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Molecular structure of amylopectin/amylose from *Solanum lycocarpum* starch after enzymatic hydrolysis

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ABSTRACT

The fruit of *Solanum lycocarpum* is a rich source of starch which remains unexploited despite its promising chemical and rheological properties. In this study, the molecular structures of amylopectin and amylose from *S. lycocarpum* starch were evaluated by HPAEC-PAD, being determined their chain length and degree of polymerization (DP). In addition, the starch was submitted to enzymatic hydrolysis using immobilized α-amylase (PANI-AMY) and glucoamylase (PANI-GLU). The enzymes immobilization onto polyaniline-glutaraldehyde derivatives was optimized through a Central Composite Rotatable Design (CCRD) and the hydrolysis products were characterized by Thin Layer Chromatography (TLC) and HPAEC-PAD. Results showed that amylopectin from *S. lycocarpum* starch had a maximal DP of 86, being observed a predominance of chains with type B1 (DP 13–24). On the other hand, the amylose is composed by short chain polymers, presenting DP of 57. Results from serial hydrolysis evidenced that the PANI-AMY reactor produced a set of malto-oligosaccharides, with predominance of pentoses (348.11 μg mL^{-1}), while in the PANI-GLU reactor it was produced high levels of glucose. In addition, hydrolysis tests using high starch concentration (10% w/v) evidenced that the reactors can be efficiently used to hydrolyze starch at the similar concentration to that used in industrial processes, being able to efficiently produce starch liquefaction. Therefore, the results obtained in this work contribute to improve the information about the properties and potentialities of native and hydrolyzed starches from *S. lycocarpum*.

1. Introduction

The search for new sources of starch have attracted considerable research interest in recent years due to their set of applications in the food industry as well as other industrial segments such as textile, paper, chemical, pharmaceutical and biotechnological industries (Falade & Okafor, 2013; Menzel et al., 2015; Zhang, Zhang, Xu, Li, & Tan, 2018). The selection of starch for industrial uses is made by considering its availability and physicochemical characteristics, that can vary depending on the source (Das & Kayastha, 2019; Joshi et al., 2013).

The Solanaceae family contains many species with agronomical and medicinal importance, with members widely distributed worldwide. *Solanum lycocarpum* (St. Hill) is a common and abundant Solanaceae member found in the Brazilian Cerrado. It presents a high resistance to hydric and climatic stress, surviving and fructifying along the year, with fruits weighing from 400 to 900 g, making this plant a very attractive target for biotechnological exploitation (Silva-Filho, Torralbo, Di-Medeiros, Batista, & Fernandes, 2012; Torralbo, Batista, Di-Medeiros, & Fernandes, 2012). A previous work stated that *S. lycocarpum* fruits are good source of starch, yielding 51% by dry weight, which is a very high value compared to those obtained from other traditional sources (Pascoal et al., 2013). Characterization of *S. lycocarpum* starch revealed a 34.7% of amylose and 38% crystallinity (Pascoal et al., 2013). Another study showed that 89.8% of this starch was hydrolyzed under simulated gastric digestion, suggesting that it may be a good raw material for production of hydrolysates, such as glucose syrup and its derivatives (Di-Medeiros et al., 2014).

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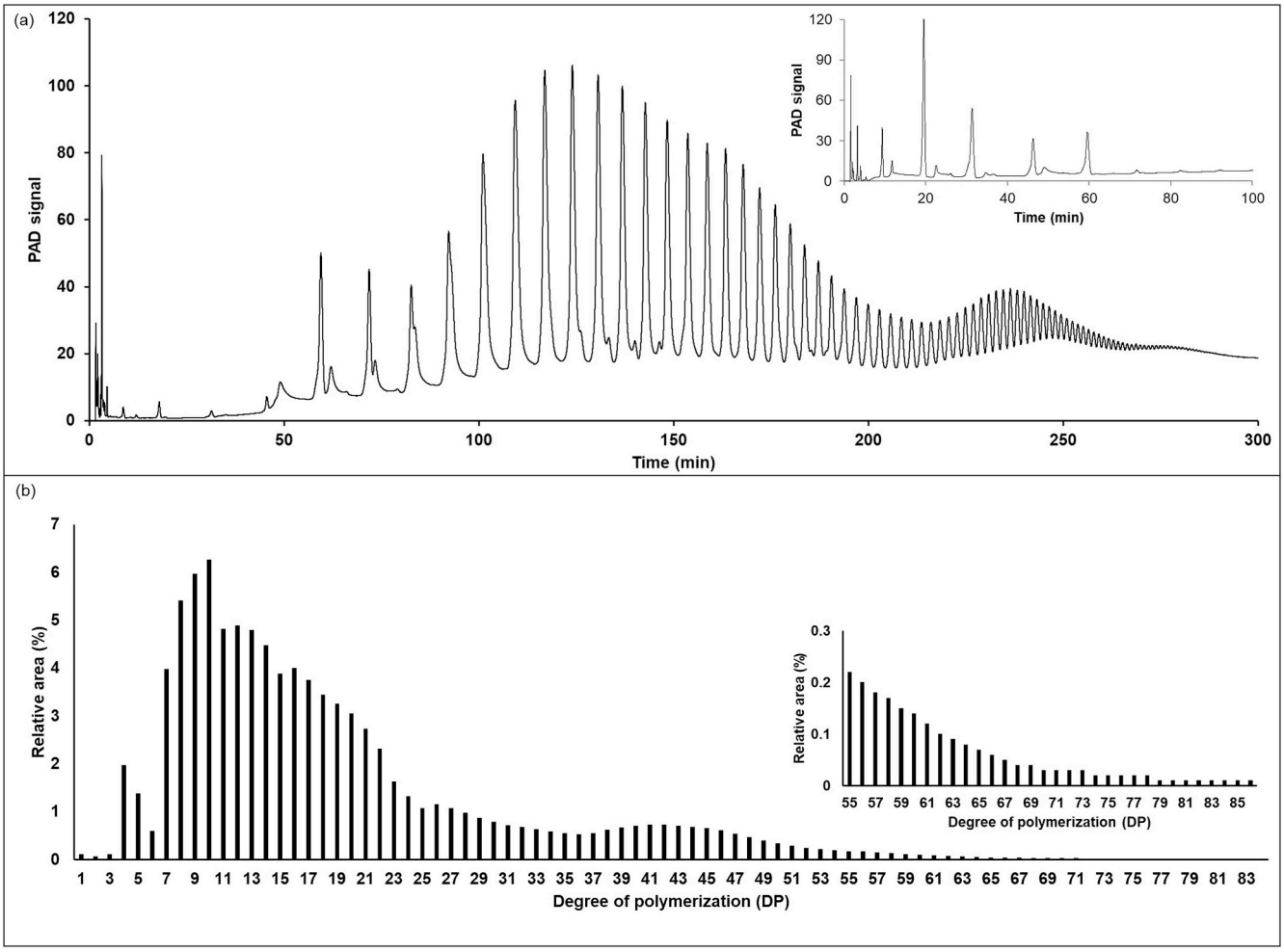

Fig. 1. (a) High-performance anion-exchange chromatogram (HPAEC-PAD) of isoamylase-debranched amylopectin from *S. lycocarpum* starch. The experiment was performed in co-chromatography with standard malto-oligosaccharides with DP 3–6 (insert). (b) Chain length distribution of isoamylase-debranched amylopectin from *S. lycocarpum* starch.

In food industry, the hydrolysis of starch followed or not by fermentation is a field that presents a constant increasing demand of raw material. Furthermore, the production of oligosaccharides is a new field of the food and pharmaceutical industries which search for new sources of starch to produce hydrolysates with adequate physicochemical properties for new applications (Lin et al., 2016; Wang & Copeland, 2015).

Amylases and glucoamylases have attracted considerable interest with respect to their application in the food and fermentation industries for starch hydrolysis and the production of oligosaccharides (Das & Kayastha, 2019; Guo et al., 2017; Lin et al., 2016). Considerable efforts have been made to produce amylolytic enzymes on a large-scale with low cost for use in such industrial processes. In this context, the use of immobilized enzymes in industrial scale has become more common because of its advantages related to the higher stability and the possibilities of repeated use for longer periods (Dicosimo, Maculiffe, Poulose, & Bohlmann, 2013; Es, Vieira, & Amaral, 2015; Mohamad, Marzuki, Buang, Huyop, & Wahab, 2015). In this field, a previous study showed the successful use of a derivative containing α-amylase (Pascoal, Mitidieri, & Fernandes, 2011) and glucoamylase (Silva, Asquieri, & Fernandes, 2005) immobilized onto polyaniline polymers to hydrolyze starch from different sources with high oligosaccharides and glucose production.

In the present study, the molecular structure of amylopectin and amylose from *S. lycocarpum* starch was analyzed before and after hydrolysis using immobilized α-amylase and glucoamylase. The enzyme immobilization onto glutaraldehyde-modified polyaniline was optimized by using a central composite rotatable design (CCRD) and the optimized system was used as sequential reactor for starch hydrolysis. The produced oligosaccharides were finally characterized by chromatographic methods.

2. Materials and methods

2.1. Materials

Aniline, 3,5-dinitrosalicylic acid (DNS), sodium acetate ($C_2H_3NaO_2$) and isoamylase (EC3.2.1.68) were purchased from Sigma Aldrich (St. Louis, MO, USA); α-amylase (Termamyl® 2X) and glucoamylase (AMG 300L®) were kindly provided by Novozymes (Araucaria, Brazil). All other reagents were of analytical grade. Solutions were prepared using distilled water. Starch used in this work was extracted from *S. lycocarpum* fruits as described by Pascoal et al. (2013).

2.2. Separation of amylose and amylopectin

The separation of amylopectin and amylose was performed as described by Mua and Jackson (1998), using 4% starch aqueous dispersion at 60 °C. The starch dispersion was submitted to other two cycles of leaching. After each leaching cycle, the dispersion was centrifuged (1430 × *g*, 5 min), and the presence of amylose in the supernatant was determined according to the standard method ISO 6647–2:2015 (ISO, 2015), using an iodine binding procedure. The supernatant containing amylose were combined, precipitated with butanol (3:1, v/v) for 24 h,