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FULL LENGTH ARTICLE

Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch

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Abstract The effect of autoclaving (120 °C/30 min), debranching (2% pullulanase/1 h) and storage at 4 °C (DS4) or 32 °C (DS32) or 60 °C (DS60) for 24 h on starch fractions, functional, pasting, thermal and structural properties of sweet potato starch was investigated. Results showed that DS4 sample displayed the lower functional properties than other modified starches. Debranching showed a significant increase in the apparent amylose content of native starch from 18.56% to 25%. A higher yield of RS (28.76%) was observed in debranched starch stored at 4 °C (DS4) due to the higher degree of retrogradation. All debranched starches showed a substantial decrease in pasting profile and higher gelatinization temperatures than in native starch. B + V X-ray diffraction pattern was observed in debranched starches with increased crystallinity value. The scanning electron micrographs of debranched starches showed rough plate-like surfaces with irregularly shaped structures were observed due to debranching and retrogradation during storage. The study concludes that a combination of autoclaving, debranching and subsequent storage at 4 °C is best technique to produce a higher amount of resistant starch in the sweet potato starch.

1. Introduction

Resistant starch (RS) is defined as the starch fraction that escapes digestion in the small intestine and might be fermented in the colon (Haralampu, 2000). There are four different types of RS: they are RS1 – Starch, which is physically inaccessible and locked within cell walls, RS2 – Granular starch that is resistant to digestive enzymes, RS3 – Retrograded starch and RS4 – Starch that is chemically modified (Eerlingen et al., 1993). Types 1 and 2 RS will get destroyed during the processing of food. RS3 is stable while heating until 100 °C since RS3 melts at ≈155 °C (Shamaia et al., 2003). Recently, RS5 has been characterized by a lipid component that has complexed with amylose to form a helical structure that contains a fatty acid tail within the central cavity (Hasjim et al., 2013). Generally, it is known that RS3 is formed when the linear amylose fraction of starch is retrograded or recrystallized after the gelatinization of starch and debranching enzymatic conversion of

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amylopectin to linear molecules (Eerlingen and Delcour, 1995).

The debranching enzyme, pullulanase is gaining popularity in the processes of starch conversion. Berry (1986) reported that amylopectin of potato starch when debranched using pullulanase before applying heating and cooling cycles considerably improved the RS3 content. The increased degree of debranching would give chains a more opportunity to align and aggregate to form perfect crystalline structure, thereby leading to the formation of more RS. Recently, various studies have been carried out to find the effect of debranching, autoclaving- cooling cycles, high temperature–pressure and storage temperature on resistant formation in cassava, potato, corn and rice starches (Lee et al., 2012; Hung et al., 2012). The sweet potato starch has a limited industrial application. The physical and chemical modification of sweet potato starch would make it more suitable for use in traditional food products that normally use other types of starches. Even though there have been few reports on the modification of sweet potato starch (Singh et al., 2005; Shariffa et al., 2009; Das et al., 2010; Mu et al., 2013; Hung et al., 2014; Song et al., 2014; Huang et al., 2015; Yu et al., 2015), technological aspects of sweet potato starch are scarce in the scientific literature. An awareness of its potential uses can help in large-scale cultivation and extraction of starch from the sweet potato crop in India.

Though there have been many reports on the preparation of RS in different tuber starches, very little information exists regarding the preparation and characterization of RS from sweet potato starch. Therefore, the objective of this study was to investigate the effects of autoclaving, debranching and subsequent storage temperature on the resistant starch formation and further on the functional, pasting and structural properties of debranched stored starches. This information would be beneficial for the better designing starch based food ingredients with improved health benefits.

2. Materials and methods

2.1. Materials

Sweet potato was purchased from Tamil Nadu Horticultural Producers Co-op. Enterprises Ltd., Procurement Center Salem, Suramangalam Uzhavar Santhai Campus, Salem, Tamil Nadu, India. All reagents used in the study were purchased from Sigma–Aldrich. Glucose Oxidase–Peroxidase (GOD–POD) kit was obtained from Beacon Diagnostics, Navsari, India. α-Amylase (22 U/mg), amyloglucosidase was from *Aspergillus niger* (300 U/mL), pullulanase was from *Bacillus acidopullulyticus* (EC-232-983-9) and all reagents were purchased from Sigma–Aldrich.

2.2. Isolation of sweet potato starch and preparation of debranched retrograded starch

Starch was isolated from the sweet potato by the method of Wickramasinghe et al. (2009). An edible part of sweet potato was cut into small pieces and homogenized with distilled water. The slurry was allowed to pass through the double-layered cheesecloth and then the filtrate was made to settle for a minimum of 3 h at a room temperature. The starch that was settled at the bottom was washed three times with distilled water, dried at room temperature (20–25 °C) for 48 h and then the dried starch was kept in an oven at 50 °C for three hours and ground into fine powder and named as Native sweet potato starch (NS). A starch suspension (20%, w/v) was gelatinized on a boiling water bath for 15 min under stirring. This gel was autoclaved at 120 °C for 30 min and then the gel was re-dissolved in distilled water to obtain a 10% (w/v) gel solution. The gel was cooled to 50 °C. Debranching was carried using an enzyme pullulanase with a concentration of 2% for 1 h. Later the debranched starch (DS) was heated at 95 °C for 20 min, cooled down to room temperature and stored for 24 h at 4 or 32 or 60 °C and was represented as DS4, DS32 and DS 60 respectively according to their storage temperature. The samples were lyophilized and stored in closed glass containers (Milašinovic et al., 2010).

2.3. Functional properties

2.3.1. Water absorption index (WAI) and water holding capacity (WHC)

Water absorption index and water holding capacity were determined by the method of Niba et al. (2001). Starch sample (1 g) was suspended in 5 mL of distilled water in a centrifuge tube. The slurry was shaken on a test tube shaker for 1 min at room temperature (20–25 °C) and then centrifuged at 3000*g* for 10 min. The supernatant was separated and poured carefully into a tared evaporating dish.

Water absorption Index was calculated as follows (g/g):

Weight of wet sediment/Initial weight of starch sample

Water holding capacity was calculated as follows (g/g):

Mass of water added to sample
– Mass of water removed from sample/Mass of the starch sample

2.3.2. Swelling power and solubility

Swelling power and solubility of starch samples were determined at 90 °C according to the method of Leach et al. (1959). About 0.35 g of starch sample was taken in a 15 mL centrifuge tube. To this 12.5 mL of distilled water was added and the tube was stirred on a vortex mixer. The tube was then kept in a water bath maintained at 90 °C for 15 min. Later the tube was cooled rapidly in an *ice water* bath to approximately 25 °C and centrifuged at 2200 rpm for 20 min. The supernatant was carefully pipette out and transferred into a Petri dish and dried at 105 °C for 5 h till constant weight. Swelling power and solubility were calculated using the formulae:

$$\text{Solubility} = \text{Weight of the soluble starch (g)} / \text{Weight of the sample (g)}$$

$$\text{Swelling power} = \text{Weight of the sediment (g)} / \text{Weight of the sample (g)}$$

2.4. Dextrose equivalent (DE)

The reducing sugar value of starch samples was measured using the dinitrosalicylic acid method of Miller (1959) to determine its DE. Different concentrations of dextrose standard

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