Journal of the Saudi Society of Agricultural Sciences (2016) xxx, xxx–xxx

King Saud University

Journal of the Saudi Society of Agricultural Sciences

www.ksu.edu.sa
www.sciencedirect.com

FULL LENGTH ARTICLE

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

A. Surendra Babu, R. Parimalavalli *

Department of Food Science and Nutrition, School of Professional Studies, Periyar University, Salem 636011, Tamil Nadu, India

Received 30 December 2015; revised 16 April 2016; accepted 18 April 2016

KEYWORDS

Pullulanase;
Functional properties;
Resistant starch;
RVA;
XRD;
SEM

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

* Corresponding author. Tel.: +91 9486980481.
E-mail address: parimalavalli.dr@gmail.com (R. Parimalavalli).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jssas.2016.04.005

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

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005

solution were taken in test tubes and DNS reagent was added in each of the test tubes. The test tubes were heated in a boiling water bath for 5 min. The Rochelle salt solution was added to each of the test tubes while the contents were still warm. The test tubes were cooled and the absorbance at 560 nm was noted and percentage of reducing sugar was determined. DE was calculated as follows:

$$DE = \text{g reducing sugar/g dry weight of starch} \times 100\%$$

2.5. Apparent amylose

Apparent amylose content determination was carried out using a colorimetric iodine affinity procedure (Williams et al., 1958) briefly a mixture of 0.1 g of the starch sample, 1 mL of ethanol and 9 mL 1 N sodium hydroxide were boiled for 10 min in a boiling water bath and allowed to cool. To a portion (5 mL) of the mixture, 1 mL of 1 N acetic acid and 2 mL of iodine solution were added and Absorbance (A) was read using a Spectrophotometer at 620 nm. The apparent amylose content was calculated as follows:

$$\text{Apparent amylose content } (\%) = 3.06 \times A \times 20;$$

where A = Absorbance value

2.6. Determination of starch fractions (RDS, SDS, and RS)

The starch fractions in the samples were determined by previously described method of Englyst et al. (1992). Briefly, starch sample (200 mg, dry base) was hydrolyzed by a mixed enzyme solution of porcine pancreatic α-amylase (290 U/mL) and amyloglucosidase (15 U/mL), where U is defined as the amount of enzyme that liberates 1.0 mg glucose from starch in 1 min at pH 5.2 and 37 °C. Phosphate buffer (15 mL, 0.2 mol/L, pH 5.2) was added to each of the conical tubes containing starch samples (200 mg, dry base). After equilibration at 37 °C for 5 min, the enzyme solution (5 mL) was added to the sample tube, followed by incubation in a water bath at 37 °C with shaking (170 rpm). Aliquots (0.5 mL) were taken at intervals of 20 and 120 min and mixed with 4 mL of 80% ethanol to deactivate the enzymes. The mixed solution was centrifuged at 2000 rpm for 10 min, and the total glucose concentrations of the 20 min-digested and 120 min-digested hydrolysates (G20 and G120, respectively) were determined using a glucose oxidase–peroxidase kit. The percentages of RDS, SDS, and RS in the samples were calculated by following equations:

$$RDS = G20 \times 0.9$$

$$SDS = (G120 - G20) \times 0.9$$

$$RS = 100 - RDS - SDS$$

2.7. Pasting properties

Pasting profile of starch was recorded using a Rapid Visco Analyser (RVA Tech Master, Perten Instruments, and Japan) as described by the Babu et al. (2016). The viscosity profiles were recorded using starch suspensions (12% w/v). The Std1 profile of the Perten Instruments was used, where the samples were held at 50 °C for 1 min, heated from 50 °C to 95 °C at 12.16 °C/min, held at 95 °C for 2.30 min, cooled from 95 °C to 50 °C at 11.84 °C/min, and held at 50 °C for 2 min. The peak viscosity (PV), breakdown (BD) trough viscosity (TV), setback (SB), final viscosity (FV), pasting time (PT), and pasting temperature (PT) were recorded.

2.8. Thermal analysis

The gelatinization characteristics of starch samples were determined by the method described by Babu et al. (2016) using a differential scanning calorimeter (DSC) (TA Instrument, Q2000, New Castle, NJ, USA). A 2 mg sample (dry basis) was weighed in an aluminum pan and 10 μL of deionized water was added. The pan was sealed tightly and then it was allowed to stand for 1 h before carrying out the analysis. An empty aluminum pan was used as a reference. The sample was subjected to a heating program over a range of temperature from 10 to 125 °C and a heating rate of 5 °C/min. The onset, peak, and final temperatures (To, Tp, and Tc, respectively) and transition enthalpy (ΔH) were determined.

$$\Delta H = KA/m$$

where ΔH is the enthalpy change of the reaction, m is the mass of the sample at the beginning of the experiment, K is the calibration coefficient, and A is the area under the peak (Artiaga et al., 2005). For retrogradation tests, the gelatinized samples were stored at 4 °C for 1 week and then rescanned with the same conditions used in the gelatinization test.

Furthermore, the percentage of retrogradation was calculated as

$$\%R = \frac{\Delta H_R}{\Delta H}$$

where $\%R$ is the percentage of retrogradation; ΔH_R is the enthalpy of retrograded starch and ΔH is the gelatinization enthalpy of native starch

2.9. Structural properties

2.9.1. Powder X-ray diffractometer (XRD)

X-ray diffraction pattern of starch samples was obtained using a Powder X-ray diffractometer (Rigaku Mini Hex-II, Japan). Since X-ray intensity of starch was affected by moisture content samples were conditioned to 75% relative humidity before taking the X-ray diffraction pattern. X-ray diffractometer was operated at 40 kV and 30 mA with 0.154 nm CuK radiation (Ni filter). Diffractograms were plotted between the 2θ angles of 10 and 60 at a scan rate of 2°/min and smoothed with the software PowderX (Chinese Academy of Sciences, Beijing). The degree of crystallinity of samples was quantitatively estimated following the method of Nara and Komiya (1983) with the Origin – version 6.0 software (Microcal Software Inc., Northampton, MA, USA).

2.9.2. Scanning electron microscope (SEM)

Starch granules were observed using a Scanning Electron Microscope (SEM) (JEOL-Model 6390, Japan). Granule size was determined by using ImageJ 1.46r (National Institute of Health, USA) software.

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005

2.10. Statistical analysis

Triplicate determinations were performed for each test and the results reported were average values. One Way Analysis of Variance (ANOVA) was conducted using a commercial Statistical Package for Social Sciences (SPSS version 16.0) (SPSS Inc, Chicago, USA). When statistical differences were found, a least significant difference (LSD) was performed to separate means at the 5% significant level.

3. Results and discussion

3.1. Functional properties

3.1.1. WAI and WHC

The result WAI of native and debranched sweet potato starches is represented in Table 1.

Native starch had the lowest WAI (Table 1) among the starches. It can be attributed to the better molecular organization of NA granules. DS4 displayed lower WAI of 3.61% than DS32 and DS60 and this might be ascribed to the higher RS content in DS4 starch (Gonzalez-Soto et al., 2007). The WHC value of the native starch was lower than the counter debranched starches. DS4 starch showed a lower WHC followed by DS60 and DS32 samples which probably attributed to the higher RS content (Ozturk et al., 2009). The retrograded resistant starch (RS3) might have resulted in the enhanced interaction between amylose–amylose and amylose–amylopectin chains, which led to the decrease in the available hydroxyl groups for hydrogen bonding with water molecules consequently decreasing the water absorption index and water holding capability of starch (Hoover and Manuel, 1996). The pattern of change observed in the WAI and WHC of the present study was comparable with some of the earlier reports (Ozturk et al., 2009; Gonzalez-Soto et al., 2007).

3.1.2. Swelling power and solubility

The swelling power and solubility of native and modified starches are presented in Table 1. The swelling power of native sweet potato starch was 13.43% and a reduced swelling power was registered for debranched starches. Similar reduced swelling power for debranched rice starch was reported by Lee et al. (2010). Yadav et al. (2007) observed that the enzyme-modified potato and sweet potato starches showed reduced swelling values. The authors suggested that high amylose content and the presence of a stronger or higher number of intermolecular bonds can reduce swelling. Storage temperature had a prominent impact on the swelling power of debranched starches. The low swelling power of DS4 and DS60 starches might be due to the existence of a huge number of crystallites formed by the association between long amylopectin chains during storage. Starch granular stability is increased as a result of crystallite formation and swelling decreases (Singh et al., 2004). The solubility of debranched starches was higher than the native starch as shown in Table 1. Water solubility is often used as a degradation indicator of molecular components. The increase in solubility may occur as a result of changes in the molecular structure or as an independent mechanism that leads to the mobility of the starch components, resulting in the leaching of carbohydrates from molecules involved (Govindasamy et al., 1996). The enzymatic hydrolysis enhances the starch solubility by reducing the molecular mass of the starch.

3.2. Dextrose equivalent (DE)

The degree of enzymatic debranching of sweet potato starch using 2% pullulanase was determined in terms of DE (Table 1). All the three debranched and stored starches (DS4, DS32, and DS60) exhibited a DE value ranging between 3.70% and 3.79%. Nevertheless, a statistically significant difference was not found among the samples. In a study Milašinovic et al. (2010) optimized the autoclaving and debranching of maize starch with pullulanase (400 PUN/mL) for resistant starch production. They observed a DE value around 2% while debranching the maize starch with 2% pullulanase for 1 h reaction time.

3.3. Apparent amylose

The apparent amylose content of native sweet potato starch was found to be 18.56% (Table 1); nevertheless, the apparent amylose in debranched samples was considerably higher than that of native starch. There was no noticeable difference in amylose between DS2, DS32, and DS60 samples. The enzyme pullulanase hydrolyzes the α-1, 6-glucosidic linkages of the amylopectin, subsequently producing linear amylose chains. Wong et al. (2007) reported that linear long chain dextrin debranched with pullulanase may act as amylose, depending on the degree of polymerization. A similar enhanced trend of amylose in debranched starch was noticed by few authors in their investigations (Lee et al., 2010, 2013). No obvious effect of storage temperature on the amylose content in the debranched and stored starches was noted.

3.4. Starch fractions (RDS, SDS, and RS)

Starch fractions (RDS, SDS, and RS) of native and debranched starches were presented in Table 2. The native sweet potato starch contained a higher amount of RDS (73.49%)

Table 1 DE, apparent amylose and functional properties of NS and debranched retrograded starches.

Samples	DE (%)	Apparent amylose (%)	Water holding capacity (g/g)	Water absorption index (g/g)	Swelling power (g/g)	Solubility (g/g)
NS	–	18.56 ± 1.0a	0.78 ± 0.0a	1.82 ± 0.1a	13.43 ± 0.9a	6.13 ± 0.4a
DS4	3.79 ± 0.0a	25.89 ± 0.3b	1.78 ± 0.2b	3.61 ± 0.2b	4.55 ± 0.1b	8.37 ± 0.0b
DS32	3.70 ± 0.0a	25.97 ± 0.6b	2.38 ± 0.1c	4.21 ± 0.1c	4.86 ± 0.0b	8.47 ± 0.0b
DS60	3.74 ± 0.0a	25.77 ± 1.0b	2.27 ± 0.0b	3.72 ± 0.0b	4.54 ± 0.1b	8.39 ± 0.0b

Mean values followed by the different letters within the column are significantly different ($p < 0.05$).

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005

Table 2 Starch fractions, crystallinity, crystallinity type and diffraction peaks of NS and debranched retrograded starches.

Samples	Starch fractions (%)			Crystallinity (%)	Crystallinity type	Diffraction peaks
	RDS	SDS	RS			
NS	73.49 ± 3.6a	12.97 ± 2.7a	13.52 ± 1.6a	35.33 ± 0.0a	C	15.48, 17.65, 20.15,23.38
DS4	61.09 ± 1.4b	10.13 ± 3.2a	28.76 ± 1.8b	43.86 ± 0.4b	B + V	14.32, 17.09, 19.49, 22.15, 23.95
DS32	60.39 ± 1.6b	20.90 ± 1.5b	18.71 ± 0.8c	42.25 ± 0.1c	B + V	14.52,17.29, 19.69, 22.25, 24.05
DS60	62.27 ± 3.5b	13.58 ± 4.8a	24.14 ± 2.7d	44.81 ± 0.0d	B + V	14.72, 16.99, 19.39, 21.95, 23.85

Mean values followed by the different letters within the column are significantly different ($p < 0.05$).

and a lower amount of SDS (12.97%) and RS (13.52%). This result is in agreement with the previous report on starch fractions of native sweet potato starch (RDS, SDS, and RS) (Hung et al., 2014). In comparison with the RS content of the native starch the enzyme treated starches displayed an increase in the RS content probably due to the combined effect of debranching and retrogradation process. After debranching of starches and storing at different temperatures (4 or 32 or 60 °C) for 24 h, the RS content of the starches was considerably increased when compared to the native starch. This result is consistent with the prior studies on the formation of RS by pullulanase debranching and retrogradation at different temperatures (Gonzalez-Soto et al., 2007; Hung et al., 2012). The increased RS is primarily owed to an increased level of amylose through debranching amylopectin molecules and followed by retrogradation. Retrogradation permits the amylose molecules to form a more tightly packed crystalline structure, which is in turn difficult for enzymatic hydrolysis. Therefore, aggregation and arrangement of double helices of the short chain amylose molecules increase the RS content (Hung et al., 2012).

Storage temperature showed a predominant effect on the level of RS content in starch samples. Storage of debranched starch at lower (4 °C) and higher (60 °C) temperatures presented a superior level of RS. On the other hand sample stored at moderate temperature (32 °C) showed higher levels of SDS. These results confirm that the higher retrogradation of starch was found in the following descending order at, 4 < 60 < 32 °C storage temperature and this is in evidence from the result of the percentage of retrogradation (Table 4). The highest RS3 was noticed in DS4 (28.76%) followed by DS60 (24.14%) and DS32 (18.71%). The result shows that DS4 might undergo rapid retrogradation than DS60 and DS32. Storage of debranched starch at higher (60 °C) temperature presented a superior level of RS. The higher RS contents of oven-stored samples are probably due to retrogradation of starch during drying at 60 °C. Throughout the drying period in an oven, starch molecules can re-associate and form tightly packed structures stabilized by hydrogen bonding. These compact molecular structures limit the accessibility of digestive enzymes (Haralampu, 2000). Retrogradation permits the amylose molecules to form a more tightly packed crystalline structure, which is in turn difficult for enzymatic hydrolysis. Therefore aggregation and arrangement of double helices of the short chain amylose molecules increase the RS content (Baik et al., 1997).This result is in agreement with Gonzalez-Soto et al. (2007) who investigated the effect of storage temperature on the RS formation in debranched banana starch. However, the authors noticed that the RS content in starches stored at 32 °C was higher than starch stored at 60 °C. In another study, Lee et al. (2013) investigated the optimum conditions of pullulanase concentrations, storage temperature, and autoclaving-cooling cycles to produce slowly digestible starch (SDS) and RS from rice starch. They proposed that optimum storage temperature for debranched rice starch for the production of SDS and RS was 36 and 63 °C respectively. Our results were comparable with the results of Lee et al. (2013) as DS32 exhibited a higher SDS while DS60 showed higher RS than DS32. Ozturk et al. (2009) in his study found that storage temperature had significant effects on RS levels as amylotype corn starch, and HylonV (H5) samples that received 9 autoclave and cooling cycles and were stored at 4 °C showed the RS levels of 42.2%. Nevertheless, samples stored at 95 °C had levels of 47.7%. Similarly, in HylonVII (H7) samples, RS was 51.7% and 57.8% in the samples stored at 4 or 95 °C, respectively. The results from the above studies convey the information that RS3 production primarily depends on the amount and length of its amylose chains, with more and longer chains being beneficial.

3.5. Pasting properties of starch

Peak viscosity of native starch (6338.00 cP) was greater than the enzyme-modified starches. The lowest peak viscosity might have resulted from the gelatinization of debranched starch samples; lower peak viscosity has been reported for pregelatinized starch as compared to native starch (Ozturk et al., 2011). All RVA parameters including trough, breakdown, final and setback viscosity of the RS preparations were found to be significantly ($p \leqslant 0.05$) lower than those of the native starch (Table 3). Storage temperature was found to have no significant effect on pasting properties of the samples. The decreases in the viscosity values in the present study might be due to the disrupted starch granules and partial solubilization caused by high autoclaving temperature and debranching (Eerlingen and Delcour, 1995). According to Gelencser et al. (2008), the higher the RS content, the lower the viscosity is. This statement holds true to the results of the present study, where DS4 with highest RS content displayed a lowest viscosity profile while DS32 represented higher viscosity with lower RS content. A similar decrease in peak and breakdown viscosity in RS samples was reported by Lee et al. (2012). The difference in setback viscosity between DS4, DS32 and DS60 may be due to the amount and the molecular weight of amylose leached from the granules and the remnant of the gelatinized starch (Loh, 1992). The pasting temperature was not detected for the debranched starches and the possible reason might be due to the loosened granule structure.

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005

Table 3 Pasting properties of NS and debranched retrograded starches.

Parameters	NS	DS4	DS32	DS60
Peak viscosity (cP)	6338.00 ± 340.5a	41.50 ± 1.5b	76.50 ± 14.5b	59.00 ± 19.0b
Trough viscosity (cP)	3288.00 ± 189.5a	40.00 ± 1.0b	65.00 ± 10.0b	52.00 ± 13.0b
Break down (cP)	3050.00 ± 186.3a	1.50 ± 0.5b	11.50 ± 4.5b	7.00 ± 2.0b
Final viscosity (cP)	4290.66 ± 168.3a	65.00 ± 2.0b	116.50 ± 25.5b	86.50 ± 23.5b
Set back (cP)	1002.66 ± 24.6a	25.00 ± 1.0b	51.50 ± 15.5b	34.50 ± 10.5b
Peak time (min)	4.00 ± 0.0a	5.73 ± 0.1b	3.73 ± 0.2c	4.50 ± 1.1a
Peak temperature (°C)	70.81 ± 0.4	nd	nd	nd

nd – Not Detected. Mean values followed by the different letters within the row are significantly different ($p < 0.05$).

3.6. Thermal properties

The native sweet potato starch exhibited a clear gelatinization peak at 81.25 °C with an endothermic enthalpy of 12.96 J/g (Table 4). Results showed higher gelatinization temperatures and enthalpy (ΔH) in debranched retrograded starches (DS4, DS32 and DS60) than in NS. Similar higher gelatinization temperatures with an endothermic peak ranged from 80 to 140 °C were observed for debranched waxy wheat, waxy maize and waxy potato starches, revealing the formation of crystalline structure during the starch debranching (Cai and Shi, 2010). The DS4 sample displayed a higher gelatinization temperature than its counterparts. This higher endothermic transition might be attributed to its higher crystallization of amylopectin (Eerlingen et al., 1994). The To, Tp, Tc, and ΔH values of stored starch (4 °C) increased markedly as the storage time prolonged due to the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (Miao et al., 2009). A broad range of endotherm (87.12–125.30 °C) was noticed in DS60 sample. It indicates that the crystallites formed under 60 °C storage were more homogeneous than those formed under 4 °C and 32 °C. Retrogradation is a slow recrystallization of starch components (amylose and amylopectin) upon cooling or dehydration (Shamaia et al., 2003). The retrogradation (%R) percentage of DS4 stored at 4 °C was 72.37% significantly higher than DS 32 (60.87%) and DS60 (68.59%). Amylose is the main component of starch, which undergoes retrogradation, or recrystallization of gelatinized starch. In this process, the long chains of amylose form helices, either singly or doubly (with other amylose chains), which then align to form insoluble crystallites resistant to enzymatic action (BeMiller and Whistler, 1996). Results suggest that a higher degree of retrogradation occurred in the starch stored at a lower temperature (4 °C) than at higher temperatures (32 °C and 60 °C).

3.7. Structural properties

3.7.1. X-ray diffraction and relative crystallinity

The XRD of native and pullulanase treated, stored starches is presented in Fig. 1. Native sweet potato starch displayed a C-type crystalline pattern with strong diffraction peaks at 15.48, 17.65, and 23.38 and a small peak at 20.15° 2θ (Table 2). Retrograded resistant starches (DS4, DS32, and DS60) showed a single strong diffraction peak in the region of 17° 2θ and illustrated three board peaks around 22° and 23° 2θ. Interestingly, an additional peak was observed around 19° and it tends to get sharpen in the debranched starch stored at a higher temperature (60 °C). The peak in this location is a characteristic of V-type polymorph (Shamaia et al., 2003). After samples were subjected to enzymatic treatment and storage, all starches showed a combination of B- and V-type diffraction pattern with a sharp peak at 17° 2θ and few small peaks at around 2θ values of 19, 22 and 24 which is a characteristic feature of the B- and V-type crystallinity (Sievert et al., 1991). This transformation of diffraction pattern was attributed to debranching and retrogradation which reorganized the structure of starch into a helical complex to that of V-amylose (Cui and Oates, 1999). Gonzalez-Soto et al. (2007) proposed that, retrograded resistant starch developed peaks, because of the process of starch chain re-ordering or retrogradation. Apart from recrystallization of amylose chains, linear debranched amylopectin chains would also associate to form an RS crystalline structure during the period of heating and cooling (Pongjanta et al., 2009). Shamaia et al. (2003) have shown that retrogradation of high amylose maize starch (HAMS) 40 °C resulted in B-type polymorph, while storage at 95 °C, produced a mixture of A- and V-type crystalline pattern. In a later work Pongjanta et al. (2009) revealed that native high amylose rice starch has possessed A-type crystallinity whereas debranched and retrograded high amylose

Table 4 Thermal properties of native and debranched retrograded starches (DS4, DS32 and DS60).

Sample	Gelatinization temperature (°C)			ΔH (J/g)	%R
	To	Tp	Tc		
NS	42.31 ± 2.12a	81.25 ± 0.66a	116.12 ± 2.01a	12.96 ± 0.05a	–
DS4	113.67 ± 1.05bc	122.64 ± 3.29b	131.27 ± 4.37b	9.38 ± 0.68b	72.37a
DS32	75.66 ± 6.06b	94.38 ± 2.94b	99.01 ± 5.35b	7.89 ± 0.59b	60.87b
DS60	87.12 ± 0.57c	98.36 ± 3.28b	125.30 ± 1.31b	8.89 ± 0.70b	68.59c

To, Tp and Tc stand for onset, peak, and conclusion temperatures respectively. ΔH (J/g) indicates enthalpy and %R represents the percentage of retrogradation. Mean values followed by different letters within the column indicate significant difference ($p < 0.05$).

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005

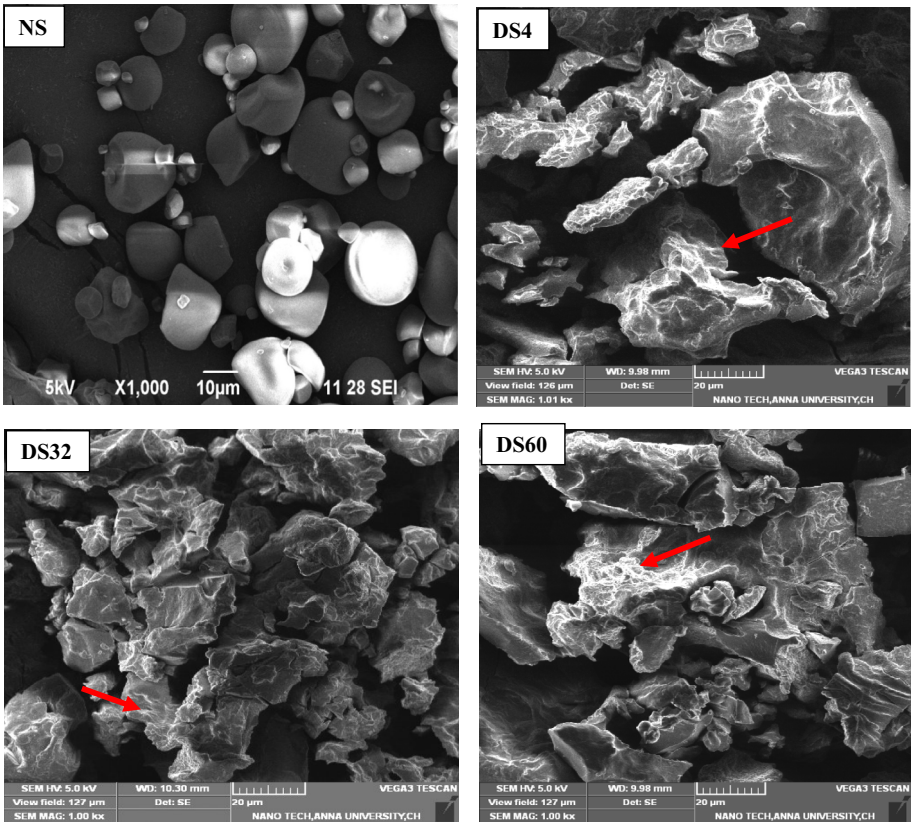

Figure 1 X-ray diffraction pattern of native sweet potato starch (NS) and debranched retrograded starches (DS4, DS32, and DS60).

rice starch presented V-type pattern. There was no considerable effect of storage temperatures on the diffraction pattern of debranched starch yet showed a sharpening of a peak at 19° with higher storage temperature (DS60) resulting in larger ordered crystalline regions, and thus the debranched and stored starches exhibited somewhat sharper X-ray patterns than its native starch. Miao et al. (2009) observed that when autoclaved debranched maize starches were stored at 4 °C for up to 4 days, the X-ray diffraction pattern changed from B-type to B-type and V-type complex. The degree of crystallinity of NS was 35.33% (Table 3). Enzyme modified starches showed a higher crystallinity values than their counter native starch. This is because the hydrolysis increases the number of linear chains or less branched or of lower molecular mass that, after thermal and cooling process, associates more easily to form a more crystalline structure (Polesi and Sarmento, 2011). DS60 has showed a higher degree of crystallinity (44.81%). Miao et al. (2009) postulated that long time (8 days) storage at 20 °C increased the degree of crystallinity in debranched maize starch.

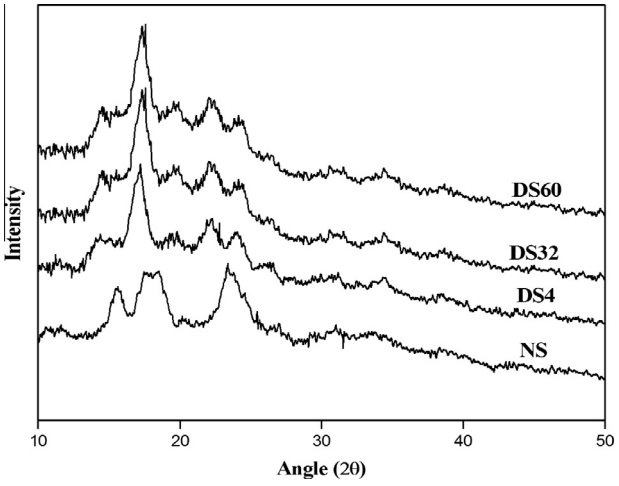

Figure 2 Scanning electron micrographs of native sweet potato starch (NS) and debranched retrograded starches (DS4, DS32, and DS60).

3.7.2. Scanning electron micrographs (SEM)

Scanning electron micrographs of starch granules are illustrated in Fig. 2. Native sweet potato starch granules were polygonal in shape; however, round and irregular shapes were also noted. No obvious defects or signs of damage on the surface of the native starch granule were noted. Nevertheless, the granular structure of starch was disappeared after being autoclaved, debranched and stored. Starches with a rough plate-like surface, bigger, irregularly shaped structures were observed after modification. The native granular structure of sweet potato starch was destroyed after being autoclaved, debranched and stored. All starch samples showed irregularly shaped fragments (Fig. 2). The huge changes in the morphology of the treated samples might be attributed to the debranching and retrogradation during storage. Zhang and Jin (2011) postulated that the retrogradation of amylose chains would result in an organization of the starch structure into a helical

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005

complex. This phenomenon would enhance the density of the crystalline structure, thus improving its resistance to enzyme digestibility. A numerous fissures and concentric layers (depicted by arrows) were visible on the outer surface of the debranched and retrograded starches (DS4, DS32, and DS60) in the SEM photomicrograph (Fig. 2), and these layers correspond to a sequence of organized and disorganized material, often referred to as crystalline and amorphous zones by French (1984). These concentric layers were ascribed to the action of autoclaving and debranching by pullulanase.

4. Conclusion

In the present study, the degree of enzymatic debranching of sweet potato starch using 2% pullulanase for 1 h was found to be about 3.70%. Due to the presence of elevated RS level, debranched starch samples stored at different temperatures showed a noticeable increase in WAI and WHC. The fractions of RDS, SDS and RS varied after debranching and retrogradation process. Storage of debranched starch at lower (4 °C) and higher (60 °C) temperatures presented a superior level of RS. However, storage represented no noticeable effect in the apparent amylose content after debranching. All RVA parameters including trough, breakdown, final and setback viscosity of the RS preparations were found to be significantly ($p \leqslant 0.05$) lower than those of the native starch. The To, Tp, Tc, and ΔH values of debranched and stored starch (4 °C) increased markedly than their counterparts. The diffraction study revealed that all modified starches showed a combination of B- and V-type diffraction pattern with a higher crystallinity due to the reorganization of the starch chain by retrogradation. Autoclaving and debranching render morphological changes in the granule as evident by SEM images. Autoclaving, debranching and low-temperature storage of sweet potato starch can increase the RS content with adequate functional properties.

Conflict of interest

The authors have declared no conflict of interests.

Acknowledgments

The authors gratefully acknowledge Department of Science and Technology (DST), New Delhi for providing financial support to carry out this research work. The authors wish to thank Dr. P. Indra Arulselvi (Assistant Professor, Plant and Microbial Biotechnology Lab, Department of Biotechnology, Periyar University) for providing the lyophilizer facility.

References

Artiaga, R., Nayab, S., Garcia, A., Barbadilloa, F., Garcia, L., 2005. Subtracting the water effect from DSC curves by using simultaneous TGA data. Thermochim. Acta 428, 137–139.

Babu, A.S., Parimalavalli, R., Jagannadham, K., Rao, J.S., Gaur, R. S., 2016. Fat mimicking properties of citric acid treated sweet potato starch. Int. J. Food Prop. 19, 139–153.

Baik, M.Y., Kim, K.J., Cheon, K.C., Ha, Y.C., Kim, W.S., 1997. Recrystallization kinetics and glass transition of rice starch gel system. J. Agric. Food Chem. 45, 4242–4248.

BeMiller, J.N., Whistler, R.L., 1996. Carbohydrates. In: Fennema, O. R. (Ed.), Food Chemistry. CRC Press Taylor Francis Group, Boca Raton, Fl, pp. 157–223.

Berry, C.S., 1986. Resistant starch formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fiber. J. Cereal Sci. 4, 301–314.

Cai, L., Shi, Y.-C., 2010. Structure and digestibility of crystalline short chain amylose from debranched waxy wheat, waxy maize and waxy potato starches. Carbohydr. Polym. 79, 1117–1123.

Cui, R., Oates, C.G., 1999. The effect of amylose–lipid complex formation on enzyme susceptibility of sago starch. Food Chem. 65, 417–426.

Das, A.B., Singh, G., Singh, S., Riar, C.S., 2010. Effect of acetylation and dual modification on physico-chemical, rheological and morphological characteristics of sweet potato (*Ipomoea batatas*) starch. Carbohydr. Polym. 80, 725–732.

Eerlingen, R.C., Delcour, J.A., 1995. Formation, analysis, structure and properties of type III enzyme resistant starch. J. Cereal Chem. 22, 129–138.

Eerlingen, R.C., Crombez, M., Delcour, J.A., 1993. Enzyme-resistant starch. I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. Cereal Chem. 703, 339–344.

Eerlingen, R.C., Van den Broeck, I., Delcour, J.A., Levine, H., 1994. Enzyme resistant starch. VI. Influence of sugars on resistant starch formation. Cereal Chem. 70, 345.

Englyst, H., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr. 46, 33–50.

French, D., 1984. Organization of starch granules. In: Whistler, R.L., Bemiller, J.N., Paschall, E.F. (Eds.), Starch, Chemistry and Technology. Academic Press, Orlando, pp. 183–247.

Gelencser, T., Juhasz, R., Hodsagi, M., Gergely, S., Salgo, A., 2008. Comparative study of native and resistant starches. Acta Aliment. 37 (2), 255–270.

Gonzalez-Soto, R.A., Mora-Escobedo, R., Hernandez-Sanchez, H., Sanchez-Rivera, M., Bello-Perez, L.A., 2007. The influence of time and storage temperature on resistant starch formation from autoclave debranched banana starch. Food Res. Int. 40, 304–310.

Govindasamy, S., Campanella, O.H., Oates, C.G., 1996. High moisture twin screw extrusion of sago starch: 1. Influence on granule morphology and structure. Carbohydr. Polym. 30, 215–286.

Haralampu, S.G., 2000. Resistant starch: are view of the physical properties and biological impact of RS3. Carbohydr. Polym. 41, 285–292.

Hasjim, J., Ai, Y., Jane, J., 2013. Novel applications of amylose–lipid complexes as resistant starch type 5. In: Shi, Y.C., Maningat, C.C. (Eds.), Resistant Starch: Sources, Applications and Health Benefits. IFT Press, Oxford, UK, pp. 79–94.

Huang, T., Zhou, D., Jin, Z., Xu, X., Chen, H., 2015. Effect of debranching and heat–moisture treatments on structural characteristics and digestibility of sweet potato starch. Food Chem. 187, 218–224.

Hoover, R., Manuel, H., 1996. The effect of heat–moisture treatment on the structure and physicochemical properties of normal maize, waxy maize, dull waxy maize and amylomaize V starches. J. Cereal Sci. 23, 153–162.

Hung, P.V., Lan-Phi, N.T., Vy-Vy, T.T., 2012. Effect of debranching and storage condition on crystallinity and functional properties of cassava and potato starches. Starch/Starke 64, 964–971.

Hung, P.V., My, N.T.H., Phi, N.T., 2014. Impact of acid and heat–moisture treatment combination on physicochemical characteristics and resistant starch contents of sweet potato and yam starches. Starch/Stärke 66, 1013–1021.

Leach, H.W., McCowen, L.D., Schoch, T.J., 1959. Structure of the starch granule. 1. Swelling and solubility patterns of various starches. Cereal Chem. 36, 534–544.

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005

Lee, K.Y., Lee, S., Lee, H.G., 2013. Influence of storage temperature and autoclaving cycles on slowly digestible and resistant starch RS formation from partially debranched rice starch. Starch/Starke 65, 694–701.

Lee, K.Y., Lee, S., Lee, H.G., 2010. Effect of the degree of enzymatic hydrolysis on the physicochemical properties and in vitro digestibility of rice starch. Food Sci. Biotechnol. 195, 1333–1340.

Lee, C.J., Kim, Y., Choi, S.J., Moon, T.W., 2012. Slowly digestible starch from heat–moisture treated waxy potato starch: preparation, structural characteristics, and glucose response in mice. Food Chem. 133, 1222–1229.

Loh, J., 1992. The effect of shears and strain on pasting behaviour of food starches. J. Food Eng. 16, 75–89.

Mu, T., Abegunde, O.K., Sun, H., Deng, F., Zhang, M., 2013. Physicochemical characterization of enzymatically hydrolysed heat treated granular starches. Starch/Stärke 00, 1–9.

Miao, M., Jiang, B., Zhang, T., 2009. Effect of pullulanase debranching and recrystallization on structure and digestibility of waxy maize starch. Carbohydr. Polym. 76, 214–221.

Milašinovic, M., Radosavljevic, M., Dokic, L.J., 2010. Effects of autoclaving and pullulanase debranching on the resistant starch yield of normal maize starch. J. Serb. Chem. Soc. 75, 449–458.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

Nara, S., Komiya, T., 1983. Studies on the relationship between water-saturated state and crystallinity by the diffraction method for moistened potato starch. Starch/Starke 35, 407–410.

Niba, L.L., Bokanga, M.M., Jackson, F.L., Schlimme, D.S., Li, B.W., 2001. Physicochemical properties and starch granular characteristics of flour from various manihot esculenta cassava genotypes. J. Food Sci. 67, 1701–1705.

Ozturk, S., Koksel, H., Kahraman, K., Ng, P.K.W., 2009. Effect of debranching and heat treatments on formation and functional properties of resistant starch from high amylose corn starches. Eur. Food Res. Technol. 229, 115–125.

Ozturk, S., Koksel, H., Ng, P.K.W., 2011. Production of resistant starch from acid-modified amylotype starches with enhanced functional properties. J. Food Eng. 103, 156–164.

Polesi, L.F., Sarmento, S.B.S., 2011. Structural and physicochemical characterization of RS prepared using hydrolysis and heat treatments of chick pea starch. Starch/Starke 63, 226–235.

Pongjanta, J., Utaipattanaceep, A., Naivikul, O., Piyachomkwan, K., 2009. Debranching enzyme concentration effected on physicochemical properties and a-amylase hydrolysis rate of resistant starch type III from amylose rice starch. Carbohydr. Polym. 78, 5–9.

Shamaia, K., Bianco-Peled, H., Shimonic, E., 2003. Polymorphism of resistant starch type III. Carbohydr. Polym. 54, 363–369.

Shariffa, Y.N., Karim, A.A., Fazilah, A., Zaidul, I.S.M., 2009. Enzymatic hydrolysis of granular native mildly heat-treated tapioca sweet potato starches at sub-gelatinization temperature. Food Hydrocolloids 23, 434–440.

Sievert, D., Czuchajowska, Z., Pomeranz, Y., 1991. Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylomaize VII starch and enzyme-resistant starch residues. Cereal Chem. 68, 86–91.

Singh, N., Sandhu, K.S., Kaur, M., 2004. Characterization of starches from Indian chickpea Cicer arietinum L cultivars. J. Food Eng. 63 (4), 441–449.

Singh, S., Raina, C.S., Bawa, A.S., Saxena, D.C., 2005. Effect of heat–moisture treatment and acid modification on rheological, textural and differential scanning calorimetry characteristics of sweet potato starch. J. Food Sci. 70, E373–E378.

Song, H.Y., Lee, S.Y., Choi, S.J., Kim, K.M., Kim, J.S., Han, G.J., et al, 2014. Digestibility and physicochemical properties of granular sweet potato starch as affected by annealing. Food Sci. Biotechnol. 23, 23–31.

Wickramasinghe, H.A.M., Takigawa, S., Matsura-Endo, G., Yamauchi, H., Noda, T., 2009. Comparative analysis of starch properties of different root and tuber crops Sri Lanka. Food Chem. 112, 98–103.

Williams, V.R., Wu, W., Tsai, H.Y., Bates, H.G., 1958. Varietal differences in amylose content of rice starch. J. Agric. Food Chem. 6, 47–48.

Wong, C.W., Muhammad, S.K.S., Dzulkifly, M.H., Saari, N., Ghazali, H.M., 2007. Enzymatic production of linear long-chain dextrin from sago (*Metroxylon sagu*) starch. Food Chem. 100, 774–780.

Yadav, A.R., Mahadevamma, S., Tharanathan, R.N., Ramteke, R.S., 2007. Characteristics of acetylated and enzyme-modified potato and sweet potato flours. Food Chem. 103, 1119–1126.

Yu, S., Mu, T., Zhang, M., Ma, M., Zhao, Z., 2015. Effects of retrogradation and further acetylation on the digestibility and physicochemical properties of purple sweet potato flour and starch. Starch/Stärke 67, 892–902.

Zhang, H., Jin, Z., 2011. Preparation of resistant starch by hydrolysis of maize starch with pullulanase. Carbohydr. Polym. 83, 865–867.

Please cite this article in press as: Babu, A.S., Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. Journal of the Saudi Society of Agricultural Sciences (2016), http://dx.doi.org/10.1016/j.jssas.2016.04.005