Food Hydrocolloids 87 (2019) 101–107

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Physicochemical and structural properties of starch from young bamboo culm of *Bambusa tuldoides*

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ARTICLE INFO

Keywords:
Carbohydrate
Grass
Industrial application
Morphology
Properties
Sustainability environment

ABSTRACT

Starch is used in a wide variety of food and non-food industrial applications. However, in some cases, it is necessary to apply physical and/or chemical treatments to alter some properties, besides being an expensive process, it is environmentally unfriendly. So, the objective of this study was to characterize the morphology, structure, physicochemical and thermal properties of starch extracted from three different parts of the young culm (bottom-SB, middle-SM and top-ST) of *B. tuldoides*. The obtained data were evaluated by ANOVA and Scott-Knot test ($p < 0.05$). The starches presented pale yellow coloration and high luminosity (on average $a^* = 0.65$, $b^* = 11.26$ and $L = 89.39$), besides polyhedral shape and small size (on average 4.64 μm) observed by optical (OM) and scanning electron (SEM) microscopies. The starches showed apparent amylose content similar to starches from cereals and isolated from bamboo seeds. The molecular size distribution of amylose chains corroborates with SM (33.35%) and ST (24.48%) amylose content, since they presented the highest values. The highest proportion of chains of amylopectin were observed for DP 13–24, without significant difference between the samples, which sustain with A-type polymorph presented for all the samples. Transition temperature above 81 °C was observed and agree with the higher proportions of amylopectin short chains. The obtained starches presented characteristics of native starches and were similar to the starches traditionally used. Thus, starch from the young culm of bamboo would be an alternative source to traditional starches, such as rice, wheat or corn, sustaining with its potential for industrial applications.

1. Introduction

Starch, which is stored in the seeds and tubers of various agricultural crops, is the major reserve of carbohydrate in plants. The production of corn, cassava, wheat and potatoes has expanded in several countries, because their starches has been used in the production of food and beverages, manufacture of adhesives, cosmetics, detergents, paper, textiles, biodegradable packing materials and biodegradable plastics (BeMiller & Whistler, 2009; Davis, Supatcharee, Khandelwal, & Chibbar, 2003; Ellis et al., 1998).

This increased demand for starch on the market has expanded the search for new sources of starches, and for chemically or physically modified starches. Recently, great attention has been given to the advances made by genetic engineering technologies, which could allow changes in the synthesis of starch *in planta*, reducing or eliminating post-harvest modifications, as cited by Davis et al. (2003).

Some crops, such as bamboo, have great potential for the application of genetic engineering, since despite their low commercial value, their starch could present properties of commercial interest, as has already been done in the production of starches with high amylose content and waxy starches using maize, wheat, rice and potato starches (Gao, Wanat, Stinard, James, & Myers, 1998; Hovenkamp-Hermelink et al., 1987; Murata, Sugiyama, & Akazawa, 1965; Nakamura, Yamamori, Hirano, Hidaka, & Nagamine, 1995).

The genetic engineering technique is very versatile and could be applied in various sources for production of different types of starch, reducing not only the processing costs of the industry, but also the damages to the environment, by reducing the need for the chemical

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https://doi.org/10.1016/j.foodhyd.2018.07.032
Received 20 April 2018; Received in revised form 12 June 2018; Accepted 19 July 2018
Available online 20 July 2018
0268-005X/

treatments. Thus, the greater the types and sources of known starches, the greater the production of modified starches by transgene technology in the future, which can present the most varied possibilities of industrial application.

The use of the culm of *Bambusa tuldoides* Munro would be a great option for the application of transgene technology to modify starches. It is a perennial crop that grows fast, without the need of replanting, and that does not require pesticides (Silva & Costa, 2012). It is a high-yield renewable resource, cheap and abundant across the globe (Akinlabi, Anane-Fenin, & Akwada, 2017). Moreover, the use of bamboo starch would be a better way to use the whole culm, since it could be obtained as a co-product of the fiber extraction, which can be used for civil or textile application, production of biomass and bioethanol, biofilms (Kobayashi, Take, Asada, & Nakamura, 2004; Littlewood, Wang, Turnbull, & Murphy, 2013; Peng & She, 2014), as well as extraction of bioflavonoids and purified celluloses and hemicelluloses for use in food products among others (He et al., 2014; Scurlock, Dayton, & Hames, 2000; Tsuda, Aoyama, & Cho, 1998).

The young bamboo culm has a higher content of starch, which is more easily extracted (Felisberto, Miyake, Beraldo, & Clerici, 2017) when compared to bamboo seed starch (Ai et al., 2016), although the young bamboo culm starch from this specie, *B. tuldoides*, has not yet been characterized as to its morphological and physicochemical properties. So, the objective of this study was to characterize morphology, structure, physicochemical and thermal properties of starch extracted from young bamboo culm of *B. tuldoides*, as an alternative to take advantage of the whole culm of bamboo and to subsidize new industrial applications.

2. Material and methods

2.1. Obtainment of young bamboo culm chips

The young bamboo culms of *B. tuldoides* were harvested from January to March, 2016, on average three years old, at experimental field from School of Agricultural Engineering – UNICAMP, Campinas, São Paulo, Brazil, on geographic coordinates 22°82′ of south latitude and 47°07' of west longitude. The procedure of separation of the young culm in three parts (bottom, middle and top) was the same described by Felisberto, Beraldo, and Clerici (2017), and the processing of each of these parts in chips, for subsequent extraction of the starch, was according to methodology described by Felisberto et al. (2018).

2.2. Starch extraction

The chips from three different parts of the young bamboo culm were obtained to extract the starch, according to methodology described by Felisberto et al. (2018). The chips were disintegrated with sodium metabisulphite solution (200 ppm at 5 °C for 60 s), left to rest for 16 h/5 °C, passed through a sieve (80 μm), and the filtrate was centrifuged (17,000 g/10 min/10 °C). The supernatant was discarded, and the precipitate was resuspended with distilled water. The extracted starch was then vacuum filtered, washed with ethanol (99.5%), acetone, and dried (45 °C/16 h). The obtained starch from the bottom (SB), middle (SM) and top (ST) of the young culm were packed in plastic hermetically sealed, until analyses.

2.3. Morphological characterization of young bamboo culm starch

2.3.1. Instrumental color

Instrumental color was performed with a 50 mm port size, illuminant D65, SCI and a 10° standard observer angle, on CR-10 colorimeter (Konica Minolta, Tokyo, Japan). L* (luminosity: 0 = black; 100 = white), a* (+a = redness; -a = greenness), and b* (+b = yellowness; -b = blueness) parameters were determined by tristimulus CIELab color space method.

2.3.2. Morphological evaluation of starch granules

Optical Microscopy (OM) – The granule morphology was observed in an optical microscopy (BX51, Olympus, Tokyo, Japan), coupled with a camera (E-330-ADU1.2X, Olympus, Tokyo, Japan). The sample was placed on a glass slide and mixed with 1–2 drops of water, and then covered with a glass cover slip, and the micrographs were captured at 100X magnifications, without light polarized.

Scanning Electron Microscopy (SEM) – SEM (JEOL JSM 5800 LV, Tokyo, Japan) at 10 kV acceleration, was used to observe the morphology of starch granules, which was placed on carbon adhesive tape attached to the stub and sputter-coated with 20 nm of gold (SCD 050 Sputter Coater, Balzers). Diameters of starch granules were measured, at least 65 starch granules, on SEM micrographs (1000 and 3000X) using ImageJ software, according to Abramoff, Magalhães, and Ram (2004).

2.4. Physicochemical characterization of young bamboo culm starch

2.4.1. Chemical composition

The obtained starches were evaluated, according to AACCI (2010), to moisture (method 44–15.02), protein (method 46–13.01, 6.25 as conversion factor), ether extract (method 30–25.01) and ash (method 08–01.01) contents. Total carbohydrates content was calculated by difference [100 – (moisture + protein + ether extract + ash)].

2.4.2. Apparent amylose content

The apparent amylose content was determined according to ISO (2007) methodology, and calculated from standard potato amylose (A-0512) (Sigma Chemical Co., St. Louis, EUA) curves.

2.4.3. Molecular size distribution

Starch molecular weight distribution profiles were determined according to Felisberto et al. (2018), using a gel-permeation chromatography (GPC) column (1.5 cm × 70 cm - Pharmacia Biotech) packed with Sepharose CL-2B gel.

2.4.4. Branch chain length distribution of amylopectin

The branch chain length distributions of amylopectin from young bamboo culm were analyzed according Costa, Volanti, Grossmann, and Franco (2017). The enzyme isoamylase used was from *Pseudomonas* sp. (EC 3.2.1.68) (Megazyme International, Wicklow, Ireland).

2.4.5. X-ray diffraction

The X-ray diffraction pattern was determined according to Felisberto et al. (2018) using a benchtop X-ray diffractometer (MiniFlex 300, Rigaku, Tokyo, Japan) equipped with Cu-Kα monochromatic radiation (λ = 0.1542 nm). The relative crystallinity was expressed in percentage of crystallinity, and it was quantitatively estimated based on the relationship between the peak area and the total area of the diffractogram according to the method described by Nara and Komiya (1983).

2.5. Thermal properties of young bamboo culm starch

The thermal properties were analyzed according to Felisberto et al. (2018), using a Differential Scanning Calorimeter (DSC - Pyris 1, Perkin Elmer, Norwalk, USA) and Pyris 1 software (Perkin Elmer, Norwalk, USA) to determine transition temperatures (Onset - T_0, Peak - T_p, and End - T_e), enthalpy change (ΔH) and the percentage retrogradation (% $R = \Delta H_{retrogradation}/\Delta H_{gelatinization}$) of the starches.

2.6. Statistical analysis

All analyzes were performed, at least, in triplicate, and data obtained were evaluated by analysis of variance (ANOVA) of the means, using the Sisvar software, version 5.6 (Federal University of Lavras,