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Physicochemical and Structural Properties of Starch Isolated from Fresh and Dried Chestnuts and Chestnut Flour

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Summary

Particle size distribution, colour, morphology and chemical composition of chestnut (Castanea sativa Mill.) starches isolated from fresh chestnut fruits (S1), semi-dried chestnut fruits at room temperature (S2) and commercial chestnut flour (S3) were determined using several experimental techniques. All starches had a bimodal particle size distribution, particularly S1 showed two types of starch granules – small (1.5 μm diameter) and large granules (10.5 μm). Starch granule sizes depended on the moisture content of the samples, decreasing slightly in the following order S1>S2>S3; however, no significant differences were observed in the morphological analysis. Most of the granules exhibited round or oval shapes, and exceptionally, some of them featured trefoil shape, which is not usually found in other starches. Colour results indicated that S3 samples had the darkest colour, followed by S2 and S1. Tested chestnut starches showed significant differences in total starch content, with starch isolation being more selective in dried samples. All samples showed low damaged starch (<2.91 %) and intermediate amylose (from 17.0 to 25.8 %) content on dry mass basis. The lowest amount of amylose was obtained in S1, even though it was within the range of common commercial starches.

Key words: gluten-free diet, optical microscopy, particle size, amylose, damaged starch

Introduction

The use of starch powders as a basis for the production of gluten-free puddings or sweet desserts is currently rising. The employed formulations to develop these products involve a large number of alternative starches to wheat starch such as corn, cassava, rice, soybean, chickpea or amaranth starches as well as their blends (1). Nevertheless, new sources like chestnut starch can provide similar products with interesting nutritional and taste characteristics (2). The research of this source of starch is motivated by the increasing demand for food industry to develop new high quality gluten-free products.

Chestnut fruits (Castanea sativa Mill.) contain relatively high fractions (on dry mass basis) of starch (50–60 %) and sugars, mainly sucrose (20–32 %), proteins with a high content of essential amino acids (4.0–7.0 %), low amount of fats (2.0–4.0 %), appreciable levels of dietary fibre (7.0–12 %), and high vitamin E, vitamin B, potassium, phosphorous and magnesium content (3,4). Physicochemical and structural properties of starches are necessary to understand how these systems behave under food processing conditions. Pasting characteristics, structural and textural properties of gluten-free formulations strongly depend on starch composition and its content (5). Many factors can greatly influence this behaviour:

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the origin of cultivars, starch composition and structure, processing methods, and storage conditions (6).

One of the most important physical properties of starch is the size of granules because of its key role in processes like milling, mixing and pasting, even in determining colour properties (7). The microstructure of starch noticeably influences the appearance, texture, and stability of the final product. Bright-field and polarised light microscopy methods are frequently used for morphological characterisation of starch (8).

The colour and chemical composition of starch are also important for the raw material suitability in bakery processes (9). Standard methods (10) are commonly followed for these determinations. Starch is one of the most responsible compounds that determines bread and pastry characteristics (11). It has high impact on flour dough behaviour when mixing or when thermal processes are involved. It is remarkable that chemical characteristics of starch (damaged starch content, amylose/amylopectin ratio, total starch content or colour) depend on the processes of the production of starch itself, i.e. extraction, drying or milling procedures (5). While cereal starches have been well-studied, studies on chestnut starch isolated from starchy materials processed under different conditions are scarce.

The main aim of this work is to provide a physicochemical characterisation of chestnut starches isolated from the same source processed under different conditions. For this purpose, the particle size distribution, colour, morphology and chemical composition of chestnut starches extracted from fresh chestnut fruits (S1), semi-dried chestnut fruits at room temperature (S2) and commercial chestnut flour (S3) were determined.

Materials and Methods

Raw materials

Chestnut fruits (Castanea sativa) and commercial chestnut flour from the same chestnut variety (Longal) were acquired in a local market (Galicia, Spain). Moisture content of chestnut starch designated S1 isolated from fresh chestnut fruits was 52.5 %, of starch labelled S2 from semi-dried chestnut fruits at room temperature 25.3 %, and of starch labelled S3 from commercial chestnut flour 10.2 %, determined following a method described previously (12).

Starch granule size analysis: laser diffraction

Particle size of the tested starches was evaluated by laser diffraction (Mastersizer 2000, Malvern Instruments, Worcestershire, UK) using the following protocol. Isolated wet starches (0.1 g) were slurried with 1 mL of water and mixed with the vortex before use. Water and ethanol (refractive indices of 1.33 and 1.36, respectively) were employed as dispersing agents at 20 °C. Sieving and laser diffraction experiments were performed in triplicate for each sample.

Colour characterisation: colorimetry

Colour measurements were performed using a Chroma Meter CR-400 (Minolta, Osaka, Japan). Colour parameters (L’, a’, b’) were assessed by CIELAB, where L’ (lightness), a’ (redness or greenness) and b’ (yellowness or blueness) are the chromaticity parameters. Total colour difference (ΔE*) was calculated by Eq. 1 using S1 as reference. At least ten measurements were carried out for each sample.

\[ \Delta E^* = \sqrt{(L_1 - L_0)^2 + (a'_1 - a'_0)^2 + (b'_1 - b'_0)^2} \]

Morphological characterisation of starches determined by microscopy

Morphological characterisation of starches was carried out by bright-field and polarised light microscopy using a BX61-P microscope (Olympus, Center Valley, PA, USA) and the cell-PA software (Olympus). Starch samples (0.2 g of each isolated starch; S1, S2 and S3) were slurried with water in Eppendorf® microfuge tubes (2.0 mL). The tubes were capped and gently agitated. The images were captured using a Hamamatsu ORCA-AG camera (Hamamatsu Photonics, Hamamatsu, Japan), and then analysed by means of free image analysis software (UTH-SCSA Image Tool v. 2.0, University of Texas, Health Science Center, San Antonio, TX, USA). Surface area (A), boundary perimeter (p), elongation, roundness (R), and compactness (c) were determined (13). Elongation is the ratio of major axis length (L) over minor axis length. Roundness and compactness are defined by:

\[ R = \frac{4pA}{p^2} \]

\[ c = \sqrt{\frac{4A}{L}} \]
Table 1. Size and shape parameters of the tested chestnut starches

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation</td>
<td>0.61±0.05a</td>
<td>0.63±0.05a</td>
<td>0.56±0.06a</td>
</tr>
<tr>
<td>Roundness</td>
<td>0.79±0.02a</td>
<td>0.79±0.02a</td>
<td>0.79±0.02a</td>
</tr>
<tr>
<td>Compactness</td>
<td>0.79±0.02a</td>
<td>0.79±0.02a</td>
<td>0.79±0.02a</td>
</tr>
<tr>
<td>Size range</td>
<td>0.01–0.215a</td>
<td>0.01–0.210a</td>
<td>–</td>
</tr>
<tr>
<td>(d_{0.1t}/\mu m)</td>
<td>(5.3±0.1)b</td>
<td>(5.3±0.1)b</td>
<td>(3.1±0.1)c</td>
</tr>
<tr>
<td>(d_{0.5t}/\mu m)</td>
<td>(27.7±0.3)b</td>
<td>(16.9±0.2)c</td>
<td>(10.5±0.4)d</td>
</tr>
<tr>
<td>(d_{0.9t}/\mu m)</td>
<td>(42.7±1.4)b</td>
<td>(34.6±0.5)c</td>
<td>(23.3±0.5)d</td>
</tr>
</tbody>
</table>

Data are presented as mean values±standard deviations. Values with different superscripted letters in rows are significantly different, p<0.05. S1=starch from fresh chestnut, S2=starch from semi-dried chestnut, S3=starch from commercial chestnut flour

Table 2. Experimental colour parameters (\(L^*, a^*, b^*\)) and total colour difference (\(\Delta E^*\)) of chestnut starches

<table>
<thead>
<tr>
<th>Starch</th>
<th>(L^*)</th>
<th>(a^*)</th>
<th>(b^*)</th>
<th>(\Delta E^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>(95.7±0.2)a</td>
<td>(0.27±0.02)b</td>
<td>(2.1±0.1)c</td>
<td>0c</td>
</tr>
<tr>
<td>S2</td>
<td>(94.4±0.1)b</td>
<td>(0.23±0.02)b</td>
<td>(3.5±0.1)b</td>
<td>(1.9±0.06)b</td>
</tr>
<tr>
<td>S3</td>
<td>(88.7±0.2)c</td>
<td>(1.00±0.02)c</td>
<td>(5.2±0.1)a</td>
<td>(7.7±0.08)a</td>
</tr>
</tbody>
</table>

Data are presented as mean values±standard deviations. Values with different superscripted letters in columns are significantly different, p<0.05. S1=starch from fresh chestnut, S2=starch from semi-dried chestnut, S3=starch from commercial chestnut flour, \(L^*\)=lightness, \(a^*\)=redness or greenness, \(b^*\)=yellowness or blueness
Low fractions of damaged starch were identified; this fact was clearly observed in the largest particles, Figs. 2c and 2d (black and white ovals, respectively). Damaged starch amount slightly increased in S3 samples. Other authors found that chestnut starch granules from dried chestnuts showed more fractures than the starch from fresh samples (23).

The size range of chestnut starch granules decreased slightly in the following order S1>S2>S3 (Table 1). Chestnut processing modified slightly the morphology of starch granules along with other properties. Length and width of starch granules showed high variability. Semi-dried chestnut samples S2 did not show significant differences in the length and width of starch granules in comparison with S1 and S3 (same area and perimeter values, data not reported). The values of elongation, roundness and compactness remained constant for all starches. These outcomes were consistent with particle size data obtained by means of laser diffraction (Table 1). Laser diffraction values were in the same range as those achieved by microscopy, although the average values were slightly higher. Microscopy assays allowed the identification of bigger agglomerates of starch granules.

**Chemical composition of starches**

Chemical properties of the tested starches (total starch, damaged starch and amylose content) are given in Table 3. The tested chestnut starches showed some differences between them. Particularly, all samples presented significant differences in total starch content. Starch isolation was improved by drying. S1 showed the lowest starch content and starch isolation was better in the case of dried samples. Other authors also observed that samples with higher moisture content gave lower starch content (23).

Damaged starch levels varied in a narrow range from 2.1 up to 3.1%. These values are in the range of soft flour types according to a wheat flour classification in which damaged starch values corresponding to soft and hard flour types were <3.0 and >5.0%, respectively (24). The starch damaged fraction slightly increased with previous chestnut drying (S3>S1). The results confirm the morphological analyses.

Amylose content of S3 (20.2±0.4%) was intermediate when compared with data reported for corn (25.3±1.7%), wheat (24.9±2.1%) and rice (19.6%) flour (4). These values are also in agreement with those previously found in chestnut starch (21.5%) (12). Previous study on Castanea crenata starch determined amylose content of 19.6% (25). S1, S2 and S3 samples showed a random trend of amylose content. The lowest fraction was obtained in S1 ((17.0±0.4)%), whereas S2 ((25.8±0.3%) exhibited the highest content. Other authors studied the effect of drying temperature on chestnut fruits and found that amylose content increased with the increase of drying temperature (to 60 °C) and by enzymatic processes (23). A lower increase in amylose content at above 70 °C could be probably owing to the enzyme inactivation. Low amylose content of S3 could be related to high drying temperatures (>70 °C) during commercial chestnut flour processing.

**Conclusions**

The average size of chestnut starch granules depends on the moisture content of the raw material, following S1>S2>S3 order. Other parameters (colour, morphology and composition) of chestnut starch varied in a narrow range. S3 showed the darkest colour, even though its value was within the common range of the colour parameters determined for other commercial starches. The tested chestnut starches presented two types of granules – small (1.5 μm diameter) and large granules (10.5 μm) – which were smaller than those identified in wheat flour. The isolation of chestnut starch was better from dried samples, achieving the largest total starch content in S3. The fractions of damaged starch were low (below 2.91%), within the typical range of soft flour types, while intermediate values of amylose content (above 17.0%) were similar to those in commercial cereal (rice, corn and wheat) starches commonly used in the food industry. Assays with the flour manufactured under well-controlled conditions should be performed in order to determine the influence of processing conditions on the physicochemical properties of chestnut flour.
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