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Morphological, thermal and physicochemical characteristics of small granules starch from *Mirabilis jalapa* L

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ABSTRACT

Some physicochemical and thermal properties of starch from *Mirabilis jalapa* L, seeds were evaluated. The starch was extracted after hulling and grinding the seeds and the flour obtained was suspended in 0.1% (*m*/*v*) NaOH solution for 12 h at 30 °C; it was then centrifuged, re-suspended, washed with deionised water and dried in an oven with circulating air at 40 °C for 12 h. The micro-images of starch granules were performed by using scanning electron (SEM) and non-contact atomic force microscopy (NC-AFM) techniques; X-ray diffraction and mid-infrared spectroscopy were both used to evaluate the relative crystallinity of the starch granules. Thermal analyses TG/DTG and DSC, were applied for the analysis of thermal behaviour of this starch and the cooking behaviour of its aqueous solution was studied by using a viscometer RVA. Thermogravimetry showed that once dehydrated, the starch was stable up to 292 °C after which two steps of decomposition occurred, which were attributed to decomposition and oxidation of organic matter, respectively. The gelatinisation temperature and enthalpy, as assessed by DSC analysis, were 82.1 °C and 5.67 J g^{-1}, respectively. RVA analysis showed pasting temperature of 76.4 °C, with a low viscosity peak at 95 °C, low breakdown, and high tendency to retrograde during cooling. Microscopic results reveal that the starch granules had a spherical shape and 67.4% of them presented diameters smaller than 890 nm. The X-ray diffractogram showed a typical A-type pattern and a relative crystallinity of 34% with a FTIR of 1047/1022 cm^{-1} and a ratio of 1.38.

1. Introduction

The *Mirabilis jalapa,* known in Brazil by the name 'Marvel' (*Maravilha*, in Portuguese) is an ornamental plant that has its origin in Latin America. Nowadays, it is cultivated almost all over the world as an ornamental plant, but in some countries it is also employed as a popular medicine due to its antibacterial and healing properties. Some studies that examined the active components of this plant reported two small proteins that presented strong antibacterial activity [1,2]. These proteins were characterised [3] and tested against different types of bacteria [4] and moulds of rice [5], and their properties were confirmed, making this ornamental plant a potential industrial raw material. The seeds of this plant also present high amounts of starch.

The objective of the present paper was to extract and characterise the starch from the seeds of this plant and to study the granule morphology, particle size, crystalline structure, thermal and pasting properties.

2. Materials and methods

2.1. Materials

Seeds from *M. jalapa* (white, yellow and red flowers) were collected from plants in the the city of São José do Rio Preto (20°49′12″ S, 49°22′44″ W), SP, Brazil. The seeds were dried at room temperature (25 °C) and stored in plastic bags until processing for starch extraction. The seeds were hulled and their fractions were observed under stereo light microscope. Some images of broken seeds were made by using a scanning electron microscope (SEM) in order to observe the internal structure and organisation, as well as the distribution of the starch granules.

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Standard amylose, AM (A9262) and amylopectin, AP (A8515) were from Sigma Chemical Co. (USA).

2.2. Starch isolation

The *Mirabilis* seeds were manually hulled and the tegument was discarded. It was milled by using a knife mill (IKA Universal Mill M 20, Germany). The material was sieved and fine flour was produced mesh no. 80; (177 μm). Starch extraction was performed by washing this flour with excess of standard NaOH 0.1% (*m*/*m*) solution and the precipitate was recovered by centrifugation (2200 × *g*). After several successive precipitations, the starchy pellet was washed with water until pH reached 7.0. The starch dispersion was sieved through a 53 μm mesh and then dried at 45 °C for 24 h. The dried starch lumps were disintegrated in a knife mill and sieved through mesh no. 80 (177 μm). This was then stored in sealed plastic bags until further use.

2.3. Chemical analysis

The moisture content was calculated based on weight loss after the samples were heated in an oven at 105 °C/12 h. The ash content was determined by incineration in a furnace at 550 °C/6 h. The fats were obtained by extraction using hexan in a Soxhlet apparatus. Protein content was estimated from the nitrogen content, which was obtained by the micro-Kjeldahl method, using a conversion factor of 6.25 as AOAC protocols and as previously reported [29]. The samples were tested in triplicate.

The amylose contents were determined using the method known as the hot NaOH procedure [6] as follows: the starch sample was packed in a paper filter and dipped in hexan; it was kept for three days at 4–8 °C for complete lipid extraction. After that period, the starch was recovered by filtration and oven dried at 40 °C/12 h for amylose determination. The de-fatted starch samples (100 mg) were dispersed with 1 mL of ethanol and suspended in 9 mL of 0.5 mol L^{-1} NaOH standard solution. The suspensions were heated for 30 min in a water bath at a temperature of 95 °C and then left overnight.

The solution was diluted to 100 mL, and a 5 mL quantity was transferred into a 100 mL volumetric flask with 25 mL of distilled water. Then 1.0 mL of 1 mol L^{-1} acetic acid, and 2 mL of standard iodine solution (0.2% iodine in 2% potassium iodide) were added and the mixture was then completed to 100 mL with distilled water.

The solution was mixed and then left for approximately 20 min to develop colour in a dark room. The optical absorbance was recorded at 620 nm using a spectrophotometer (Shimadzu UVmini-1240, Tokyo, Japan). A blank, containing all the reagents except starch, was prepared for the reference cell. Solutions of intermediate AM proportions, which were obtained by mixing the standard AM and AP (20, 15, 10, 5, and 0% of AM), were prepared as described above. A straight-line plot was used to determine the contents of AM in the samples. Triplicate AM content measurements were performed for each sample.

2.4. Scanning electron microscopy (SEM) and atomic force microscopy (AFM)

Scanning electron microscopy makes it possible to characterise starch granules in relation to their shape and size. Atomic force microscopy makes it possible to measure the diameter of starch granules as well as their surface characteristics. As reported by [7], a topographical analysis of the surface of starch may be useful for calculating the average diameters and two types of rugosity; the average rugosity (R_a) and its average quadratic root (R_q) by using the following Eqs. (1) and (2):

$$R_a = \frac{1}{L}\int_0^L |Z(x)|\mathrm{d}x \quad (1)$$

$$R_q = \frac{1}{L}\int_0^L |Z^2(x)|\mathrm{d}x \quad (2)$$

where $Z(x)$ is the function that describes the surface profile analysed in terms of height (Z) and position (x) of the sample over the evaluation length (L).

The morphology of the granules was analysed using a scanning electron microscope, model S-3000N SEM (Hitachi Ltd., Tokyo, Japan). A double-sided strip was affixed to the conductive support of electrons and the starch was spread on strips. These were coated with gold using an E 102 ion sputter (Hitachi Ltd., Tokyo, Japan) for 60 s at 50 mA. The granules were then examined under the following conditions: voltage of 15.0 kV, emission current of 100 mA, high vacuum (10.4 Pa), working distance of 18.9–19.9 mm with 5000× and 18,000× magnification.

For the atomic force microscopy (AFM) analysis of roughness and average diameters, a suspension was prepared with 1% starch. A droplet was placed on a glass slide and left to dry at room temperature. The analysis was performed in an atomic force microscope (AFM), model SPM 9600 (Shimadzu) by the non-contact method with scan rate (0.8 Hz) in areas of 2 × 2 μm and 5 × 5 μm.

2.5. Particle size distribution

The particle size distribution was evaluated by using a laser diffraction particle size analyser (Zetasizer, model Nano ZS90, Malvern Instr., UK). Starch samples of 50 mg were dispersed in 25 mL of distilled water and mixed in an ultrasound sonicator for 10 min at room temperature (~25 °C) until the complete breakdown of agglomerates.

2.6. X-ray diffraction powder patterns (XRD)

X-ray powder diffraction patterns (XRD) were obtained by using an X-ray diffractometer, model Ultima 4 (Rigaku, Japan), employing Cu Kα radiation (λ = 1.541 Å) and settings of 40 kV and 20 mA. The scattered radiation was detected in the angular range of 5–80° (2θ), with a scanning speed of 8° min^{-1} and a step of 0.06°.

2.7. FT-IR spectroscopy

Fourier transform mid-infrared spectroscopy was employed to collect starch spectra (FT-IR PRESTIGE-21, Shimadzu, Japan) using the transmission technique. KBr pellets were made with approximately 1 mg of starch and 100 mg of KBr for IR spectroscopy, using a hydraulic press. The number of scans adopted was 64 for each sample, and a 4 cm^{-1} resolution considering the 4000–400 cm^{-1} range; the temperature during the analysis was 25 °C. The Excel© (Microsoft) software programme was employed to analyse the spectral data. According to Smits et al. [8] and van Soest et al. [9] the absorbances at wavenumbers 1047 and 1022 cm^{-1} correspond to the crystalline and amorphous zones, respectively. The ratio of 1047/1022 cm^{-1} with values over 1.0 indicates a higher proportion of crystalline material and lower values indicate a higher proportion of amorphous material, as reported elsewhere [10].