obtained from Province of Rodríguez de Mendoza (Region of Amazonas) from Peru. Seeds (21 × 20 mm) were manually cleaned and selected. The seeds were roasted in an oven with air circulation at temperatures of 100, 120, 140, and 160°C for 10, 20, and 30 min. During roasting, seeds were periodically stirred. Each batch of seeds was composed of 1 kg and three replicates per condition were used. After each treatment, seeds were allowed to cool down to room temperature and the skin was manually removed to obtain the kernel. The kernels are referred to as seeds in this study. Cryogenic milling of the seed with liquid nitrogen to obtain a particle size <1 mm was carried out. Then, the seeds were kept in sealed bags with gas nitrogen and stored at −20°C in dark conditions until analysis. A control sample (seed without roasting treatment) was evaluated. Humidity according to AOAC (1995) [14] and oil yield (%) using the solvent extraction method (Section 2.2.1) were evaluated in all samples.

2.2. Sample Analysis

2.2.1. Oil Extraction. Seeds were submitted to 6 h extraction with petroleum ether using Soxhlet equipment. After the extraction process, the flask content was filtered and the filtrate was rapidly concentrated under nitrogen flow in a 30°C water bath. The obtained oil was placed in an oven at 30°C for 1 h, weighed, and stored in sealed amber glass vials. The vials were kept at −20°C until analysis. The oil extracted was used in the following analysis described in Sections 2.2.2, 2.2.3, and 2.2.5.

2.2.2. Physicochemical Characteristics. The free fatty acid (FFA) and peroxide value (PV) were determined by standard procedures [14]. The *p*-anisidine (*p*-AV) value was determined according to the recommended methods of IUPAC [15]. All these analyses were carried out in the *P. huayllabambana* oil.

2.2.3. Fatty Acid Content and Composition. The FA composition was determined by gas chromatography according to the method employed by Chirinos et al. [7]. The FAs of the oil samples were converted into methyl esters (FAMEs). FAMEs were separated by injecting 1 μL of the solution into a GC-2010 plus Shimadzu (Kyoto, Japan) equipped with a flame ionization detector FID-2010 and an autoinjector AOC-20i. The column used was a Restek Rt-2560 (Bellefonte, PA) (0.2 μm, 100 m × 0.25 mm ID). The oven temperature was programmed as follows: initially the temperature was 100°C (for 4 min), it then increased to 240°C at 3°C/min, and there was an isothermal period of 25 min at 240°C. The injector and detector temperatures were set at 225 and 245°C, respectively. High purity helium was used as carrier gas. FAMEs were identified and quantified by comparing their retention times to known previously injected standards. Results were expressed as g of fatty acid per 100 g of seed in dry matter (DM).

2.2.4. Tocopherol Content and Composition. The seeds were prepared following the methodology reported by Amaral et al. [16] with slight modifications. Briefly, 300 mg of sample and 100 μL of butylated hydroxytoluene (BHT) (10 mg in 1 mL of *n*-hexane) were homogenized for 1 min by vortex mixing, after the addition of each of the following reagents: ethanol (2 mL), extracting solvent (*n*-hexane, 4 mL), and saturated NaCl solution (2 mL). Then, the mixture was centrifuged at 4000 g for 4 min at 1°C and then the clear upper layer was recovered. The sample was reextracted twice using 2 mL of *n*-hexane. The combined extracts were taken to dryness under nitrogen, and the residue was reconstituted in a volume of 1.5 mL with *n*-hexane. The extract was dried with anhydrous sodium sulphate (0.5 g), centrifuged at 4000 g for 20 min at 1°C, and transferred to a dark vial for the subsequent HPLC analysis.

Samples were separated using a normal phase HPLC column on a Waters 2695 Separation Module (Waters, Milford, MA) equipped with a Waters 2475 multifluorescence detector and the Empower software. A YMC-Pack Silica Col (3 μm, 250 × 4.6 mm column (Kyoto, Japan)) and a 4.0 × 2.0 mm guard column were used for tocopherol separation at 35°C. The mobile phase was composed of *n*-hexane/2-propanol/acetic acid (1000/6/5, v/v/v). A solvent flow rate of 1.4 mL/min under isocratic conditions was used. 10 μL of sample was injected. The fluorescence detector was programmed at the excitation and emission wavelengths of 290 and 330 nm, respectively. Tocopherols were identified and quantified by comparing their retention time to known

Table 1: Yield, free fatty acids, peroxide, and *p*-anisidine values of *P. huayllabambana* oil[1,2] submitted to different roasting conditions.

Roasting conditions	Oil yield (w/w, %)	Free fatty acids (as oleic acid, %)	Peroxide value (meq O_2/kg)	*p*-anisidine value
Control	44.1 ± 4.7^{f}	0.33 ± 0.03^{h}	6.3 ± 0.40^{g}	$0.21 \pm 0.02^{f,g}$
100°C				
10 min	$55.9 \pm 1.0^{c,d,e}$	0.56 ± 0.03^{g}	10.0 ± 0.49^{f}	0.25 ± 0.04^{f}
20 min	$61.2 \pm 3.1^{a,b}$	0.82 ± 0.07^{f}	11.6 ± 0.69^{e}	0.28 ± 0.04^{f}
30 min	62.4 ± 1.3^{a}	2.53 ± 0.22^{c}	16.5 ± 0.77^{d}	$0.69 \pm 0.08^{d,e}$
120°C				
10 min	54.2 ± 2.6^{e}	0.82 ± 0.07^{f}	19.8 ± 1.06^{c}	$0.21 \pm 0.07^{f,g}$
20 min	$55.8 \pm 2.3^{c,d,e}$	1.04 ± 0.08^{e}	$21.4 \pm 0.22^{b,c}$	$0.91 \pm 0.08^{c,d}$
30 min	$57.6 \pm 0.6^{b,c,d,e}$	$2.65 \pm 0.21^{b,c}$	$21.7 \pm 1.81^{b,c}$	$1.02 \pm 0.09^{b,c}$
140°C				
10 min	54.4 ± 4.5^{e}	0.84 ± 0.04^{f}	20.0 ± 0.51^{c}	$1.07 \pm 0.02^{b,c}$
20 min	$59.1 \pm 1.4^{a,b,c,d}$	1.15 ± 0.07^{e}	22.1 ± 0.30^{b}	$1.19 \pm 0.16^{a,b}$
30 min	$59.4 \pm 0.6^{a,b,c}$	$2.77 \pm 0.07^{a,b}$	22.5 ± 1.60^{b}	1.31 ± 0.04^{a}
160°C				
10 min	$55.0 \pm 1.0^{d,e}$	0.87 ± 0.03^{f}	25.6 ± 1.52^{a}	0.78 ± 0.08^{d}
20 min	$60.6 \pm 0.7^{a,b}$	1.39 ± 0.08^{d}	22.1 ± 2.51^{b}	$1.09 \pm 0.11^{a,b,c}$
30 min	62.1 ± 0.8^{a}	2.92 ± 0.03^{a}	22.1 ± 1.17^{b}	$1.21 \pm 0.09^{a,b}$

[1]Average ± SD of three replicates.
[2]Values within the same column followed by different letters stand for significant differences ($p < 0.05$).

previously injected standards. Results were expressed as mg per 100 g of seed (DM).

2.2.5. Phytosterol Content and Composition. Samples were prepared using the methodology reported by Duchateau et al. [17]. Briefly, 100 mg of oil was saponified with ethanolic KOH solution (1 mL) at 70°C for 50 min. The internal standard (1 mL of β-cholestanol, 10 mg/L in *n*-heptane) was added to each sample. The unsaponifiable fraction was extracted using liquid-liquid partitioning into 1 mL of distilled water and 5 mL of *n*-heptane. The organic phase was transferred to a test tube containing Na_2SO_4, and the extraction was repeated two times with 5 and 4 mL of *n*-heptane, respectively. The *n*-heptane extracts were combined and homogenized before injection into a gas chromatography system.

The phytosterol composition was determined by GC. Phytosterols were separated by injecting 2 μL of the extract to a GC-2010 plus Shimadzu (Kyoto, Japan) equipped with a flame ionization detector FID-2010. The column used was a Supelco SACTM–5 (St. Louis, MO, USA) (0.2 μm, 30 m × 0.25 mm ID). The oven temperature was programmed as follows: initially the temperature was 250°C (for 2 min), it then increased to 285°C at 25°C/min, there was an isothermal period of 285°C for 32 min, and there was a split ratio of 10. The injector and detector temperatures were set at 300°C. Helium was used as carrier gas. Phytosterols were identified and quantified by comparing their retention times to known previously injected standards. Results were expressed as mg per 100 g of seed (DM).

2.2.6. Total Phenolics Content. Total phenolics content (TPC) was determined following the method of Singleton and Rossi [18]. 0.5 g of defatted seeds was homogenized with 10 mL of 70% acetone to a uniform consistency and left at 4°C for 20 h before filtration. 500 μL of samples was combined with 1250 μL of 7.5% sodium carbonate solution and 250 μL of 1 N Folin-Ciocalteu reagent and allowed to react for 30 min at room temperature. Absorbance of the mixture was measured at 755 nm. Gallic acid was used as standard. TPC were expressed as milligram of gallic acid equivalents (GAE)/100 g of seed (DM).

2.3. Statistical Analysis. Quantitative data are presented as mean values with the respective standard deviation values corresponding to three replicates. All analyses were processed by the One-Way Analysis of Variance (ANOVA). A Duncan test was used to determine significant differences. Differences at $p < 0.05$ were considered as significant. Statgraphics Plus 5 (Statistical Graphics, Herndon, VA, USA) was used for all statistical tests.

3. Results and Discussion

3.1. Oil Yield, Free Fatty Acids, Peroxide, and p-Anisidine Values. Initial oil content in *P. huayllabambana* seed was 44.1%, and this value is close to the value reported by Muñoz Jáuregui et al. [9] (45.8–48.8%) and is higher than the value reported by Chirinos et al. [7] for 16 cultivars of *P. volubilis* (33.4–37.6%). Roasting at different conditions resulted in oil contents of 54.2–62.4% (Table 1). The highest oil yields were obtained at 100 and 160°C, respectively. Perren and Escher [19] indicated that dehydration destroys the native microstructure of the plant cellular compartments, increasing their porosity and thus increasing the intracellular diffusion of the oil and the compounds present in the oil.

The initial acidity of the oil of *P. huayllabambana* corresponded to 0.33% (as oleic acid). As the roasting conditions got more severe (higher temperature and longer times), the acidity of the oil progressively increased (Table 1), being an indication of the oxidative damage of the oil. The highest acidity values were obtained at temperatures of 100, 120, 140, and 160°C for 30 min (2.53–2.92%). Epaminondas et al. [20] reported that heat exposure and the associated oxidative processes with a hydrolytic rancidity affect the refraction index and contribute to the increase of the acidity of the oil.

PV indicates the oxidation stage of an oil or fat (mainly as evidence of primary oxidation) after processing or storage. The PV of the oil of *P. huayllabambana* in the control corresponded to 6.3 meq O_2/kg. The PV progressively increased with the roasting conditions, with the highest values at 160°C (Table 1). Roasting conditions of 100°C for 10 min resulted in a PV value of 10.0 meq O_2/kg; this value coincides with the PV values suggested by CODEX [21] as maximum limit in refined oils. Cisneros et al. [13] found that the PV of the oil obtained from the kernel of *P. volubilis* increased when roasted at 77°C for 9 min (9 meq O_2/kg) and decreased at 100°C for 9 min (0.5 meq O_2/kg). Vujasinovic et al. [22] found that roasting of pumpkin seeds at temperatures between 90 and 130°C increased the PV and Epaminondas et al. [20] found that roasting of linseed increased the oxidative processes associated with the oil provoking changes in the physicochemical characteristics including the PV, which increased after exposure to roasting conditions of 160°C for 15 min. The high values of PV might be the result of the degradation of the FA, especially the PUFA present in the oil of *P. huayllabambana*. Shahidi [23] indicates that oxidation of 0.4% of PUFA to hydroperoxides represents a change of hydroperoxides of 16 meq O_2/kg oil.

p-AV provides information related to the nonvolatile carbonyl compounds present in the oil during the oxidative process. This value is usually used to detect secondary oxidation products [24]. The oil obtained from the control seeds of *P. huayllabambana* control presented *p*-AV of 0.21. The *p*-AV slowly increased as the roasting temperature reached 160°C (Table 4). These results indicate that severe roasting conditions produce an increase of secondary oxidation products due to degradation of hydroperoxides.

3.2. Fatty Acids Profile and Content. The content and fatty acid profile of the seeds of *P. huayllabambana* submitted to the different roasting conditions are presented in Table 2. The most representative fatty acids in order of importance corresponded to α-linolenic acid > linoleic acid > oleic acid > palmitic acid > stearic acid with percentages of participation of 54.1, 25.8, 10.3, 4.3, and 5.3% of total FA, respectively. These same fatty acids in this species have been previously reported [3, 4, 9] with percentages of participation of 51.3–54, 26.6–29.3, 9.33–9.80, 1.9–3.7, and 5.1–6.6%, respectively. Roasting triggered an increase in the FA content of the seeds compared to the control with values of 20.0–52.1, 0–10, 18.7–39.5, 25.8–44.1, and 23.4–35.7% for palmitic, stearic, oleic, linoleic, and α-linolenic acids, respectively. Similarly, SFA and PUFA increased reaching values of 26.6–48.8 and 26.9–38.8%, respectively. The evaluated roasting conditions favoured the dilatation of the plant cells of the seeds facilitating the availability of the oil for extraction and thus of the fatty acids (FAs). Higher contents of FA in the seeds were found in those treatments which involved 100°C for 10–30 min. The percentage (%) of participation of the FA in the control submitted to the different roasting conditions remained unaltered with contents of palmitic, stearic, oleic, linoleic, and α-linolenic acids, SFA, and PUFA within the range of 5.6–6.0, 3.8–4.2, 10.2–10.6, 25.8–27.2, 52.8–54.2, 9.0–9.7, and 79.6–80.8%, respectively. Similar results were reported in previous studies with different oleaginous seeds. In a previous reported study on *P. volubilis* kernel destined to obtain oil and submitted to 77, 85, and 100°C for 9 and 10 min, it was observed that these conditions did not trigger substantial changes in the fatty acid profile of the oil in comparison with the sample without roasting [13]. Additionally, Epaminondas et al. [20] found that roasting of linseed (~43.2% α-linolenic acid in the oil) at 160°C for 15 min did not induce substantial changes in the fatty acid composition. Lee et al. [25] found that safflower seeds (~81% linolenic acid in the oil) submitted to temperatures of 140, 160, and 180°C for 16–24 min did not induce any significant change in the fatty acid composition. The slight changes in the FA might be related to the cover of the seed during roasting which might have acted as a protective layer avoiding the direct contact of oxygen with the substrates that initiate the oxidative processes that trigger the changes in the oil. Additionally, the natural antioxidants present in the seeds might have exerted a protective effect in the integrity of the FA. In relation, natural compounds such as tocopherols and phenolic compounds have been effective as antioxidants in different oily systems [26, 27]; these compounds have been previously reported in *P. huayllabambana* [3].

3.3. Tocopherol Profile and Content. α-Tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol were found in the seed of *P. huayllabambana* (Table 3) the last two being very important representing 99.7% of the total tocopherol content. The same tocopherols have been previously reported in this species [3]. Tocopherols are recognized as potent lipophilic antioxidants. Schmidt and Pokorný [26] indicate that the tocopherol antioxidant activity in lipid systems follows this order: $\gamma > \delta > \alpha > \beta$; thus, the presence of high percentage of γ-tocopherol and δ-tocopherol constitutes a factor of antioxidant protection for the seed and the respective extracted oil. Tocopherol behavior after exposure to different roasting conditions is presented in Table 3. α-Tocopherol was negatively affected at roasting conditions of 140°C and 120°C for 30 min with respect to the control sample. However, a notorious increase of α-tocopherol was observed at 120°C for 20 min. β-Tocopherol increased after exposure to roasting conditions of 140°C for 30 min and to 160°C for all evaluated times, with values similar to the control for all the other roasting conditions. At roasting conditions of 100 and 120°C for 10 min, higher contents of γ-tocopherol with respect to the control were observed. Lower contents of γ-tocopherol were observed at 160°C. δ-Tocopherol followed similar behavior to

Table 2: Fatty acids content[1,2] of *P. huayllabambana* seed (g/100 g, DM) submitted to different roasting conditions.

Fatty acid	Control	Roasting conditions											
		100°C			120°C			140°C			160°C		
		10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
Palmitic	2.5 ± 0.0^{f}	3.4 ± 0.4^{b}	3.6 ± 0.4^{a}	3.8 ± 0.2^{a}	$3.0 \pm 0.1^{c,d,e}$	$3.1 \pm 0.1^{b,c,d}$	3.3 ± 0.1^{b}	$3.0 \pm 0.1^{c,d,e}$	3.3 ± 0.3^{b}	3.4 ± 0.3^{b}	$3.2 \pm 0.5^{b,c}$	3.6 ± 0.3^{a}	3.6 ± 0.0^{a}
Stearic	$2.0 \pm 0.4^{c,d,e}$	$2.0 \pm 0.1^{d,e}$	$2.2 \pm 0.1^{a,b}$	2.4 ± 0.0^{a}	$1.9 \pm 0.1^{e,f}$	$2.0 \pm 0.2^{d,e}$	$2.0 \pm 0.0^{c,d,e}$	$1.9 \pm 0.0^{d,e,f}$	$2.0 \pm 0.2^{c,d,e}$	$2.1 \pm 0.2^{a,b,c}$	$2.0 \pm 0.4^{c,d,e}$	2.3 ± 0.2^{a}	2.2 ± 0.0^{a}
Oleic	4.8 ± 0.1^{i}	6.3 ± 0.8^{d}	$6.6 \pm 0.7^{a,b}$	6.7 ± 0.4^{a}	$5.8 \pm 0.4^{e,f,g}$	$5.9 \pm 0.0^{e,f}$	$6.1 \pm 0.0^{d,e}$	$5.7 \pm 0.0^{f,g}$	$6.3 \pm 0.2^{b,c}$	$6.2 \pm 0.2^{c,d}$	$5.9 \pm 0.6^{e,f,g}$	$6.4 \pm 0.5^{b,c}$	$6.4 \pm 0.1^{a,b,c}$
Linoleic	12.0 ± 1.0^{i}	$16.0 \pm 2.7^{b,c,d}$	17.3 ± 3.0^{a}	17.3 ± 0.2^{a}	$15.3 \pm 0.8^{f,g}$	$15.7 \pm 0.7^{d,e,f}$	$15.9 \pm 0.1^{c,d,e}$	15.1 ± 0.1^{g}	$16.2 \pm 0.2^{b,c,d}$	$16.3 \pm 0.2^{b,c}$	$15.2 \pm 1.0^{f,g}$	$16.1 \pm 1.2^{b,c,d}$	16.5 ± 0.2^{b}
α-Linolenic	25.2 ± 0.1^{i}	$31.9 \pm 0.8^{c,d,e}$	34.2 ± 3.9^{a}	33.9 ± 0.1^{a}	30.8 ± 1.3^{e}	30.7 ± 1.3^{e}	31.5 ± 0.0^{d}	30.2 ± 0.1^{f}	$32.1 \pm 0.5^{b,c}$	$31.9 \pm 0.5^{c,d}$	$29.9 \pm 1.8^{f,g}$	$31.8 \pm 2.1^{c,d}$	32.6 ± 0.2^{b}
SFA*	$4.5 \pm 0.3^{f,g}$	$5.3 \pm 0.8^{b,c}$	$5.8 \pm 0.7^{a,b}$	6.1 ± 0.4^{a}	$4.9 \pm 0.4^{d,e,f}$	$5.1 \pm 0.0^{b,c,d,e}$	$5.3 \pm 0.0^{b,c}$	$4.9 \pm 0.0^{c,d,e,f}$	$5.3 \pm 0.2^{b,c,d}$	$5.5 \pm 0.2^{a,b}$	$5.1 \pm 0.6^{b,c,d,e}$	$5.9 \pm 0.5^{a,b}$	$5.8 \pm 0.1^{a,b}$
PUFA**	37.1 ± 1.0^{h}	$47.9 \pm 6.4^{c,d}$	51.5 ± 6.8^{a}	51.1 ± 0.1^{a}	46.1 ± 2.1^{e}	46.4 ± 2.0^{e}	47.4 ± 0.0^{d}	45.3 ± 0.0^{f}	48.3 ± 0.7^{c}	48.1 ± 0.7^{c}	45.1 ± 2.8^{f}	$47.9 \pm 3.3^{c,d}$	49.1 ± 0.3^{b}

[1] Average ± SD of three replicates.
[2] Values within a row followed by different letters stand for significant differences ($p < 0.05$).
* Saturated fatty acids.
** Polyunsaturated fatty acids.

Table 3: Tocopherol content[1,2] of *P. huayllabambana* seed (mg/100 g of seed, DM) submitted to different roasting conditions.

Tocopherol	Control	Roasting conditions											
		100°C			120°C			140°C			160°C		
		10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
α-Tocopherol	$0.090 \pm 0.01^{b,c}$	$0.083 \pm 0.0^{b,c,d,e,f}$	$0.075 \pm 0.0^{c,d,e,f,g}$	$0.068 \pm 0.02^{d,e,f,g}$	0.114 ± 0.03^{a}	$0.079 \pm 0.01^{c,d,e,f,g}$	$0.072 \pm 0.01^{e,f,g}$	$0.084 \pm 0.02^{d,e,f,g}$	$0.072 \pm 0.01^{e,f,g}$	$0.075 \pm 0.02^{b,c,d,e}$	$0.080 \pm 0.00^{b,c,d,e,f}$	0.095 ± 0.01^{b}	$0.089 \pm 0.01^{b,c,d}$
β-Tocopherol	0.005 ± 0.00^{d}	0.005 ± 0.00^{d}	0.005 ± 0.00^{d}	0.005 ± 0.00^{d}	0.008 ± 0.00^{d}	0.005 ± 0.00^{d}	0.005 ± 0.00^{d}	0.006 ± 0.00^{d}	0.005 ± 0.00^{d}	$0.025 \pm 0.00^{b,c}$	0.021 ± 0.00^{c}	$0.027 \pm 0.01^{b,c}$	$0.029 \pm 0.01^{a,b}$
γ-Tocopherol	$32.52 \pm 3.40^{a,b,c}$	40.12 ± 3.91^{a}	$36.25 \pm 4.50^{a,b}$	$32.92 \pm 2.64^{a,b,c}$	41.16 ± 4.80^{a}	$35.10 \pm 2.19^{a,b}$	$28.76 \pm 0.80^{b,c}$	$33.25 \pm 1.74^{a,b,c}$	34.46 ± 0.33^{c}	$32.35 \pm 4.94^{a,b,c}$	23.63 ± 1.94^{d}	24.21 ± 0.32^{d}	$28.29 \pm 1.45^{b,c}$
δ-Tocopherol	$14.96 \pm 0.96^{b,c}$	17.57 ± 1.57^{a}	$16.03 \pm 2.11^{a,b}$	$14.51 \pm 0.84^{a,b}$	17.42 ± 1.09^{a}	$15.46 \pm 0.81^{b,c}$	12.43 ± 0.35^{d}	$13.70 \pm 0.30^{c,d}$	$15.07 \pm 0.39^{b,c}$	$14.01 \pm 1.65^{c,d}$	4.64 ± 1.13^{e}	5.22 ± 0.78^{e}	5.94 ± 0.35^{e}
Total tocopherols	$47.58 \pm 2.46^{b,c}$	57.79 ± 5.48^{a}	$52.36 \pm 6.62^{a,b}$	$47.51 \pm 3.48^{b,c}$	58.70 ± 5.76^{a}	50.65 ± 3.01^{b}	$41.26 \pm 1.16^{c,d}$	$47.05 \pm 1.82^{b,c}$	49.62 ± 0.70^{b}	$46.46 \pm 6.60^{b,c}$	28.37 ± 3.07^{f}	29.55 ± 0.52^{f}	$36.33 \pm 3.87^{d,e}$

[1] Average ± SD of three replicates.

[2] Values within a row followed by different letters stand for significant differences ($p < 0.05$).

Table 4: Phytosterols (mg/100 g, DM) and total phenolic contents (mg GAE/100 g, DM)[1,2] of *P. huayllabambana* seed submitted to different roasting conditions.

Compound	Control	Roasting conditions											
		100°C			120°C			140°C			160°C		
		10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
						Phytosterols							
Campesterol	$5.5 \pm 0.6^{a,b,c}$	$5.7 \pm 0.3^{a,b}$	$5.2 \pm 0.8^{a,b,c,d,e}$	$5.5 \pm 0.2^{a,b,c,d}$	$4.7 \pm 0.2^{c,d,e,f}$	6.0 ± 0.7^{a}	$4.8 \pm 0.2^{b,c,d,e,f}$	$4.4 \pm 0.4^{e,f}$	$4.5 \pm 0.5^{e,f}$	$4.8 \pm 0.4^{c,d,e,f}$	3.9 ± 0.2^{f}	$4.6 \pm 0.4^{c,d,e,f}$	$4.8 \pm 0.7^{b,c,d,e,f}$
Stigmasterol	$24.8 \pm 1.8^{d,e}$	$31.8 \pm 1.0^{a,b,c}$	$33.1 \pm 2.0^{a,b}$	34.9 ± 1.0^{a}	$33.2 \pm 0.2^{a,b}$	$33.5 \pm 0.7^{a,b}$	$32.0 \pm 1.4^{a,b,c}$	$29.1 \pm 4.8^{b,c}$	$29.2 \pm 4.9^{b,c}$	$30.2 \pm 0.8^{b,c}$	$28.4 \pm 0.6^{c,d}$	$29.6 \pm 2.3^{b,c}$	$30.2 \pm 1.3^{b,c}$
β-Sitosterol	$60.5 \pm 5.1^{d,e,f,g}$	$70.1 \pm 6.2^{a,b,c}$	$63.7 \pm 2.9^{a,b,c}$	$66.3 \pm 4.0^{a,b}$	$61.0 \pm 0.2^{b,c,d,e}$	73.0 ± 4.9^{a}	$61.5 \pm 3.1^{c,d,e}$	$56.0 \pm 5.0^{d,e,f,g}$	$55.0 \pm 8.5^{e,f,g}$	$60.3 \pm 4.2^{c,d,e,f}$	$51.9 \pm 1.4^{f,g}$	$57.2 \pm 5.6^{d,e,f,g}$	$61.0 \pm 3.6^{c,d,e,f}$
Sum of evaluated phytosterols	$90.8 \pm 7.2^{d,e,f,g}$	$107.6 \pm 6.4^{a,b,c}$	$102.0 \pm 3.0^{a,b,c,d}$	$106.3 \pm 4.8^{a,b}$	$98.9 \pm 0.2^{b,c,d,e}$	112.5 ± 6.3^{a}	$98.3 \pm 4.4^{c,d,e}$	$89.6 \pm 9.5^{d,e,f,g}$	$88.7 \pm 13.9^{e,f,g}$	$95.4 \pm 5.3^{c,d,e,f}$	$84.3 \pm 1.7^{f,g}$	$91.5 \pm 6.0^{d,e,f,g}$	$96.0 \pm 3.1^{c,d,e,f}$
						Total phenolics							
Total phenolic content	$91.5 \pm 4.5^{b,c,d}$	104.7 ± 9.4^{a}	$100.6 \pm 6.5^{a,b}$	$99.9 \pm 6.9^{a,b,c}$	$92.1 \pm 3.2^{b,c,d}$	$96.9 \pm 6.2^{a,b,c}$	$94.5 \pm 4.6^{a,b,c}$	$95.9 \pm 6.5^{a,b,c}$	$90.0 \pm 3.4^{b,c,d}$	$97.9 \pm 5.3^{a,b,c}$	$90.8 \pm 1.8^{b,c,d}$	83.7 ± 2.4^{d}	$89.7 \pm 0.5^{c,d}$

[1] Average ± SD of three replicates.
[2] Values within a row with different letters stand for significant differences ($p < 0.05$).

γ-tocopherol. A considerable decrease of γ-tocopherol and δ-tocopherol at 160°C was obtained. The decrease in the content of γ-tocopherol has been previously reported in *P. volubilis* oil obtained from roasted seeds at 99–102°C with respect to a raw seed [13]. Total tocopherols exhibited the same trend displayed by γ-tocopherol and δ-tocopherol because they were the two most representative tocopherols of the seed. The increase of the tocopherols in the roasted seeds of *P. huayllabambana* could be due to thermal degradation of the cellular structures which led to better extraction conditions making the tocopherols more available in the oil [12]. Different trends have been reported in the literature regarding the effect of roasting on the tocopherol content in seeds and nuts rich in these compounds. Lee et al. [25] found that α-tocopherol, β-tocopherol, and γ-tocopherol content gradually increased in canola oil when seeds were roasted at 140 and 180°C, while Durmaz and Gökmen [28] reported that seeds of *Pistacia terebinthus* roasted at 180°C for 5–30 min presented losses of α-tocopherol, β-tocopherol, and δ-tocopherol, but γ-tocopherol slightly increased. The divergence in the different results implies that roasting can affect the structure and content of tocopherols in different ways, depending on the seed type, cellular constituents, and intensity of the thermal treatment, among others. Finally, it was observed that α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol participated with percentages of 0.14–0.19, 0.01–0.05, 68.4–70.7, and 29.1–30.6%, respectively, when the roasting conditions were within 100 and 140°C, being close to the control (0.18, 0.10, 68.3, and 31.4%, resp.). Increasing the roasting temperature to 160°C resulted in a notorious change in the degree of participation of the different tocopherols with increased amounts of α-tocopherol, β-tocopherol, and γ-tocopherol (0.22–0.28, 0.07–0.11, and 77.9–85.5%) in relation to the control, while δ-tocopherol significantly reduced its participation (15.0–17.7%).

3.4. Phytosterol Profile and Content. β-Sitosterol, campesterol, and stigmasterol were present in *P. huayllabambana* seed in quantities of 60.1, 24.8, and 5.5 mg/100 g seed (DM), respectively, summing up 90.8 mg/100 g (DM), β-sitosterol being the most representative. The three determined phytosterols have been previously reported in the same species [3] and in *P. volubilis* [6]. Roasting at different conditions resulted in differences in the content of the evaluated phytosterols (Table 4). Campesterol suffered slight changes as the roasting temperature was increased (140–160°C); higher contents were observed at 100°C and 120°C for 10 min. Stigmasterol content increased in all treatments compared to the control, with the conditions of 100 and 120°C for the different evaluated times (10–30 min) being the most favourable ones. β-Sitosterol displayed a marked increase at 100°C for 10–30 min and at 120°C for 10–20 min in comparison to the control and slightly decreased as the intensity of the thermal treatment was increased. Finally, the trend was followed by the sum of the three phytosterols submitted to different conditions of roasting, with increases at temperatures of 100 and 120°C but losses at temperatures close to 160°C with values close to the ones displayed by the control. Murkovic et al. [29] found that the content of sterols in the seeds of roasted pumpkin at 150°C up to 60 min resulted in a decrease at the beginning (10–20 min) but then progressively increased close to 60 min of roasting reaching values much higher than the control sample. The authors indicated that the observed trend might be due to the changes in humidity during roasting of the sample (results were presented in FW, different than in the present study) facilitating the extractability of the phytosterols. Finally, changes related to the participation of the different evaluated phytosterols revealed that the participation was maintained with respect to the control: campesterol, stigmasterol, and β-sitosterol representing 4.6–6.2, 29.6–36.9, and 54.1–69.1%, respectively, in comparison to the control (6.0, 27.3, and 66.6%, resp.).

3.5. Total Phenolic Content. The results related to the changes observed in the TPC of the seeds of *P. huayllabambana* submitted to different roasting conditions are displayed in Table 4. The control displayed a TPC content of 91.5 mg GAE/100 g seed (DM) or 84.6 mg GAE/100 g fresh weight (FW), with this value being higher than the values reported for almond, macadamia, and pine nuts (32–47 mg GAE/100 g, FW); however, higher values have been reported for Brazil and cashew nuts, hazelnuts, pecans, pistachios, and nuts (from 112 to 1625 mg/100 g, FW) [30, 31], and even higher values were reported for flax and safflower seeds (383 and 559 mg GAE/100 g, FW) [32]. Roasting did not significantly affect the integrity of the phenolic compounds. The amounts were either similar or slightly increased in comparison with the control sample (Table 4). The highest TPC was obtained at 100°C for 10 min (104.7 mg GAE/100 g seed, DM) and gradually decreased to values of 83.7 and 90.8 mg GAE/100 g (DM) as the temperature increased to 160°C. Vujasinovic et al. [22] found that the roasting process of pumpkin seeds at temperatures within the 90–130°C range for 30 and 60 min resulted in an increase in the phenolic content in the pumpkin oil. Similarly, Durmaz and Gökmen [28] observed that the TPC in the obtained oil from *Pistacia terebinthus* seeds at 180°C for 5–40 min decreased as the roasting time was prolonged. Both studies attributed the obtained results to the fact that roasting softens the cellular structures favouring the extraction of the phenolic compounds in the oil. Finally, as with the tocopherols, severe roasting conditions (140–160°C) resulted in lower values of TPC possibly due to the consumption of phenolic compounds acting as antioxidant compounds against oxidation.

4. Conclusions

The study of the roasting conditions of *P. huayllabambana* seeds to obtain snacks showed a marked effect in the fatty acids, tocopherols, phytosterols, and total phenolics compounds, showing similar, higher, or lower values than the control. Roasting favoured the extractability of bioactive compounds; however, temperatures higher than 120°C did not guarantee the integrity of these bioactive molecules. Additionally, the values of acidity, peroxide value, and *p*-anisidine value indicative of oxidative stability of the seed of

P. huayllabambana indicated that it is not advisable to roast the seeds at temperatures higher than 100°C for 10 min as to minimize the oxidative processes. Thus, roasting conditions of *P. huayllabambana* seed to obtain snacks which guarantees the integrity of the fatty acids, tocopherols, phytosterols, and total phenolic compounds and an adequate oxidative stability must not incur in processing conditions more severe than 100°C for 10 min under the evaluated conditions of this study. Same conclusion can be taken for oil extraction, given that the highest oil extraction yields were obtained at that condition (100°C for 10 min).

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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