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Physicochemical, Structural and Rheological Properties of Chestnut (*Castanea sativa*) Starch

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Abstract Chestnuts have high starch content, which makes them an alternative source of starch for the food industry. Brazil is a country where the production of chestnuts has been increasing in recent years. The aim of this study was to extract starch from chestnuts (*Castanea sativa*), to characterize its physicochemical, structural and rheological properties, and to compare the results with corn starch. Chestnut starch presented light color, and granules smaller than corn starch, with various dimensions, suggesting a bimodal distribution. Chestnut starch showed 20.48% absolute amylose, higher amylopectin branched-chain length and B-type crystallinity. The infrared spectra of chestnut starch showed characteristic peaks at 1647, 1157, 1079, and 1018 cm^{-1}. Chestnut starch presented higher peak viscosity, breakdown and setback, and lower pasting and gelatinization temperatures than corn starch. The swelling power and the solubility of chestnut starch were significantly higher than those of corn starch. Chestnut starch showed characteristics of a gelling and thickening agent, with potential for use as an ingredient in the food industry, as an unconventional starch from an alternative source.

Keywords: *chain length, molecular structure, morphology, pasting properties*

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1. Introduction

Species of chestnut tree were introduced in Brazil, and have adapted well to the climate in the higher altitudes of the south and southeast of the country, which resemble the temperate zone of the Northern Hemisphere. In São Paulo, there are commercial field of chestnut tree species including Chinese chestnuts (*Castanea mollissima*), Japanese chestnuts (*Castanea crenata*), American chestnuts (*Castanea dentata*) and, mainly, Portuguese chestnut (*Castanea sativa*) [1].

One of the main problems concerning trade of chestnuts in natura is related to its high moisture content (~50%) [2] and fungal development. To deal with the microorganism development, some countries use the frozen peeled or dehydrated to become an economic advantage which enables the diversification of products offered to consumers, including chestnut paste and flour, which are incorporated into a wide variety of food products [2,3].

According to reference [4], chestnut flour has about 6.0% moisture, 5.6% protein, 5.4% fat, 2.3 % fiber, 2.1% ash, and high starch content (78.6%). These authors reported chemical composition for chestnut starch obtained from *Castanea sativa*, Mill. for 0.83% protein, 1.51% fat, 1.09% fibers, 0.51% ash, and 96.06% starch, in dry basis (d.b.). Furthermore, the authors studied the viscoamylographic pattern of chestnut starch and determined paste clarity and elasticity, and gel strength, comparing the results with those of corn starch and cassava starch. They concluded that chestnut starch had properties that were intermediate between both conventional starch sources studied.

Considering the high starch content of chestnuts, and the need for a solution for the problem of preservation of chestnuts *in natura*, this study aimed to extract chestnut starch and characterize it for its physicochemical, structural and rheological properties, for its application as an ingredient in the food industry, using corn starch as the basis for comparison, due to the high use of this type of starch in the food industry. Furthermore, the use of analytical methods with high resolution could explaining better the chestnut starch performance for possible application.

2. Material and Methods

2.1. Raw Material

The chestnuts (*Castanea sativa*) were provided by CATI - Integral Technical Assistance Coordination (São Bento do Sapucaí, BRA) and regular corn starch (Amidex 3001) was donated by Corn Products (Mogi Guaçu, BRA).

2.2. Cleaning and Manual Dehusking

The healthy chestnuts were sanitized by immersion in chlorinated water (2 ppm) in a ratio of chestnut and water equal to 1:2 (w:v) for three minutes. Then, the chestnuts were dehusked manually and the inner pellicle was removed by abrasion under running water in a R6025 abrasive peeler (Bufalo, São Paulo, BRA) for four minutes at 1720 rpm.

2.3. Starch Extraction

The starch extraction from chestnuts followed the methodology proposed by [5]. Starch was extracted by grinding 500 g of chestnuts and 750 mL distilled water in a OBL10/2 blender (OXY, Santana de Parnaíba, BRA), at 35000 rpm for three minutes. The resulting material was filtered using an 88 µm sieve. The retentate was subjected to the extraction process twice more. An aliquot of 200 mL permeate was centrifuged in a FR22 refrigerated centrifuge (FANEM®, Piracicaba, BRA), for 10 minutes at 20°C and 1700 xg. The supernatant was discarded, the upper layer (containing fiber and protein) was removed manually with the aid of a spatula, and the pellet was re-suspended in 200 mL distilled water. This step was repeated four times. At the end, the starch was re-suspended in 100 mL of 99.5% ethanol, vacuum filtered on Whatman N°5 filter paper, and dried in a TE394/2 forced-air drying oven (TECNAL®, Piracicaba, BRA), at 40±0.2°C for 15 hours. The dried starch was ground in a blender until particle size was less than 250 µm.

2.4. Physicochemical Properties

2.4.1. Chemical Composition of Starch Samples

Chestnut and corn starch samples were characterized for moisture, protein, ash, and lipid contents according to AACC methods 44-15.02, 46 13.01, 08 01.01 and 30-25.01 [6], respectively. The total dietary fiber (TDF) was determined by AOAC method 991.43 [7]. Analyses were performed in triplicate and the results expressed as percentage. Digestible carbohydrates (starch and sugars) were calculated by difference.

2.4.2. Instrumental Color

Color measurements were performed in triplicate by the CIELab system, using a 45/0-L colorimeter (XE MiniScan, Reston, USA), through direct reading on the sample in the power form. The test conditions were: illuminant D65, observer angle of 10° and calibration mode Reflectance Specular Included.

2.5. Structural Properties

2.5.1. Scanning Electron Microscopy

The starch samples were fixed on stubs and coated with a 92Å thick gold layer in a SC7620 Polaron Sputter Coater (VG Microtech, Uckfield, GBR), and analyzed using a 440i scanning electron microscope (LEO Electron Microscopy, Oxford, GBR), with a 6070 dispersive energy X-ray detector (LEO Electron Microscopy, Cambridge, GBR). The accelerating voltage was 10kV and the beam current was 50pA.

2.5.2. High Performance Anion Exchange Chromatography with Pulse Amperometric Detection (HPAEC-PAD) for Analysis of Amylopectin Branched-Chain Length Distribution

The amylopectin of the chestnut and corn starch was debranched using isoamylase (Megazyme, 3U) enzyme according to [8] and the branched-chain length distribution was determined according to the method proposed by [9]. Briefly, it was used a ICS 3000 HPAEC-PAD system (Dionex Corporation, Sunnyvale, USA) equipped with an AS40 automatic sampler. Samples were filtered (0.22 µm membrane) and injected into the HPAEC-PAD system (20 µL sample loop). The flow rate was 0.8 mL/minute at 40°C. The standard quadruple potential (E) waveform was employed with the following periods and pulse potentials: E1=0.10V (t1=0.40 s); E2=2.00 V (t2=0.02 s); E3=0.60 V (t3=0.01 s); E4=0.10 V (t4=0.06 s). All eluents were prepared with ultrapure water (18 mΩ.cm) with N2 sparging. Eluent A was 150 mM NaOH and eluent B was 500 mM sodium acetate and 150 mM NaOH. The branched chains of amylopectin were separated using a Dionex CarboPacTM PA-100 guard column (4 mm x 50 mm) and a Dionex CarboPacTM PA-100 column (4 mm x 250 mm). The gradient of eluent B was 28% at zero minutes, 40% at 15 minutes, and 72% at 105 minutes. The data were analyzed using the Chromeleon software, version 6.8 (Dionex Corporation). The samples were analyzed in duplicate.

2.5.3. Absolute Amylose

The absolute amylose content was determined by using the Amylose/Amylopectin assay procedure of Megazyme (K-AMYL 07/11). The absorbance was measured by absorption using a DU-70 spectrophotometer (Beckman, Fullerton, USA), at 510 nm. Analyses were performed in triplicate, and the results were expressed in percentage.

2.5.4. X-ray Diffraction

The starch samples were conditioned at 100% relative humidity for seven days at room temperature in a desiccator containing 300 mL distilled water with 1% sodium azide to inhibit microorganism growth. The XRD patterns were obtained in a RU200B XRD equipment (Rigaku Rotaflex, Tokyo, JAP), with Cu rotating anode at 40 kV and 80 mA, and diffraction angles from 3° to 30° (2theta), with a step size of 0.02° at a scan speed of 2°/minutes. The relative crystallinity was calculated by the ratio of the total area and the area of the peaks, according to the method proposed by [10] using the software Origin Microcal Inc., version 6.0 (Northampton, USA), with a 5-point smoothing FFT Filter. The analysis was performed in triplicate, and the results expressed in percentage crystallinity.

2.5.5. Thermal Properties

The thermal properties of the starch samples were determined in a TA60 differential scanning calorimeter (Shimadzu, Kyoto, JAP), based on the methodology

described by [11], with modifications. Aluminum pans containing 3 mg sample (d.b.) and 7 µL deionized water were hermetically sealed and equilibrated at room temperature for one hour before measurements. An empty pan was used as reference. The scanning temperature ranged from 30 to 95°C, at a heating rate of 5°C/minute. Based on the thermograms, the values of the To, peak temperature (Tp), temperature range (ΔT), and ΔH were obtained in triplicate, and the results were expressed in °C for To, Tp and ΔT, and in J.g-1 for ΔH.

2.5.6. Fourier Transform Infrared Spectroscopy

The methodology for obtaining the FTIR spectra was based on the study of [12]. The samples were dried in a TE-395 vacuum oven (Tecnal®, Piracicaba, BRA), at -600 mmHg and 50°C for 24 hours, ground in an agate mortar, and filtered through a sieve of 100 µm. The potassium bromide (KBr) pellets were prepared by mixing 2 mg sample and 200 mg KBr, and analyzed by a IRPrestige 21 Fourier Transform Infrared Spectroscope (Shimadzu, Kyoto, JAP). The absorbance ranged from 4000 to 400 cm^{-1} at a 4 cm^{-1} resolution and 40 scans. The proportion of crystalline area was also calculated according to the method described by [13].

2.6. Rheological Properties

2.6.1. Pasting Properties

The viscoamylographic profiles of the starch samples were determined according to the method 162 of ICC [14] using a RVA-4500 Rapid Visco Analyser (Warriewood, AUS), and the curves were analyzed by the software TCW3.15.1.255. The parameters evaluated were: pasting temperature, peak viscosity, breakdown, final viscosity and setback. The analysis was performed in triplicate, and the results expressed in °C for the pasting temperature and in cP for the other parameters.

2.6.2. Gel Strength

The gel strength of the starch samples was determined by the method proposed by [15], with modifications. The gels obtained from the pasting property analysis were poured into a cylindrical tube of polyvinyl chloride with 25 mm diameter and 20 mm depth, and stored at 7°C for 24 hours. The samples were kept at room temperature for 1 hour before analysis. The texture was determined in a TA-XT2i texture analyzer (Stable Micro Systems-Haslemere, GBR), with a load of 25 kg. The conditions were the following: pre-test, test and post-test speeds of 5.0, 1.0, and 1.0 mm.s-1, respectively; penetration distance 10.0 mm; detection limit 0.05N; and cylinder probe Derlin P/10. The analysis was performed with six replicates and the results expressed in N.

2.6.3. Swelling Power And Solubility

The swelling power and solubility were determined at 50, 60, 70, 80 and 90°C, according to the methodology described by [16], with modifications. Samples of 0.02 g (d.b.) were weighed in 50 mL conical centrifuge tubes. Then, 20 mL distilled water was added, and the tubes were placed in a water bath under mechanical stirring at 100 rpm. After 30 minutes of incubation, the tubes were centrifuged at 2200 xg for 15 minutes. The supernatant was collected and oven dried to constant weight to quantify the soluble fraction, and the results were expressed as percentages. The tubes containing the residual material were weighed to determine the swelling power. The analyses were performed in triplicate.

2.7. Statistical Analysis

The data were analyzed using the software Statistica 7.0 (Statsoft, Tulsa, USA) for analysis of variance and comparison between means by Student's t test, with a significance level of 5%.

3. Results and Discussion

3.1. Chemical Composition

The fat contents of chestnut and corn starch samples were 0.15±0.03 and 0.10±0.02%, respectively, and did not differ from each other. Both ash and total dietary fiber contents of chestnut starch were higher ($P \leq 0.05$) than those of corn starch, with ash contents of 0.05±<0.01 and 0.02±<0.01%, and total dietary fiber of 1.36±0.11 and 0.58±0.42 %, respectively. According to Demiate et al. [4], chestnut starch has characteristics similar to corn starch, especially with respect to lipids, ash and fiber. Reference [17] reported that purified starch showed protein content lower than 0.6%, which occurred with the starch samples of this study, with values of 0.14±0.02% for chestnut starch and 0.26±0.03% for corn starch. The total carbohydrate content of chestnut starch was 87.56%, similar to that obtained for corn starch (87.21%).

3.2. Instrumental Color

The color parameters of chestnut and corn starch samples were 99.11±0.30 and 99.58±0.43 for L*; 0.58±0.02 and 0.64±0.01 for a*; and 3.16±0.12 and 5.65±0.02 for b*, respectively. Although no difference was observed for the lightness of the starch samples, a* and b* parameters were statistically different ($P \leq 0.05$). Chestnut starch showed a greater tendency to red, probably due to the pigment found in the peel, while corn starch showed a greater tendency to yellow, probably because of corn pigments (zeaxanthin).

3.3. Morphology of the Starches Granule by Scanning Electron Microscopy

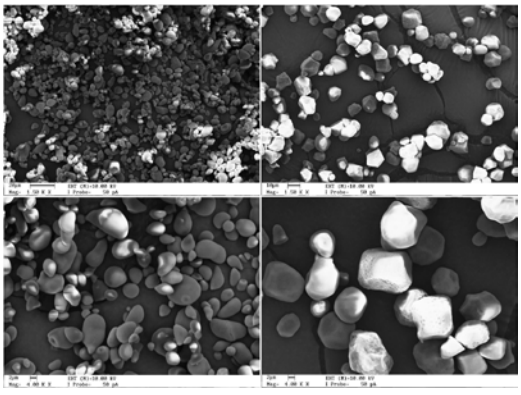

Figure 1. Micrographs of chestnut starch (left) and corn starch (right) at 1500 x (top) and 4000 x (bottom) magnifications

The micrographs of chestnut starch (Figure 1) showed oval and irregular shaped granules, presenting predominantly smooth surfaces, different to corn starch, which showed circular and polyhedral shaped granules, and porous surfaces. A few chestnut starch granules had holes or fissures on the surface, especially the larger ones. Chestnut starch exhibited a distinct population of smaller granules with a mean diameter from 2 to 5 µm, and a population of larger granules with mean diameters from 10 to 20 µm, suggesting a bimodal distribution. The corn starch sample showed granules of more uniform size, characteristic of a modal distribution.

3.4. Amylopectin Branched-Chain Length Distribution and Absolute Amylose Content

Chestnut and corn starch samples exhibited a profile of amylopectin branched-chain length distribution with two populations of different sizes (Figure 2). The first population showed a peak at DP 12 for both samples, and the second population showed peaks at DP 43 and DP 42 for chestnut and corn starches, respectively.

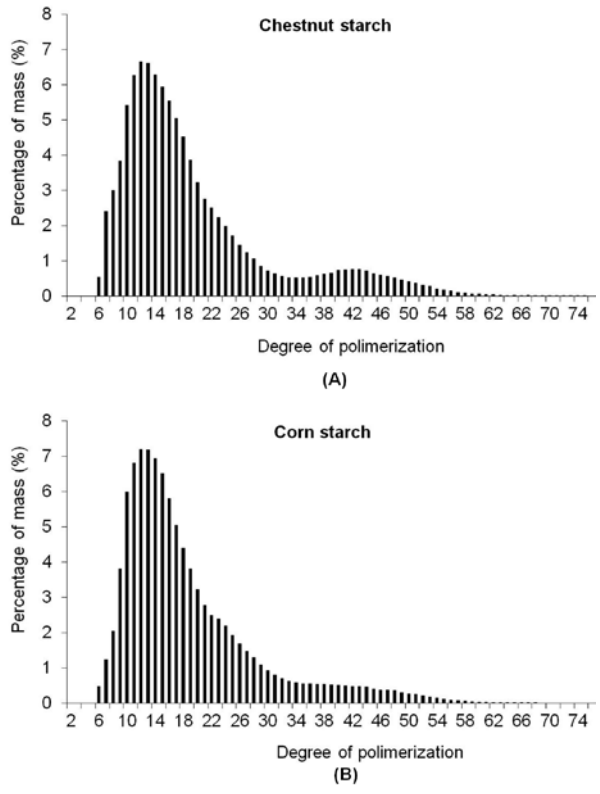

Figure 2. Amylopectin branched-chain length distribution of chestnut starch (A) and corn starch (B)

The amylopectin chains length was classified as proposed by [18], in which the amylopectin branched-chains A, B1, B2 and B3 occur at DP 6-12, 13-24, 25-36 and ≥37, respectively (Table 1). Chestnut starch showed a higher number of long (DP 25-36) and very long chains (DP ≥37), resulting in a higher DP (19.60) when compared to corn starch (18.75). The highest DP of chestnut and corn starches was 76 and 68, respectively. Chains at DP 18-21 represent the total length of the lamellar structure [19]. Thus, the amylopectin from chestnut starch has more branched-chains that participate in two or more clusters than corn starch.

The absolute amylose content was found to be 20.48±0.51% for chestnut starch and 19.94±0.56% for corn starch, and these values did not differ from each other.

Table 1. Amylopectin branched-chain length distribution of chestnut starch and corn starch in mass-basis

Starch	Degree of polymerization (DP)					
	6-12	13-24	25-36	≥37	Average	Highest detectable
Chestnut	27.96[a]	50.42[b]	10.50[b]	11.12[a]	19.60[a]	76
Corn	27.61[a]	52.78[a]	12.16[a]	7.45[b]	18.75[b]	68

Means with different letters in the same column differ statistically from each other ($P \leq 0.05$).

3.5. X-ray Diffraction

Figure 3 shows the XRD patterns for chestnut and corn starches samples. Chestnut starch showed single peaks at diffraction angles of 5, 8, 15 and 17° at 2theta, and a double peak between 22 and 24° at 2theta, which is characteristic of B-type polymorphism. Corn starch showed A-type polymorphism with single peaks at 15 and 23° at 2theta, and a double peak between 17 and 18° at 2theta, in agreement with the findings of [20]. The B type polymorphic profile is characteristic of starch containing a high population of very long amylopectin chains at DP>37 [21], which was observed for chestnut starch (Table 1). Starch presenting A-type crystallinity has smaller amylopectin branched-chain length than the B-type crystallinity [22], thus explaining the results of this study. The percentage of crystallinity of chestnut starch was 35.26±0.10%, which was similar to corn starch (36.44±0.51%).

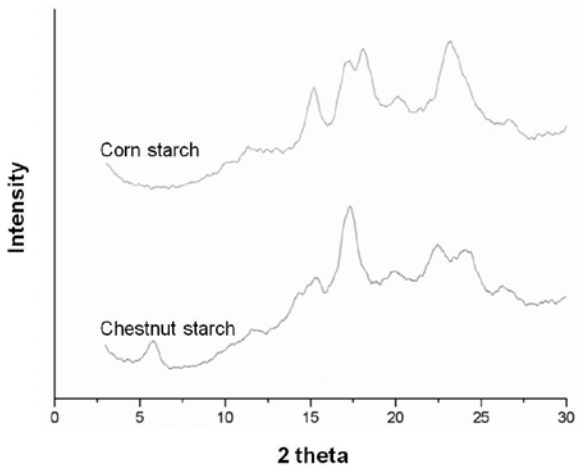

Figure 3. X-ray patterns of chestnut starch and corn starch

3.6. Fourier Transform Infrared Spectroscopy

The measurements occurred in the bands between 1800 and 450 cm^{-1}, since the organic compounds in foods have transmittance in this region [23]. The spectra of the chestnut and corn starches samples were very similar (Figure 4), differing only by the presence of a peak at 1190 cm^{-1} (indicated by the arrow) observed in the chestnut starch spectrum. Both spectra exhibited peaks characteristic of starch at wavelengths of 1647 and 1649 cm^{-1} for chestnut and corn starch, respectively, corresponding to the stretching (scissoring) of two O─H

groups absorbing a water molecule. Similar bands presenting peaks at 1157, 1079 and 1018 cm^{-1} for chestnut starch, and at 1160, 1080 and 1010 cm^{-1} for corn starch were observed, and are due to the C─O bond stretching [24].

This technique is very sensitive to structural changes at the molecular level, such as the conformation of starch chains and crystallinity, unlike the XRD technique, which is related to the packing of double helices within an organized crystalline structure [25]. The absorbance band at 1047 cm^{-1} is sensitive to the amount of crystalline structure and the band at 1022 cm^{-1} is characteristic of the amorphous starch. Therefore, the ratio of the heights of bands at 1047 and 1022 cm^{-1} expresses the relationship between the amounts of crystalline to amorphous structures in starch [25,26]. It was observed that the ratio of the crystalline to amorphous structure was 0.83±<0.01 for chestnut starch and 0.89±<0.01 for corn starch, indicating that chestnut starch showed more amorphous structure than corn starch ($p<0.001$). Higher values for the ratio between the crystalline and amorphous structures are the result of a greater structural organization of the starch granule [13].

In XRD, there was no difference in the percentage of crystallinity between the samples, which is in disagreement with the result found in FTIR, possibly due to the greater sensitivity of infrared spectroscopy in relation to XRD.

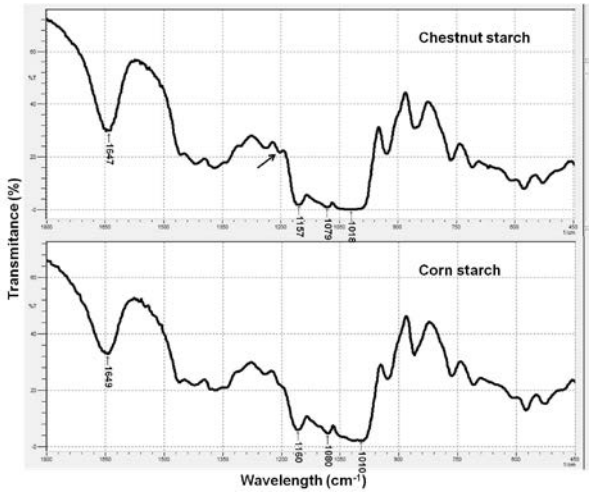

Figure 4. Fourier Transform Infrared Spectroscopy of chestnut starch and corn starch

3.7. Thermal Properties

Chestnut starch showed a lower T¬0 than corn starch. Both the T¬0 and the Tp of chestnut starch were statistically different (P≤ 0.05) from corn starch (Table 2). High T0 are related to a more densely packed crystalline structure and higher molecular order [27]. By FTIR, it was found that chestnut starch had a slightly lower molecular order than corn starch, which may have contributed to its lower gelatinization temperature. Moreover, when the absolute amylose content is similar, starch presenting B-type polymorphism such as chestnut starch, has a lower gelatinization temperature than starch presenting A-type polymorphism, which may be due to the greater amount of water molecules in the B-type polymorphism, making the double-helices unit cell more loosely packed than that in A-type polymorphism [19].

The extent of crystalline perfection is reflected in the T0 range and in the ΔH obtained by DSC. Greater ΔH expresses greater crystalline perfection and homogeneity [28]. These results suggest that despite a slight difference in the molecular order of the starch, this difference was not enough to influence the ΔH values, which were statistically equal.

Table 2. Thermal properties of chestnut starch and corn starch

Starch	T_{on} (°C)	T_{peak} (°C)	ΔT (°C)	ΔH ($J.g^{-1}$)
Chestnut	56.27±0.67[b]	60.20±0.51[b]	9.15±0.64[a]	13.49±0.56[a]
Corn	62.89±0.08[a]	67.16±0.06[a]	9.13±0.18[a]	13.64±0.62[a]

Mean of three replicates ± standard deviation; means with different letters in the same column differ statistically from each other ($P \leq 0.05$); Ton - onset gelatinization temperature; Tpeak - peak temperature; ΔT - temperature range; ΔH - enthalpy variation.

3.8. Pasting Properties and Gel Strength

The pasting temperature of chestnut starch was lower (P≤ 0.05) than that of corn starch (Table 3). Pasting temperature is influenced by several factors, including the size and shape of the starch granules, amylopectin branched-chain length distribution, degree of polymerization of amylopectin, presence of phosphate monoester derivatives and lipids [29].

Chestnut starch had higher viscosity values than corn starch, and these were statistically different (P≤ 0.05). The amylose content, lipid content, and amylopectin branched-chain length distribution influenced the starch pasting properties [19,30]. Chestnut starch had the highest average amylopectin branched-chain length, mainly due to the higher number of very long chains (DP≥37), which may have contributed to the higher viscosity values observed for this starch. The granule swelling and molecular rigidity are some key parameters to controlling starch pasting properties. The breakdown observed is the result of the disruption of the granular structure of the gelatinized starch due to mechanical stirring and high temperature. Reference [31] observed, for rice starches, that the higher the ratio of long chains of amylopectin, the lower the breakdown, since they help maintain the structure of the gelatinized starch, but this did not occur in the present study.

Chestnut starch showed higher setback, which may also be due to the higher number of long chains of amylopectin, since the absolute amylose content did not differ between both starch samples. Setback could be related to gel strength, however, the gel strength of both starch samples was similar, possibly due to temperature differences during measurements (50°C for RVA and room temperature for gel strength).

Table 3. Pasting properties and gel strength of chestnut starch and corn starch

Starch	Peak viscosity (cP)*	Breakdown (cP)*	Final viscosity (cP)*	Setback (cP)*	Pasting temperature (°C)*	Gel strength (N)**
Chestnut	2665±16[a]	670±12[a]	3121±20[a]	1127±11[a]	67.07±0.05[b]	1.55±0.18[a]
Corn	1690±10[b]	469±4[b]	1694±4[b]	483±7[b]	83.15±0.07[a]	1.53±0.08[a]

*Mean of three replicates ± standard deviation; **Mean of six replicates ± standard deviation, means with different letters in the same row differ statistically from each other ($P \leq 0.05$).

3.9. Swelling Power and Solubility

The swelling power of both starch samples increased with increasing temperature (Figure 5). Chestnut starch showed a greater increase in swelling power as a function of a higher temperature (2.23 g/g at 50°C, and 35.45 g/g at 90°C), as compared to corn starch (2.04 g/g at 50°C and 12.50 g/g at 90°C). The increase of the swelling power with temperature is a result of the increased mobility of starch molecules, which facilitates water diffusion, favoring swelling and solubility [32]. High amounts of long amylopectin branched-chains increase the gyration radius of the amylopectin molecules, helping to keep viscosity [31]. The gyration radius is related to the volume occupied by the amylopectin molecule in solution [33]. The higher ratio of long amylopectin branched-chains of chestnut starch may have resulted in a greater gyration radius of amylopectin molecules, which contributed to the higher swelling power as compared to corn starch.

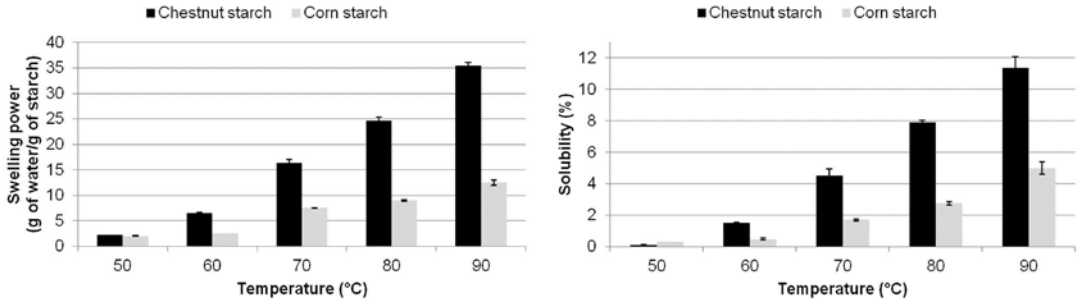

Figure 5. Swelling power and solubility of chestnut starch and corn starch

Gel formation requires hydration and swelling of starch granules, which occurs predominantly in the amorphous region of starch [32]. In FTIR analysis, chestnut starch presented higher percentage of amorphous area, which facilitated its hydration, resulting in higher swelling power.

Solubility is expressed as the percentage (in weight) of starch molecules leached into the aqueous medium after heating [16]. Chestnut starch presented higher solubility than corn starch from 60°C to 90°C. A significant increase in the solubility of chestnut starch was observed above 60°C, while corn starch showed an increase in solubility only after 70°C. This behavior is probably due to the pasting temperature and the gelatinization temperature of the starch when swelling begins, forming a disorganized structure of the starch granules, allowing linear molecules to leach from the granules. Thus, the chestnut starch granule seems to be more fragile than that of corn starch.

4. Conclusion

Chestnut starch has structural characteristics and physicochemical properties different from those presented by corn starch. Chestnut starch showed B-type polymorphism with granules of different sizes suggesting a bimodal distribution of the granules. The absolute amylose content of chestnut starch was similar to corn starch. Chestnut starch had higher pasting viscosity, swelling power and solubility than corn starch, as well as lower pasting temperature and gelatinization temperature, suggesting facility in cooking processes. Thus, chestnut starch presented thickening characteristics, with potential for application in food and non-food industries, and also for use as a raw material in studies of modified starches.

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