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Effects of partial *in vitro* digestion on properties of European chestnut (*Castanea sativa* Mill) flour

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ABSTRACT

Several enzymes can modify original properties such as pasting and termal profiles of flours. European chestnut flour was subjected to partial enzymatic hydrolysis for 12, 24 and 36 h, to study the thermal phase transition by differential scanning calorimetry (DSC), thermogravimetry and differential thermal analysis (TG-DTA), surface morphology by scanning electronic microscopy (SEM) and pasting properties by rapid visco analyser (RVA). Enzyme treatment has changed flour structural stability, since ΔH_{gel} analysis showed rising values. Concerning TG-DTA results, fibre content probably affected mass loss values. SEM images showed notable erosion on surface granules during the process due to enzyme action. Finally, treated samples showed lower peak viscosity and lower setback viscosities values. Since there were evidences of thermal, morphological and pasting modifications, partial hydrolysis allows the use of chestnut flour in wider range of applications.

1. Introduction

The European or Portuguese chestnut (*Castanea sativa* Mill) is a native fruit tree from the northern hemisphere and still plays an important social, ecological, forested and agronomic part in that region. The high nutritional value of chestnut, allied with wood extraction, guaranteed an important role from ancient times to the present day. The chestnuts contain significant concentrations of health promoting compounds and essential nutrients to the human diet, such as antioxidants L-ascorbic acid [1–4], vitamin E [4] and [5], carotenoids [4], polyphenols [4,6,7], besides free sugars, proteins, lipids (monounsaturated and polyunsaturated), fibers, other vitamins and minerals [8–11]. Among these nutrients, starch figures as the main component with content between 38% to 80% [12–14], mainly composed of around 33% of amylose and 67% of amylopectin [12,13,15]. Many studies indicate a large number of botanical sources of starch, but in spite of that not everyone has industrial value yet [16,17,19]. Chestnuts are mainly consumed fresh, but a surplus is often lost. Since there are potential applications for this crop in order to add value to underexploited non-conventional starch sources [20].

The consumer goods industry, particularly the food industry, has sought starch with specific physicochemical and structural properties when submitted to different conditions of process [21,22] and also as viable cost-effective alternatives [23]. Some characteristics of starch granules such as swelling, breakdown and retrogradation in large part determine textures and stabilities of high moisture starch based foods [24]. In order to obtain knowledge of these properties, the thermal analysis constitutes an important means to characterise the starches. This is due to the fact that when a sample is heated, chemical or physical changes may occur, depending on whether the thermal heat is less or greater than amylose and amylopectin chains stabilities. Therefore, it is necessary to know the temperature limits to which the starch can be submitted to without compromising its properties. The knowledge of material thermal properties can lead to improvements of processes, transport, conservation and applications [25]. In particular, the chestnut starch was studied by some authors and presented inherent characteristics such as low tendency to retrogradation, low gelatinsation temperature range and stability when submitted to commercial freezing cycles, making it a potential alternative to commercial starches [12,15].

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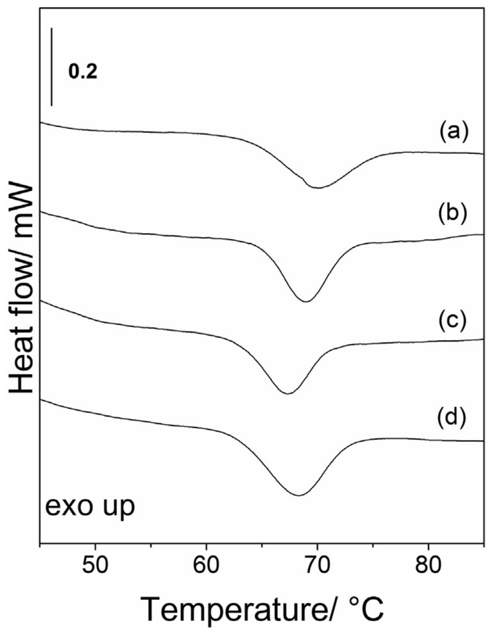

Fig. 1. DSC curves of: (a) untreated chestnut flour; (b) chestnut flour treated for 12 h; (c) 24 h; and (d) 36 h.

Since European chestnut was identified as a potential source of starch and it has a large potential for commercial use, the aim of this study was to evaluate some thermoanalytical and pasting characteristics of chestnut flour after enzyme hydrolysis.

2. Materials and methods

2.1. Materials

The European chestnut (*C. sativa* Mill) samples were collected from South cultivars in Brazil. The seeds were evaluated in terms of weight, diameter, moisture content and proportions between edible and non-edible parts considering 50 samples. For the experiment an enzymatic complex of Distillase™ (Genencor, USA), containing α-amylase (1,4-α-D-glucan glucanohydrolase—EC 3.2.1.1), glucoamylase (1,4-α-D-glucan hydrolase E.C. 3.2.1.3) and aspergillopepsin (EC 3.4.23.18) and reagents with analytical grade were used. The DSC and RVA analyses were done in triplicate and the statistical test of *Tukey* delimited by SASM-Agri v. 8.2 software was used.

2.2. Chestnut flour

The chestnut fruits harvested at mature stage and the seeds were removed manually from the fruit then peeled.The flour was obtained by dry milling the dehydrated seeds and sieving (60 USBS). Chemical composition of untreated sample was previously obtained according to Demiate, Oetterer and Wosiacki methodology [15] and presented following composition: moisture 7.65% ± 0.23, ash 2.09% ± 0.04, protein 5.72% ± 0.33, lipids 1.60% ± 0.02, fibre 20.65% ± 0.99, total carbohydrate 62.29% ± 1.60.

2.3. Enzymatic hydrolysis

In order to promote hydrolysis of studied matter, 2 g of chestnut flour were added to 0.008 L of distilled water and 15 μL of enzyme (according to enzyme data sheet instructions) in a 0.125 L erlenmeyer flask. The pH was adjusted to 5.5 with addition of HCl (0.1 mol L^{-1}) and NaOH (0.1 mol L^{-1}) solutions. After preparing the samples and covering the erlenmeyer flask, they were kept in a TECNAL shaker Model TE-420 at 30 °C under continuous stirring of 1.66 Hz for 12 h, 24 h and 36 h. In order to obtain the solid part, the aqueous medium was filtered through Whatman 42 filter paper using a vacuum pump. Solid samples remaining on paper filter were dried in an oven at 50 °C for 36 h, and then kept in a desiccator over anhydrous calcium chloride.

2.4. Thermal analysis of chestnut flour

DSC curves were recorded using a DSC 60 (Shimadzu, Japan) under synthetic air flow at 100 mL min^{-1}, heating rate of 5 °C min^{-1}. Each European chestnut flour sample (approximately 2.5 mg) was weighted directly in an aluminium pan and distilled water was added (1:4 w/v) using a micropipette. The pan was hermetically sealed and allowed to equilibrate for 2 h before analysis. In order to obtain the DSC curves, the instrument was calibrated with indium 99.99% purity, m.p. = 156.6 °C, ΔH = 28.71 J g^{-1}, and an empty aluminum crucible was used as References [26,27].

TG–DTA curves were recorded using a simultaneous TG 60 system (Shimadzu, Japan) under an oxidative atmosphere (air) flow at 100 mL min^{-1} and at a heating rate of 10 °C min^{-1}. The initial mass of sample was about 6 mg. Alumina crucibles were used for the TG–DTA experiments. All mass loss percentages were determined by TA-60WS data analysis software [17,27].

2.5. Microscopy analysis

After drying, samples of chestnut flour untreated and treated by enzyme were mounted on aluminum stubs and covered with gold foil. The scanning electron microscopy (SEM) analysis was carried out using the equipment Mini Sputter Coater SC 7620 Polaron (Quorum Technologies Ltd., East Sussex, England) and observed on scanning electron microscope LEO 440i-Leica (Wetzlar, Germany). SEM analysis has been widely used in scientific research as a useful tool to examine small particles from interesting materials in order to obtain information relative to the morphology of the samples examined [28].

2.6. Pasting behaviour

Prior to determining the pasting properties of each sample, it was necessary to determine their moisture in order to calculate the exact amount of dry matter in the suspension. An infrared moisture analyser (Sartorius AG, MA35 M, Germany) was used for the moisture determination. The pasting properties of samples were determined using a RVA-4 Viscoamylograph (Newport Scientific Pvt. Ltd., Australia). A dispersion of 2.5 g (8% moisture) flour in 0.028 L of distilled water underwent a controlled heating and cooling cycle under constant stirring where it was held at 50 °C for 2 min, heated from 50 to 95 °C at 6 °C min^{-1}, and held at 95 °C for 5 min, cooled to 50 °C at 6 °C min^{-1}, and held at 50 °C for 2 min.

3. Results and discussion

3.1. Differential scanning calorimetry (DSC)

Starch granules, when heated in excess of water, undergo significant structural and morphological changes including swelling due to water absorption, loss of crystallinity due to the amylopectin double helix dissociation, and amylose leaching to the water phase [12]. The purpose of using the technique in this study was to evaluate the gelatinisation properties, shown in Table 1, of chestnut starch portion through the energy variation due to phase transition. The DSC curves of untreated and modified chestnut flour samples are presented in Fig. 1.