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Chemical Composition and Glycemic Index of Brazilian Pine (*Araucaria angustifolia*) Seeds

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The seeds of Parana pine (*Araucaria brasiliensis* syn. *Araucaria angustifolia*), named pinhão, are consumed after cooking and posterior dehulling, or they are used to prepare a flour employed in regional dishes. Native people that live in the South of Brazil usually consume baked pinhão. As a result of cooking, the white seeds become brown on the surface due to the migration of some tinted compounds present in the seed coat. In this work, the proximate composition, minerals, flavonoids, and glycemic index (GI) of cooked and raw pinhão seeds were compared. No differences in moisture, lipids, soluble fiber, and total starch after boiling were found. However, the soluble sugars and P, Cu, and Mg contents decreased, probably as a consequence of leaching in the cooking water. Also, the boiling process modified the profile of the phenolic compounds in the seeds. No flavonols were detected in raw pinhão seeds. The internal seed coat had a quercetin content five times higher than that of the external seed coat; also, quercetin migrated into the seed during cooking. The internal seed coat had a high content of total phenolics, and seeds cooked in normal conditions (with the seed coat) showed a total phenolics content five times higher than that of seeds cooked without the seed coat. Cooking was then extremely favorable to pinhão seeds bioactive compounds content. The carbohydrate availability was evaluated in a short-term assay in humans by the GI. The GI of pinhão seeds cooked with the coat (67%) was similar to that of the seeds cooked without a coat (62%) and lower than bread, showing that cooking does not interfere with starch availability. The low glycemic response can be partly due to its high content of resistant starch (9% of the total starch).

KEYWORDS: Pinhão seeds; proximate composition; carbohydrates; flavonoids; glycemic index; *Araucaria angustifolia*; minerals

INTRODUCTION

Pinhão seeds of the tree *Araucaria angustifolia* (Parana tree), which belongs to the Coniferae group, are consumed in the South and Southeast of Brazil; they are mainly cooked in water but are also used as a raw flour for regional dishes or baked. South Brazilian natives (Caigangues and Guaranis) have consumed baked pinhão and pinhão flour for a long time, from prehistoric times until today, and especially during winter, they survived consuming only pinhão (*1*, *2*).

Besides the internal membrane that adheres to the seed, another very resistant coat involves the seed. Both of them are easily removed after the seeds are cooked in water. As a result, the white seeds become brown on their surface because of the migration of some tinted compounds present in both internal and external seed coats. The astringent taste and the brown color of the surface of cooked seeds (**Figure 1**) and of the water used in cooking indicate that the brown color could be due to oxidized polyphenolic compounds. Polyphenols occur widely throughout the plant kingdom and are important to human health, as they may have potential beneficial roles in cardiovascular disease and cancer, possibly associated with their antioxidant properties (*3*).

The literature about nutritional aspects of pinhão is very scarce. Wosiacki and Cereda (*4*, *5*) partially characterized the starch (which is supposed to be the main component) and suggested its utilization as an additive in low acid foods. Additionally, Datta, Figueiroa, and Lajolo (*6*, *7*) purified and characterized two major lectins (lectin I and lectin II) from pinhão. According to them, despite their different thermal stabilities, both lectins lost their activities as sugar-binding proteins at temperatures higher than 80 °C. Available data about the proximate composition of pinhão are not reliable because of limitations of the analytical methods used at the time of the studies. Despite being a starchy food, the glycemic response produced by the consumption of pinhão seeds has, also, never

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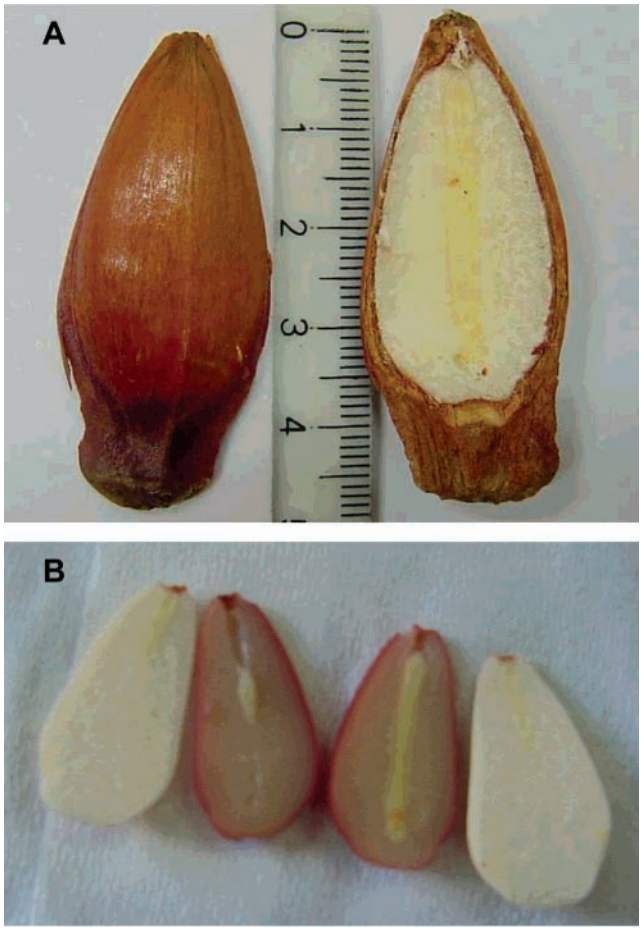

Figure 1. (**A**) External and internal view of raw pinhão seed with coat. (**B**) Pinhão cooked with coat (internal seeds) and cooked without coat (external seeds).

been evaluated. Also, there are no reports on polyphenol contents. In this work, the proximate compositions of raw and cooked pinhão seeds were compared. To estimate the influence of the migration of compounds from the coat to the seed on nutritional aspects, phenolic compounds and the glycemic index (GI) were also analyzed in seeds cooked with and without the coat.

MATERIAL AND METHODS

Material. Seeds of pinhão (*Araucaria brasiliensis* syn. *A. angustifolia*) were obtained from a local market in São Paulo, Brazil. The weight of the seeds varied between 7 and 9 g each. The seed coats (internal and external) corresponded to approximately 22% of the final weight. Pinhão seeds with (492 g, ~70 units) and without (500 g, ~90 units) coats were cooked in an open kettle, with 1 L of distilled water for, respectively, 1.5 and 1 h, until they were palatable. The water left after cooking (300 and 400 mL for seeds with and without coats, respectively) was collected for flavonoid analysis. Samples were sliced, frozen in liquid nitrogen, and stored at −80 °C. Samples for mineral analysis were lyophilized in order to concentrate the minerals.

Proximate Composition. The total protein content was determined by a semimicro Kjeldahl method according to AOAC procedure 2055 (*8*). The conversion factor used was %N × 6.25. The ash content was determined by incineration in a muffle furnace at 520 °C. The moisture content of the sample was calculated based on weight loss after the sample was heated in an oven at 105 °C. Lipids were extracted in a Soxhlet extractor without acid digestion according to an AOAC method (*8*).

Dietary Fiber (DF). The DF content was determined by the enzymatic−gravimetric method according to Prosky et al. (*9*).

Soluble Sugars. Soluble sugars were extracted three times with 80% ethanol at 80 °C. After centrifugation, the supernatants were combined and the ethanol was evaporated under vacuum by a speed-vac system. The soluble sugar content was analyzed by high-pressure liquid chromatography with pulse amperometric detection (HPLC-PAD; Dionex, Sunnyvale, CA), using a PA1 column (Dionex) in an isocratic run of 18 mM NaOH during 25 min. Total soluble sugars (TSS) were given as the sum of glucose, fructose, and sucrose values.

Total Starch. The total starch from frozen samples was solubilized in 0.5 M NaOH, and after neutralization with 0.5 M acetic acid, an aliquot was precipitated with 80% ethanol. The precipitated starch was hydrolyzed with amyloglucosidase (28 U/mL), and the resultant glucose was determined by the system glucose-oxidase/peroxidase/ABTS (450 nm), as described by Cordenunsi and Lajolo (*10*). The total starch was calculated as glucose × 0.9.

Resistant Starch (RS). The RS content was analyzed using the methodology proposed by Goñi et al. (*11*). The samples (100 mg) were hydrolyzed with 0.1 mL of pepsin (10 mg/mL of 0.1 M KCl/HCl buffer, pH 1.5) for 60 min at 40 °C. The available starch was hydrolyzed with 1 mL of α-amylase (40 mg/mL of 0.1 M Tris-Maleate buffer, pH 6.9) for 16 h at 37 °C. The RS residues were solubilized with 2 M KOH, and the soluble RS was hydrolyzed with 80 μL of amyloglucosidase (140 U/mL of 0.4 M sodium acetate buffer, pH 4.7) for 45 min at 60 °C. The glucose was determined using a glucose oxidase assay (GOD/POD/ABTS) as described in Total Starch. The RS was calculated as glucose × 0.9. A sample of cooked bean was used as the reference standard.

Amylose. Samples of isolated starch (about 6 mg) were weighed in a medium-pressure inox pan, and 30 μL of L-α-lysophosphatidylcholine 2% (w/w in water) was directly added. The pan was hermetically sealed and stored for 1 h at room temperature. The temperature was raised from 35 to 160 °C at 15 °C/min and kept at this temperature for 2 min. The temperature was then decreased to 35 °C at 10 °C/min. As a standard, 3 mg of potato amylose type III was used. The amylose content was calculated by the ratio between the exotherm measured for the sample and that of pure amylose during cooling according to Mestres et al. (*12*).

Isolation of Granule Starch. About 50 g of thickly sliced pinhão seeds was homogenized with 25 mL of extraction buffer consisting of an aqueous solution of 4% (w/v) sodium chloride, 1% (w/v) ascorbic acid, and 0.18% (w/v) ethylenediaminetetraacetic acid. The homogenate was filtered through cheesecloth. The slurry was centrifuged for 20 min at 5860*g* at 2 °C. The supernatant was discarded, and the precipitate was washed twice with a cold aqueous solution of 4% (w/v) sodium chloride. The precipitate was washed with acetone and dried at room temperature.

Minerals. Ca, Mg, Fe, Cu, and Zn contents were determined by AAE (Hitashi-540 Atomic Absorption Spectrophotometer) after wet digestion of the samples with HNO_3/H_2O_2 (5:1) at 100 °C. Tritisol Merck standards were used to give standard curves**,** and 0.1% La (as La_2O_3) was added to the samples and standard dilutions for Mg and Ca analyses.

Total Phenolics. The total phenols were determined with a modification of the method of Swain and Hillis (*13*), using the Folin−Ciocalteau reagent and catechin as the standard (Sigma Chemical Co., St. Louis, MO). A 0.25 mL aliquot of the appropriately diluted extracts (10 g ground sample/100 mL methanol) or standard solution (0.2 mg catechin/mL methanol) was mixed with 0.25 mL of Folin−Ciocalteau reagent and 2.0 mL of distilled water. After 3 min, 0.25 mL of saturated sodium carbonate was added with mixing. After the mixture was incubated for 30 min at 37 °C, the absorbance was read at 750 nm. The total phenolic contents were expressed as mg catechin equivalents (CE)/g fresh sample. All samples were analyzed in triplicate.

Flavonoids. Extraction of the flavonoids was performed according to the method of Price et al. (*14*), with some modifications. Frozen samples (~15 g, in duplicate) were extracted three times in a solvent mixture (100 mL the first time, 50 mL the next two times) containing methanol:water:acetic acid (final solvent composition 70:30:5, including the water of sample) at speed 5 for 1 min (Brinkmann Homogenizer, Polytron-Kinematica GmbH, Kriens-Luzern, Sweden), while cooled in ice. The homogenate was filtered under reduced pressure through filter

Table 1. Proximate Composition (g/100 g) (Mean ± SD of Triplicate Assays) of Raw and Cooked (with Coat) Pinhão Seeds

					DF			
pinhão	moisture	ash	protein	lipids	soluble	insoluble	starch	TSS
raw	49.50 ± 0.02	1.60 ± 0.01	3.57 ± 0.05	1.26 ± 0.07	0.63 ± 0.13	4.26 ± 0.20	36.28 ± 0.11	2.43
cooked	50.35 ± 0.71	1.41 ± 0.02	3.31 ± 0.05	1.26 ± 0.09	0.55 ± 0.18	5.17 ± 0.25	34.48 ± 0.72	0.64

Table 2. Carbohydrates (g/100 g) (Mean ± SD of Triplicate Assays) of Raw and Cooked (with Coat) Pinhão Seeds

	TSS			starch		
pinhão	glucose	fructose	sucrose	total	resistant	amylose
raw	2.25 ± 0.14	0.07 ± 0.01	0.11 ± 0.01	36.28 ± 0.11	ND[a]	29.6 ± 2.5
cooked	0.56 ± 0.11	0.03 ± 0.01	0.05 ± 0.01	34.48 ± 0.72	3.27 ± 0.05	ND[a]

[a] ND means not determined.

paper (Whatman no. 1), and the combined fractions were evaporated under vacuum at 40 °C to ~20 mL in a Rotavapor RE 120 (Büchi, Flawil, Sweden) and made up to 25 mL with water. An aliquot of 20 mL of the extract was added to a 1 g polyamide SC6 column (Macherey-Nagel Gmbh and Co., Germany) preconditioned first with methanol (20 mL) and then with water (60 mL). The column was washed with water (20 mL) and further eluted with methanol (40 mL) and with methanol:ammonia (99.5:0.5) (*15*). These fractions were evaporated to dryness under reduced pressure at 40 °C, redissolved in methanol (1 mL), and filtered through 0.22 μm PTFE filters (Millipore Ltd., Bedford, U.S.A.).

Identification and quantification of flavonoids were achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with an autosampler and quaternary pump coupled to a diode array detector. The column used was a Prodigy 5 μm ODS3 reversed phase silica column (250 mm × 4.6 mm i.d., Phenomenex Ltd.), and the elution solvents were as follows: A, water:tetrahydrofuran:trifluoroacetic acid 98:2:0.1, and B, acetonitrile. The solvent gradient was the same one used by Price et al. (*14*). The samples were injected in duplicate, and peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards and the library spectra. The flavonoids were quantified using the respective external standards, prepared by dissolving in methanol, HPLC grade. Quercetin was obtained from Apin Chemicals Ltd. (Abingdon, U.K.), and catechin was obtained from Sigma Chemical Co. The calibration was performed by injecting the standard three times at five different concentrations. The results were expressed as mg/100 g fresh weight (FW), as means ± standard deviation (SD).

Assay with Humans. Six healthy volunteers, women, at the ages of 33.6 ± 10.7 years, who had normal body mass indices (23.9 ± 1.1 kg/m^2), participated in the study. The Ethical Research Committee of the School of Pharmaceutical Science, São Paulo University, approved the experimental protocol, and the volunteers gave their written consent. The volunteers came to the laboratory once a week after a 10 h fast. White bread (standard food) was tested twice in the first 2 weeks. In the following 2 weeks, the volunteers consumed the pinhão seeds cooked with the coat or without the coat. The cooked seeds were consumed right after the cooking procedure with 150 mL of water. The blood glucose was determined from each subject on fast (time zero), and afterward, a food was consumed. The volunteers had 10 min to consume each portion. Blood samples were taken at 15, 30, 45, 60, 90, and 120 min after food ingestion in order to elaborate the glycemic curve (*16*). The glucose was measured in capillary whole blood with blood glucose sensors (Advantage Roche). The GI of each sample was calculated from the resulting area, and the white bread area was considered as the standard (100%) (*17*). The glycemic load (GL) was estimated according to Foster-Powell et al. (17).

Statistical Analysis. The GI was expressed as the mean ± SEM, and the other results were expressed as means ± SD. Comparisons of the mean values were performed by Student's *t*-test using the Minitab-Windows computer system.

RESULTS AND DISCUSSION

Proximate Composition. Moisture represents 50% of the total FW of cooked pinhão seeds, and starch is the second main compound with about 34%, which classifies the seed as a good source of complex carbohydrates. **Table 1** shows that protein (~3%) and lipid (~1.3%) contents were low and comparable to other starchy foods such as rice and beans. Besides starch, pinhão is a good source of total dietary fiber (TDF), as calculated by the sum of the soluble fiber and insoluble fiber, reaching 5.7% (**Table 1**). This value is comparable to the TDF found in cooked legumes (beans and chick peas) and vegetables (broccoli), as reported by Li et al. (*18*). Most of the TDF content of pinhão is insoluble fiber. As expected by the high amount of starch, the TSS content was very low in cooked seeds; glucose is the major soluble sugar with only marginal amounts of fructose and sucrose (**Table 2**).

A comparison of the proximate composition of raw and cooked pinhão (**Table 1**) shows that there are no differences between cooked and raw samples related to moisture, protein, lipids, soluble fiber, and total starch contents. An increase in moisture content would be expected in boiled seeds because of the high content of starch and the high capacity of water absorption of isolated pinhão starch, as mentioned by Wosiacki and Cereda (*5*). These authors found a much higher incorporation of cold water in isolated pinhão starch (150%) when compared to corn starch (101%). This apparent discrepancy between the high capacity of water absorption and no changes in the moisture content could be explained by the TDF. The high content of TDF suggests that similarly to beans (*19*), there is a more resistant cell wall that remains intact after the cooking process, which impedes the water absorption and starch swelling. In addition, the size and shape of the seed did not change after cooking.

Only 25% of the initial glucose (2.25% in raw seed) and 45% of the sucrose and fructose remained in the seed after it was cooked. Comparable reductions in soluble sugars can be found in data obtained by Li et al. (*18*) for raw and cooked broccoli and carrots, with the difference that these vegetables have only a thin skin, instead of a hard coat like that of the pinhão seed.

Insoluble DF increased in cooked seeds (4.3−5.2%, **Table 1**), which can probably be related to the not negligible amounts of RS (**Table 2**). In fact, the high content of amylose (~30%, **Table 2**) in pinhão starch may contribute to RS formation after cooling the cooked seeds. The values of RS for cooked pinhão (3.27%) are similar to the legumes, such as beans, chick peas, lentils, and peas (*20*).

Table 3. Mineral Content (mg/100 g) of Raw and Cooked Cooked Pinhão Seeds, with and without Coat (*$n = 3$)

	mg/100 g			RDA (mg/day)		0.15 × RDA
mineral	raw	cooked	cooked without coat	women	men	mg
Ca	12.8 ± 0.2*	15.8 ± 1.3	14.7 ± 1.5	1000	1000	150
P	102.7 ± 1.5	93.3 ± 2.4	54.4 ± 1.3	700	700	105
Mg	55.0 ± 0.8[a]	52.0 ± 0.5	40.7 ± 0.1	310	400	45/60
Fe	0.72 ± 0.03	0.67 ± 0.04	0.61 ± 0.02	18	8	3.0/1.2
Zn	0.81 ± 0.005	0.77 ± 0.03	0.75 ± 0.02	8	11	1.2/1.6
Cu	0.26 ± 0.02	0.23 ± 0.01	0.18 ± 0.01	0.9	0.9	0.14

[a] Highlighted values of mineral concentration (mg/100 g) are higher than 15% of the respective RDA (DRI, 2001) for men and women or for women only.

Table 4. Total Phenolics (mg/g FW) and Quercetin Content (mg/100 g FW) of Raw and Cooked Pinhão Seeds, with and without Coat

	pinhão	total phenolics[b]	quercetin
raw pinhão	with internal seed coat	3.21 ± 0.17	
	without seed coat	0.23 ± 0.01	ND
cooked pinhão[a]	with internal seed coat	0.54 ± 0.01	1.86 ± 0.01
	without seed coat	0.11 ± 0.01	ND
seed coat	internal	345 ± 7	5.71 ± 0.18
	external		1.11 ± 0.05

[a] After the seed was cooked, the seed coat (internal and external) was removed. [b] Total phenolic contents were expressed as mg CE/g fresh sample. ND, not detected.

Figure 2. GI after ingestion of pinhão cooked with and without seed coat (mean of values ± SEM, $n = 6$). Different letters between treatments show significant differences ($p < 0.05$). The GI for white bread is shown for comparison.

Minerals. Pinhão can be considered a source of Mg and Cu as 100 g of edible parts contains more than 15% of the respective mineral recommendation for adults (**Table 3**) (*21*, *22*). Cooking without the coat significantly altered the concentration of P, Cu, and Mg to 53, 69, and 74%, respectively. When cooked with the coat, the reduction varied from 3 (Cu) to 9% (P), only the last with statistical significance. New food sources are always important for nutritional orientation for some specific groups. Athletes, for instance, are frequently reported to have an Mg daily ingestion below the RDA of 400/420 mg/day, and food items with a high Mg density (mg/1000 kcal) can improve the quality of their diets.

Phenolic Compounds. Raw pinhão seeds presented a very low content of phenolics in comparison to the internal seed coat, and this value was further reduced by cooking of the peeled seeds (from 0.23 to 0.11 mg/g FW). However, in normal conditions of cooking, i.e., in the presence of the seed coats, there was a migration of phenolics from the seed coat into the seed, which had a total phenolics content of 0.54 mg/g FW when ready for consumption (**Table 4**).

No flavonoids were detected in raw pinhão seeds, but the flavonol quercetin was present in significant amounts in both internal and external seed coats (**Table 4**). No other flavonoids were identified in the external seed coat. The internal seed coat had a quercetin content (5.7 mg/100 g FW) five times higher than that of the external seed coat, and catechin was also present in high amounts (41.4 mg/100 g FW). Quercetin migrated into the seed during cooking but not into the cooking water, differently from catechin, which migrated into both the cooking water and the seeds. The catechin content of cooked pinhão seeds ready for consumption was 30 mg/100 g FW. These results showed that cooking was quite favorable to pinhão seeds in terms of total phenolics and flavonoids content.

Glycemic Response. Figure 2 shows information about the carbohydrate availability of cooked pinhão seeds. The glycemic responses produced by pinhão seeds cooked with the coat were 23% lower than for white bread. Many factors may interfere in the starch availability, such as the physical shape of the food, particle size, conditions of gelatinization and retrogradation, physical organization of cellular content, DF content, and presence of other compounds (*19*). The results showed that compounds such as phenolics that migrated into the seeds (**Table 4**) did not interfere since cooking with or without the coat had no difference on the GI (**Figure 2**). Similar results were observed by Menezes et al. (*19*) studying the consumption of cooked beans (*Phaseolus vulgaris* L.). It was observed that the presence of the seed coat that contained polyphenols has no effect on the plasmatic glucose, insulin, and GLP-1 7−36 (polypeptide like glucagon-1 7−36) response. The high content of DF in the pinhão seeds (11.4% dry weight) can make the complete swelling and gelatinization of the starch granules more difficult, producing a high concentration of unavailable RS (9% of total starch).

The importance of the GI and GL studies is linked to the physiological effects of diets with low GIs on prevention and control of chronic noninfectious diseases (*17*, *23*, *24*). Despite the evidenced positive physiological effects of the low GI foods, there is little information available about the GIs of Brazilian foods (*22*). In this context, the GI produced by the pinhão seeds (62−67%) classifies it as a food of low GI, like white spaghetti. At the same time, the GL was also low (12 per serving size of 50 g).

In conclusion, pinhão seeds can be considered a source of starch, DF, and Mg and Cu; in addition, pinhão seed intake produces a low GI. As a consequence of the cooking process (seed with a coat), a decrease in the soluble sugars content and an increase in phenolic compounds were observed. The external coat protected the seed from losing minerals during boiling, and the internal coat supplied phenolic compounds for the seed, as shown by the quercetin content of seeds cooked with and without the coat. The high content of amylose in the starch probably contributes to the formation of RS in the seed after cooking and to the low glycemic response.

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