Morphology and structure of chestnut starch isolated by alkali and enzymatic methods

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Abstract

The structure and morphology of starch from fruits of two chestnut (Castanea sativa Mill.) varieties, Martainha and Longal, isolated by alkaline (A3S) and enzymatic (ENZ) methods were assessed. Chestnut starch granules were found to be round and oval in shape, consisting of medium/small granules, with a mean granule size ranging between 9 and 13 μm. Isolated chestnut starch appeared to the naked eye as a white powder, with high values of L*, and the Longal variety produce starches duller than Martainha.

No differences between samples were observed by FTIR analysis. The X-ray patterns of isolated starches are of C-type (more specifically of Cb type) with a relative crystallinity between 31.5% and 39.8%. The 13C CP/MAS NMR spectra are similar for both varieties but different for the used isolation methods. The amorphous phase in the starch granules isolated by A3S methods was lower than that of the starch extracted by the ENZ method, making the B-type allomorph in the C-type starch granules more evident than in the A-type. Those differences in the structure of isolated starches are shown by a lower degree of damage, and a higher level of crystallinity of starches isolated by the A3S method, which means that its original structure is less affected or partially destroyed. This study would be helpful to better understand the relationships among structure and functional properties for a eventual industrial application of chestnut starches.

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1. Introduction

For centuries sweet chestnuts (Castanea sativa Mill.) represented one of the most important food resources of the European rural areas (Adua, 1999). Chestnut is one of the most ancient edible fruits in Portugal and, in the past, widely used in some way of potato and most of the tubers (Ferreira-Cardoso, Rodrigues, Gomes, Sequeira, & Torres-Pereira, 1999). In some areas, the term “bread tree” has been used for chestnut trees, where the fruit has been one of the fundamental staple foods used in human nutrition. A better knowledge of fruit composition may offer new opportunities for the chestnut market. The fruits are carbohydrates rich and low in fat content that makes it interesting to use in human diets. Starch is the main constituent of chestnut fruit ranging from 38% to 80% (Borges, Gonçalves, Carvalho, Correia, & Silva, 2008; Correia, Leitão, & Beirão-da-Costa, 2009; Miguélez, Bernárdez, & Queijeiro, 2004). There is increasing evidence showing that the consumption of chestnuts has become more important in human nutrition due to the health benefits provided by the presence of bioactive components (Blomhoff, Carlsen, Andersen, & Jacobs, 2006), including lectin, cysteine proteinase inhibitor and quercetin (Wang & Ng, 2003). It also comprises of considerable levels of vitamins, fibres, essential fatty acids and minerals (Borges et al., 2008).

Polysaccharides in general, especially starch, play an important role in the growth and development of living organisms (Yang et al., 2009). Furthermore, starch is an important material both in food and non food industries. Approximately 60 million tonnes are extracted annually worldwide from various types of cereal, tuber and root crops (www.zuckerforschung.at), of which roughly 60% is used in foods (i.e., bakery products, sauces, soups, sugar syrups, ice cream, snack foods, meat products, baby food, drinks, fat substitutes) and 40% in pharmaceuticals and non-edible purposes (Burrell, 2003).

The diversity of starch granules in size and shape with different botanical origins is quite well known. Starch granules range in size (from 1 to 100 μm diameter) and shape (polygonal, spherical, lenticular), occurring individually or as clusters, and can vary greatly with regard to content, structure and organization of the...
amylase and amylopectin molecules, the branching architecture of amylopectin, and the degree of crystallinity (Lindeboom, Chang, & Tyler, 2004). This diversity in the form of starch granules and their molecular constituents influences the starch’s functionality.

Isolation methods influence different starch extension characteristics, as previously reported by Correia and Beirão-da-Costa (2010). The best results considering the yield, purity and pasting properties were obtained with two methods: i) applying low shear and an alkali treatment and ii) using low shear and a protease digestion step. These methods lead to extraction yields of 83.9% and 79.9% and a purity level of 98.3% and 96.3%, for Longal variety, respectively (Correia & Beirão-da-Costa, 2011).

Chestnut fruit presents a large potential for commercial use since they are good sources of starch. In a previous study, starch contents of chestnut fresh fruits from Longal and Martainha varieties were 46.8% and 48.6% respectively (Correia et al., 2009).

Starch granule stability depends on the arrangement of atoms in the polysaccharide structure, according to the distribution of the intra- and intermolecular forces. The structure of amylase and amylopectin, and the form and crystallinity of starch granules, have been extensively studied using many complementary approaches, like small-angle scattering techniques that measure differences in electron density distribution, and diffraction techniques indicative of crystallinity of the material (Copeland, Blazek, Salman, & Tang, 2009). Imaging techniques such as light microscopy, scanning and transmission electron microscopy, scanning probe techniques such as atomic force microscopy (AFM), spectroscopic methods such as nuclear magnetic resonance and Fourier-transform infrared spectroscopy are other approaches used to obtain structural information on starch granules and molecules. These analytical methods provide explicit information on the molecular constituents and their organization in starch granules.

The main objectives of this research are to obtain useful morphological and structural information about chestnut starch in order to understand the molecular behaviour and evaluate the effect of the starch isolation methods on the starch properties, and therefore produce useful information for possible industrial applications.

2. Materials and methods

2.1. Materials

Chestnut (C. sativa) var. Longal and Martainha fruits were collected from Soutos da Lapa, a Protected Designation of Origin region of Portugal. Three sets of 1 kg of each chestnut fruits were randomly harvested at a mature stage, stored, dried and then milled as previously reported by Correia et al. (2009).

2.2. Starch extraction methods

Starch was isolated from the chestnut flours following two methods (Correia & Beirão-da-Costa, 2011):

1) Alkaline pH using successively three sieves (A3S)

The flours (120 g) were soaked in 2 volumes of 0.25% NaOH at 5 °C for 24 h. Suspensions were homogenized and screened through a 180 μm sieve. The procedure was then repeated twice. The precipitate was screened successively in 75 and 53 μm sieves. The mixture was centrifuged in a Universal 16 centrifuge (Hettich Zentrifugen Company, Germany) at 800 × g/15 min, the mucilaginous layer scraped away and the precipitate was then suspended in water. This last step was repeated twice. Isolated starches dried for two days at 40 °C in a FD 115 Binder ventilated drying chamber (with an air flow of 300 m³/h).

The purity of isolated starches was 98.3% and 98.0% for Martainha and Longal respectively.

2) Enzymatic method (ENZ)

Protease from Aspergillus oryzae was purchased from Sigma Chemical Co. One unit of protease was defined as the amount of enzyme that liberated 1.0 μmol of tyrosine per minute from casein at pH 7.5 at 37 °C. The protease was added (900 units) to 120 g individual flours. Water was added (360 ml) and the slurry was adjusted to pH 7.5 (with 0.1 M NaOH or 0.1 M HCl). It was incubated at 37 °C for a period of 2 h. The slurry was then centrifuged in the same conditions as previously mentioned. The starch fraction was suspended, washed with water (200 ml) and filtered through a 53 μm sieve. The filtrate was centrifuged. The supernatant and tailings were discarded and the starch dried as reported above.

In a previous study undertaken by Correia and Beirão-da-Costa (2011) these isolation methods were tested, and yield, purity and damaged starch content were determined. The total starch content for Martainha and Longal flours were 31.7% and 32.3%, respectively. The starch purity was described as the amount of starch as a starch isolate. The yields reached with these experimental conditions for A3S and ENZ methods were 83.9% and 79.9% for Longal and 85.5% and 81.9% for Martainha, respectively. Isolated starches shown to be higher in purity, presented values of 98.3% and 98.6% for Martainha, respectively for the A3S and ENZ methods. The damaged starch contents were 30.1% and 34.6% for Longal and 28.8% and 30.6% for Martainha, respectively for the A3S and ENZ methods. The final pH of the starches isolated by the two methods was 6.8 ± 0.2.

2.3. Scanning electron microscopy

Scanning electron micrographs (SEM) of isolated starches were obtained with an ISI-D 130 scanning electron microscope (International Scientific Instrument). Starch samples were suspended in ethanol to obtain a 1% suspension. One drop of the starch—ethanol suspension was applied on an aluminium stub using a double-sided adhesive tape and the starch was coated with gold-palladium (80:20). An accelerating voltage of 10 kV was used.

2.4. Particle size and distribution analysis

The sizes of starch granules were determined by a laser particle size analyzer Mastersizer X (Malvern Instruments Ltd, Malvern, United Kingdom). A polydisperse mode of analysis and a 300 mm lens were used. Starch powder was dispersed in isopropyl alcohol in the equipment circulation unit to obtain an obscuration of 15–20%. Before measuring, the sample was circulated in the equipment with mechanical shear and an ultrasound for 5 min to disperse the starch clots. Measurements were taken in intervals of 2 min. Size distributions were determined at three replications of duplicate samples (n = 6). The results were expressed in terms of volume (%) occupied by the starch granules, and the classes defined based on the Lindeboom et al. (2004) classification: large (>25 μm), medium (10–25 μm), small (5–10 μm) and very small (<5 μm).

2.5. Colour evaluation

The colour difference was analyzed using a Chroma Meter CR-300 Minolta (Osaka, Japan) colorimeter and the classification by the CIELAB (1986) system. From L’ a’ b’, the chroma (c*) and hue angle (h*) were determined. Colour lightness (value), L’ (100: white...
to 0: black), measures how light/dark the colour of the object is; chroma or saturation, \(c^*\) (0–60), measures how dull/vivid the object colour is; hue angle, \(h^*/C_14\) (0–360\(^{\circ}\)), expresses the characteristic/dominant colour (0 = red/purple; 90 = yellow; 180 = bluish/green). The colour difference \((\Delta E)\) was determined by comparison to a blank standard tile \((L^* = 97.46; a^* = -0.02; b^* = 1.72)\) and using the Eq. (1).

\[
\Delta E = \left( (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right)^{1/2} \quad (1)
\]

For reference, a commercial maize (Maizena\textsuperscript{®}, Copam, Portugal) starch was used.

Twenty five individual measurements were performed for each sample.

2.6. Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra of starches separated from the Longal and Martainha chestnut were obtained on a Mattson 7000 spectrophotometer. A finely ground sample was deeply mixed with dried potassium bromide powder (KBr) (1:100 in weight) and pressed in a die at 10,000 psi to yield a disc. The calibration was carried out using KBr as blank and the spectra recorded with a resolution of 4.0 cm\(^{-1}\) and 64 scans registered in the medium-infrared area, which extends from 4000 to 400 cm\(^{-1}\).

2.7. X-ray diffraction

The analysis of the crystalline structure of the starches was carried out in a Philips diffractometer (X'Pert MPD, Almelo, Netherlands), using CuK\(_\alpha\) radiation (\(\lambda = 0.154 \text{ nm}\)) generated at 40 kV and 20 mA. The starches had a moisture content of between 8 and 10%. The sample was scanned through the 2\(\theta\) (diffraction angle) from 3 to 50\(^{\circ}\) at a speed of 8\(^{\circ}\)/min. The degree of relative crystallinity was determined following the method previously reported by Huang et al. (2007) using the follow equation (Eq. (2)):

\[
\text{Crystallinity (\%)} = \frac{I_c}{(I_a + I_c)} \times 100 \quad (2)
\]

where \(I_a\) = amorphous area on the X-ray diffractogram, \(I_c\) = crystallized area on the diffractogram.

2.8. Solid-state NMR

The Longal and Martainha starches were characterized by \(^{13}\text{C}\) NMR using cross polarization and magic angle spinning (CP/MAS). The spectra were obtained from a Bruker 500 spectrometer (Bruker, Germany). The spectra were obtained with static magnetic field of the 11.7 T. Before NMR measurements samples were kept at 100% relative humidity for 48 h. The sample was placed in a 4 mm zirconium rotor sealed with Kel-FTM caps and placed in rotation at 9 kHz. The acquisition parameters used were as follows: proton pulse of 4\,\mu s, contact time of 2 ms, delay of 4 s and 7000 scans.

2.9. Statistical analysis

The obtained results were subject to a one-way analysis of variance (ANOVA) test using the Statistic\textsuperscript{®} vs 6 software. The separation of means or significant difference comparison of all parameters was tested by the Tukey's HSD test. The level of significance used for all the statistical tests was 95%.

3. Results and discussion

3.1. Morphology of chestnut starch

SEM images and granule size distribution of chestnut starch produced by the two isolation methods are shown in Fig. 1. Granules are round and oval in shape, exhibiting some fractures. These fractures are more evident in the ENZ method for both varieties, suggesting that this method is a more damaging one. Considering the variety, there were no significant differences detected among starch particle size distributions. The smaller-sized starch granules were

![Fig. 1. SEM (×1500) and granule size distribution of chestnut starch: L – Longal; M – Martainha; A3S – alkaline pH and using successively three sieves method; ENZ – enzymatic method.](image-url)
about 80% of the total volume (maximum size of 18 μm) of particles and the mean granule size ranged between 9 and 13 μm. The average granule sizes for Longal starches were 12.5 μm and 13.8 μm for alkali and enzymatic methods, respectively. Starch extracted from Martainha flour showed the same tendency, with mean granule sizes of 12.4 μm and 13.1 μm for alkali and enzymatic methods, respectively. Thus, chestnut starch consists of medium/small granules (Lindeboom, Chang, & Tyler). It was also observed that some of starch granules are much larger and higher than 62.5 μm in diameter. For both varieties, the isolation method seems to affect the starch granule sizes since the starch isolated by the ENZ method did not show granules larger than 60 μm. Thus, it can be stated that the alkali extraction method induces the presence of a larger range of starch granules than the enzymatic method, or rather, that during the extraction with protease, more granules are lost/destroyed and removed over to the aqueous phase by non-starch polysaccharides (Daiuto, Cereda, Sarmento, & Vilpoux, 2005).

Isolated chestnut starch appeared to the naked eye as a white powder. Both isolated chestnut starches presented high values of $L^*$, those produced by A3S method were the lighter starches (Table 1). Starches from both varieties presented a predominant yellow colour ($H^*$ values near 90°). The Longal variety produced starches duller than Martainha, this aspect was probably related to the colour characteristics of the flour, since this trend was also observed for the Longal and Martainha flours (Correia et al., 2009). However, the most appropriate colour measurement seems to be the $\Delta E$ calculation as it clearly puts into evidence major differences between the samples. According to Olange (1994), considering the colour difference ($\Delta E$) classification, chestnut starches presented $\Delta E$ values of 1.9 and 4.1 for Longal and 3.1 and 4.2 for Martainha, respectively for the A3S and ENZ methods. Thus, they are classified as distinct (1.5–3.0) to very distinct (3.0–6.0) (Table 1). Those results were also compared to those shown by a commercial maize starch sample. For the same conditions, this starch reference was also classified as very distinct ($\Delta E$ value 5.9 ± 0.12), a value higher than those found for chestnut starches. Based on these results, chestnut starch may be adequate for the same kind of products where maize starch is used. The isolation method seems to affect the colour parameters. The ENZ method presented higher values of the total colour difference parameter and $L^*$ (darker) parameter. These results could be related to the degree of purity of the isolated starches, since the A3S method presented higher purity, when compared with the ENZ method, as mention previously.

### 3.2. Fourier-transform infrared (FTIR) spectroscopy

A comparison between the FTIR spectra obtained for the starch granules isolated by A3S and ENZ methods for both varieties were assessed, in order to observe the interactions between starch molecules. The results showed that no differences can be observed in those spectra. For a better understanding of the type of interactions between starch molecules presented in chestnut starches, as an example, the spectrum of the sample Martainha starch isolated by the ENZ method is reported. The characteristic peaks of occurring starch molecule interactions are presented in Fig. 2. The absorption peaks at 3417 cm$^{-1}$ and 2935 cm$^{-1}$ visible in Fig. 2 should be attributed to the complex vibration stretches associated with free, inter- and intra-molecular hydroxyl groups and $\text{–CH}$ stretches associated with the ring methine hydrogen atoms, respectively. The absorptions at about 1653 cm$^{-1}$ may be attributed to $\text{H}_2\text{O}$ bending vibration (Luo, Huang, Fu, Zhang, & Yu, 2009; Zhang et al., 2007). Three characteristic peaks appeared between 935 and 1160 cm$^{-1}$. These peaks were attributed to $\text{C–O}$ bond stretching. The peaks at 1085 and 1031 cm$^{-1}$ were characteristic of the anhydro-glucose ring $\text{C–O}$ stretch (Fang, Fowler, Tomkinson, & Hill, 2002; Wu, Geng, Chang, Yu, & Ma, 2009) and at 868 cm$^{-1}$ were associated with the $\text{C–H}$ of residual carbons (Fleire-Pelegrin et al., 2007). The results are similar to those obtained for unmodified potato starch from Luo et al. (2009) and Wu et al. (2009).

### 3.3. X-ray diffraction

Starch is a semi-crystalline material and this structure can be identified through characteristic X-ray diffraction patterns. Four

<table>
<thead>
<tr>
<th>Variety</th>
<th>Isolation method</th>
<th>$L^*$</th>
<th>$C^*$</th>
<th>$H^*$</th>
<th>$\Delta E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longal</td>
<td>A3S</td>
<td>96.1 ± 0.17$^a$</td>
<td>3.1 ± 0.12$^b$</td>
<td>91.9 ± 0.70$^b$</td>
<td>1.9 ± 0.13$^b$</td>
</tr>
<tr>
<td></td>
<td>ENZ</td>
<td>93.5 ± 0.19$^a$</td>
<td>3.7 ± 0.11$^a$</td>
<td>83.0 ± 0.62$^b$</td>
<td>4.1 ± 0.14$^b$</td>
</tr>
<tr>
<td>Martainha</td>
<td>A3S</td>
<td>94.1 ± 0.23$^a$</td>
<td>4.3 ± 0.18$^a$</td>
<td>91.8 ± 0.37$^a$</td>
<td>3.1 ± 0.24$^a$</td>
</tr>
<tr>
<td></td>
<td>ENZ</td>
<td>93.7 ± 0.21$^b$</td>
<td>4.2 ± 0.15$^b$</td>
<td>87.3 ± 0.48$^b$</td>
<td>4.2 ± 0.15$^b$</td>
</tr>
</tbody>
</table>

Values are mean ± standard error of mean.

Means sharing the same letters in columns are not significantly different from each other (Tukey’s HSD test, $p < 0.05$).

![Fig. 2. The FTIR spectra of chestnut starch: L – Longal; M – Martainha; A3S – alkaline pH and using successively three sieves method; ENZ – enzymatic method.](image-url)
major types of X-ray patterns of native starches, designated as A, B, C, and V, have been identified (Zobel, 1988). The A-type is characteristic of most starches of cereal origin; the B-type of potato, root starches and amylomaize starches; the C-type of smooth pea and various bean starches (Liu, 2005).

The X-ray diffraction patterns of starches from chestnuts are presented in Fig. 3. This figure also shows the corresponding X-ray diffraction parameters and crystallinity level calculated from the ratio of diffraction peak area and total diffraction area.

Chestnut starch exhibits a C-type X-ray pattern, a mixture of “A” and “B” unit cells, the polymorphs were present in varying proportions (Gernat, Radosta, Damascun, & Schierbaum, 1990). The “B” X-ray pattern was characterized by peaks at diffraction angles around 5.6°, 15°, 17°, 20° and doublet peak at 22°–24°, like some legumes starches and potato starches (Jayakody et al., 2007; Singh, Nakaura, Inouchi, & Nishinari, 2008). On the other hand, pure A-type starches such as wheat and maize do not show the 2θ peak at 5.6° and exhibit a shoulder at 2θ = 18°, a unique peak around 2θ = 23° instead of the doublet 2θ = 22°–24°, and an increase in the relative intensity of the band at 2θ = 15° (Jayakody et al., 2007). The double helical structures are essentially the same, both in the A- and B-type crystalline forms, but the packing of the helices in the A-type crystalline structure is more compact than in B-type crystallites, which have a more open structure with a hydrated core (Imberty, Büléon, Tran, & Perez, 1991). These 2θ values were also encountered for other C-type X-ray pattern in legume starches, such as black beans, lentil and pinto beans and smooth peas (Zhou, Hoover, & Liu, 2004). Hizukuri, Fuji, and Nikuni (1960) classified the C-type spectrum into C_a, C_b, and C_c based on their resemblance to A and B-type or between the two types, respectively. Based on this, the X-ray spectra of chestnut starches could be classified as C_b types because the peaks related with this type of starch are more intense (Fig. 3). The presence of a peak at 2θ near 20° indicates the occurrence of crystalline amylase–lipid complexes (Yang, Jiang, Prasad, Gu, & Jiang, 2010). Furthermore, the presence of this peak has been observed in high amylose maize (V and VII) starches and low amylomaize maize starches (Shi, Capitani, Trzasko, & Jeffcoat, 1998). This pattern is also a characteristic of Longal and Martainha starches (Fig. 3).

The relative crystallinity of chestnut starch presented values between 31.5% and 36.0%. The Longal variety presented the lowest degree of crystallinity, meaning that its original structure could be changed or partially destroyed. In general, the differences in relative crystallinity between starches have been attributed to: (1) crystal size, (2) amount of crystalline regions (influenced by amylpectin content and chain length), (3) orientation of the double helices within the crystalline domains, (4) extent of interaction between double helices (Hoover & Ratnayake, 2002).

3.4. Solid-state NMR

13C CP/MAS NMR is very helpful to understand structural differences between samples that have similar nature (Billaderis & Zawistowski, 1990). The starch granule structure consists of two types of helices from amylopectin side chains: (1) helices that are packed in regular arrays form crystallinity, which can be measured by both X-ray diffraction and 13C CP/MAS solid-state NMR, and (2) helices that are not packed in regular form cannot be detected by X-ray diffraction but can still be detected by 13C CP/MAS solid-state NMR (Cooke & Gidley, 1992). It is therefore not surprising that estimates of order proportion by NMR spectroscopy are considerably higher than those obtained from X-ray diffraction.
environments for C1, therefore the C1 peak in A-type starch spectra is a triplet (~102, 101 and 100 ppm) (Atichokudomchai, Varavinit, & Chinachoti, 2004). For B-type starch, maltose is the repeat unit and the threefold screw axis generates the double helix providing two different environments for C1, thus the C1 peak in B-type starch spectra is a doublet (~101 and 100 ppm) (Gidley & Bociek, 1985; Veregin, Pyfe, Marchessault, & Taylor, 1986). Signal at 81–84 ppm is attributed to C4 and the overlapping signal around 71–75 ppm is associated with C2, C3 and C5. The 13C CP/MAS NMR Longal and Martainha chestnuts are presented in Fig. 4. From the results, it may be assessed that Longal and Martainha starch structures are similar. However, the isolation methods clearly affected both varieties. Since C-type starches have both A- and B-type crystallites, it can be suggested that the resonances in the spectrum mainly depend on the relative proportions of A- or B-allomorph in the sample (Bogracheva et al., 2001). These assignments of the resonances are based on the literature reported chemical shifts (Bogracheva et al., 2001; Gidley & Bociek, 1985; Veregin et al., 1986).

Signals at 99–104 and 58–65 are attributed to C1 and C6 in hexapyranoses, respectively. As described above, the C1 resonances for A3S method, in both chestnut varieties, indicates that B-type allomorph is actually predominant in the chestnut starch isolated by this method whereas a typical A-type characteristic can be found by the ENZ method conducted a decrease in starch crystallinity, meaning that its original structure could be changed or partially destroyed. This is in accordance with the encountered differences on polymeric forms of starch crystalline structures of starch isolated by A3S and ENZ methods. The structure of isolated chestnut starches could be related with the chain length of amyllopectin remains after starch isolation. The A-type starch crystallites are formed from shorter chain (A-chains with dp of 6–12) and the B-type starch crystallites from longer chain (Jane, Wong, & McPherson, 1997; Liu, 2005). This could be due to the high level of long chains of amyllopectin present in starch isolated by A3S method opposed to the ENZ method: the amorphous phase in the starch granules decrease and the B-type allomorph in the C-type starch granules is more evident than the A-type. Thus, the isolation conditions seem to affect the structure of starch.

Previously, for X-ray diffraction results, it was noticed that the ENZ method conducted a decrease in starch crystallinity, meaning that its original structure could be changed or partially destroyed. This is in accordance with the encountered differences on polymeric forms of starch crystalline structures of starch isolated by A3S and ENZ methods. The structure of isolated chestnut starches could be related with the chain length of amyllopectin remains after starch isolation. The A-type starch crystallites are formed from shorter chain (A-chains with dp of 6–12) and the B-type starch crystallites from longer chain (Jane, Wong, & McPherson, 1997; Liu, 2005). This could be due to the high level of long chains of amyllopectin present in starch isolated by A3S method (unpublished data), possibly caused by a higher lixiviation of short chains and/or a higher preservation of crystalline structure during starch isolation. Furthermore, the A-type starch crystallites are believed to be formed in warmer conditions with denser crystalline packing, whereas B-type starch crystallites are formed in wet and cold conditions with less dense crystalline packing (Liu, 2005). This requirements for formation of A and B-type starch crystallites are in accordance with our starch isolation conditions, on A3S method flour was soaked at 5 °C and on ENZ method flour was incubated at 37 °C. In spite of these, some authors mentioned that starch granular structure in general is not fully understood, and there is even less understanding about the granular structure of C-type starches (Wang et al., 2009).
4. Conclusion

In general, starch isolated from the two studied varieties, Longal and Martainha, seems to have similar morphology and structure. Chestnut starch granules were found to be round and oval in shape, consisting of medium/small granules classified predominantly as B-type granules. Chestnut starches could be classified as a C3 type, with a high relative crystallinity value, more than 30% and they presented similar interactions between starch molecules. In spite of the interactions between molecules in starch granule being similar for both extraction methods, the structure of the granules was significantly different. The presence of crystalline amyllose–lipid complexes were found, being more intense in starches isolated by ENZ method, which is probably due to the higher content of lipids in those starches. Generally, no significant differences were observed considering the crystallinity degree of starch granules determined by XRD, but some differences were observed in the 13C CP/MAS NMR patterns for chestnut starch isolated by the A3S and ENZ methods. The intensity of C-1 and C-4 amorphous domains resonances was smaller in ENZ method than in the A3S method. The C1 resonances indicate that B-type allomorph is predominant in the chestnut starch isolated by A3S method whereas a typical A-type characteristic can be found in the ENZ method. Letea, F. & Gidley, M. J. (2004) showed that starch isolated by the last method presented less compact crystallite structure of the double helices. Since isolation methods induce structural differences between the isolated starches, it is possible to determine that the functional properties will be also different, leading to different industrial applications.

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