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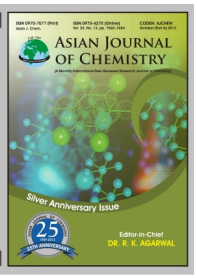

Mechanism of the Formation, Properties and Molecular Structure of Slowly Digestible Starch from the African Locust Bean *Parkia biglobosa* Collected from Conakry, Guinea

A. SANKHON[1,2], L. WANG[1], W. YAO[1,*], I. AMADOU[1], H. WANG[1], H. QIAN[1] and M. SANGARE[2]

[1]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, P.R. China
[2]Department Chimie, Faculté des Sciences, de la Nature Université Julius, Nyereré de Kankan, Guinea

*Corresponding author: Fax: +86 510 85329081; Tel: +86 510 85328726; E-mail: yaoweirongcn@jiangnan.edu.cn

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Slowly digestible parkia starch prepared by prehydrolysis with α-amylase and amyloglucosidase at different times (20, 45, 70, 95, and 120 min) were investigated. The *in vitro* Englyst test showed 52.29 % slowly digestible parkia starch content and the digestion results of the prehydrolyzed starch showed an almost constant amount of slowly digestible parkia starch, although an increase of rapidly digestible starch accompanied a reduction of resistant starch with increasing prehydrolysis time. Scanning electron microscopy revealed that slowly digestible parkia starch results from an increase of pore size until almost complete fragmentation of starch granules. Amylopectin and amylose ratio contributed to the amount of slowly digestible parkia starch at different digestion time. Prehydrolysis parkia starch with nutritional quality and functionality affected by encapsulating easily digestible material, for example, gelatinized starch. X-Ray powder between layers of SEM are resistant to enzyme digestion, thus, food products with multiple layers of this structure would likely have a slow digestion property.

Key Words: Parkia starch, Slowly digestible starch, Mechanism, Structure, Nutritional properties.

INTRODUCTION

Starch comprises as much as 70-80 % of the total carbohydrates in normal diets. It is composed almost entirely of the polysaccharides amylose and amylopectin. The physical arrangement of amylose and amylopectin and the interaction between starch molecules and other food components determine the physicochemical and functional properties of starch. These properties affect the quality of starch-based products and are essential to determine potential applications of starch as its enzymatic transformation. Many factors, including surface organization (*e.g.*, pores), granular architecture, starch composition, type of crystal polymorph, granular size and the presence of compound granules, affect the rate and extent of digestion of starch granules[1]. Starch has been classified on the basis of digestibility as rapidly digestible starch (RDS), slowly digestible parkia starch (SDS) and resistant starch (RS)[2]. Rapidly digestible starch is digested quickly and causes a rapid rise in the blood glucose levels immediately after consumption. Resistant starch is not digested in the small intestine at all and is fermented by micro-organisms in the colon to give short-chain fatty acids such as acetate, propionate and butyrate. Slowly digestible parkia starch is digested completely in the small intestine and can provide a sustained and prolonged glucose release. The nutritional properties of slowly digestible parkia starch hold potential for a dietary approach for the prevention of various diseases, such as diabetes and cardiovascular diseases. Due to the proposed beneficial physiological effects, slowly digestible parkia starch[3] and resistant starch[4] have gained much attention recently. Slowly digestible parkia starch and resistant starch can be produced from native starches using different methods such as chemical[5,6], physical[7] and enzymatic[8,9] treatments.

Rapidly digestible starch and slowly digestible parkia starch are measured after incubation with both pancreatic amylase and amyloglucosidase at 37 ºC for 20 and 120 min, respectively. Resistant starch is not hydrolyzed after 2 h of incubation[2]. On the other hand, Guraya *et al.*[10] suggested that rapidly digestible starch can be measured after incubation with porcine pancreatic amylase at 37 ºC for 1 h and slowly digestible parkia starch can be measured up to a certain time after which no further increases are noticed. Resistant starch is the starch residue that remains after rapidly digestible starch and slowly digestible parkia starch are removed. A moderate postprandial glycemic and insulinemic response of slowly digestible parkia starch implies that slowly digestible parkia starch-rich foods may provide wide health benefits in reducing common chronic diseases such as obesity, diabetes and cardiovascular disease

by reducing the stress on systems that regulate glucose homeostasis[11]. Therefore, much attention is being given to slowly digestible parkia starch as a new functional food component or ingredient in the development of novel food products.

The concept of slowly digestible parkia starch is a result-oriented definition based on the *in vitro* Englyst test[2], as the starch that is digested after the rapidly digestible starch but in no longer than 2 h under standard conditions of substrate and enzyme concentrations. To apply this concept in food production processes and provide applicable ways to utilize slowly digestible parkia starch, fundamental research is needed to understand the mechanism and structural basis of an slowly digestible parkia starch to bring health benefits to consumers.

Slowly digestible parkia starch is the fraction of starch that is digested slowly but completely in the human small intestine[12]. Slowly digestible parkia starch is defined as the starch that is digested after the rapidly digestible starch but in no longer than 2 h under standard conditions of substrate and enzyme concentration[2]. It is well known that starch digestion is affected by both intrinsic structure including macrostructure and molecular structure and extrinsic factors such as food matrixes and food processing[13-15]. In the current study, the intrinsic property of native and enzymatic prehydrolyse (α-amylase and amyloglucosidase) in different times (20, 45, 70, 95 and 120 min) and explained on the basis of changes in granular structures or granule size, the layered structure of crystalline, amorphous regions and molecularly, both amylopectin (AP) and amylose (AM) contributed to the amount of slowly digestible parkia starch as evidenced by a similar ratio of amylopectin to amylose at different digestion times are likely the fundamental structural basis for the mechanism and their slow digestion property. However, to the best of our knowledge, no work has been reported yet on the digestibility of starch fractions obtained from *Parkia biglobosa* seeds collected from Conakry, Guinea. Therefore, the objective of this study was to examine the mechanism of the formation and properties of slow digestion from *Parkia biglobosa* starch. Such information can help identify uses for this starch in food and other industrial applications related to slowly digestible parkia starch preparation.

EXPERIMENTAL

Africa locust bean (*Parkia biglobosa*) seeds were purchased from the local market in Madinah (Conakry, Guinea) in November, 2011 and shipped to Wuxi, China. Porcine pancreatic α-amylase, pepsin and amyloglucosidase were purchased from Sigma-Aldrich (Shanghai, P.R. China) and were used for analyzing the content of slowly digestible parkia starch. Chemicals and solvents in this work were of analytical grade.

Starch isolation: The isolation of starch from *Parkia biglobosa* seeds was performed according to the method of Perez-Sira and Amaiz[16] with slight modification. The flow chart for preparation of the starch from *Parkia biglobosa* seeds is shown in Fig. 1. Visible dirt and contaminants were removed from the dark-coloured parkia seed (1 kg), which was then steeped in a solution of sodium hypochlorite (35 g) and potassium hydroxide (50 g) in water (2 L) at room temperature (28 ºC) for 3 h. The pH of the steep solution was elevated to 9 and the mixture was maintained at 100 ºC in a thermostatted water bath for 3 h. The solution was then drained and the seeds were immersed in water and left overnight at ambient temperature. Finally, the seeds were thoroughly washed, manually dehulled and the cotyledon was washed repeatedly until the wash pH was neutral. The cotyledon was blended with water for 24 h using a domestic blender. The homogenate was filtered through muslin cloth and the filtrate was allowed to settle overnight. The supernatant was decanted and the sediment was centrifuged at 6750 × g for 10 min using a ZOPR-52D refrigerated centrifuge (Hitachi Koki Co. Ltd., Tokyo, Japan). The sedimented starch was resuspended in water and the process was repeated six times. The resultant starch was dried at 60 ºC in a hot air oven, then ground to powder using a mortar and pestle and stored in cellophane for analysis. The preparation of the starch from the *Parkia biglobosa* seeds is summarized in Fig. 1.

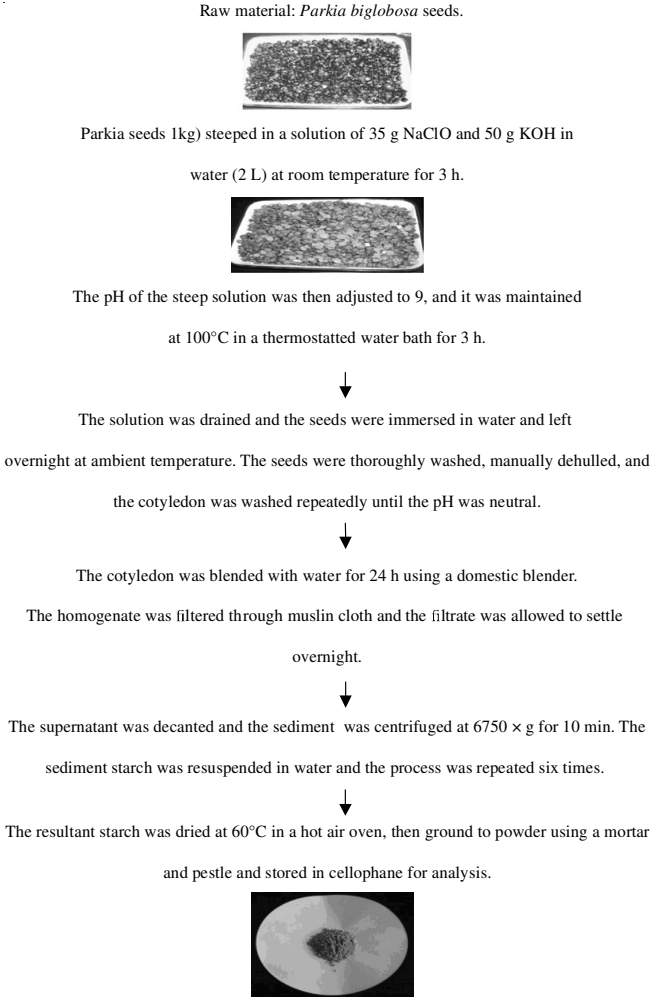

Fig. 1. Preparation of the starch from the *Parkia biglobosa* seeds

Chemical composition of parkia starch: Proximate analyses (moisture, ash, total protein and total fat content) of the native starch in parkia samples were carried out according to the standard methods of AACC[17]. Total carbohydrate content was calculated by the following equation: carbohydrate % = 100 - (% moisture + % ash + % crude protein + % crude fat)[18]. The total and reducing sugar contents were assessed according to the methods described by James[18] with a slight modification,

using glucose as a standard. The sugar content in the samples was calculated according to the standard curve and formula: Y = 0.165× - 0.0044 and R^2 = 0.9983. Sugar content (as % glucose) = C × 250/W (g), where W is the weight (g) of the pure starch used and C is the concentration of glucose per 20 mL. Each analytical determination was performed in triplicate.

Methods of enzymatic starch hydrolysis: Starch fractions of rapidly digestible starch, slowly digestible parkia starch and resistant starch were measured on the basis of the Englyst[2,19] test and the values were expressed on a dry weight basis. Starch hydrolysis kinetics was measured using an *in vitro* enzymatic hydrolysis method developed for this study. Specifically, 290 U/mL α-amylase and 6 U/mL amyloglucosidase were dissolved into 10 mL of NaOAc buffer (0.1 M, 4 mM $CaCl_2$, pH 5.2, made with benzoic acid saturated distilled water) to hydrolyze 200 mg of starch on dry weight basis (dwb) in a water bath held at 37 ºC with a shaking speed of 160 rpm. Aliquot samples (100 µL) were taken at different time intervals and the reaction was stopped with 900 µL of absolute ethanol in a 1.5 mL microcentrifuge tube. After centrifugation (6000 × g, 10 min), the glucose concentration of the supernatant was measured using a glucose oxidase/peroxidase (GOPOD) kit. The percentage of hydrolyzed starch was calculated by multiplying the glucose content by a factor of 0.9.

The above procedure was also used to prepare prehydrolyzed native parkia starch samples by stopping the reaction at 20, 45, 70, 95 and 120 min using ethanol (to 80 % concentration). After centrifugation at 3000 × g for 10 min and vacuum-drying, the precipitates were treated with pepsin (5 % w/v, pH 2.0 water by adding HCl) for 0.5 h at 37 ºC in a water bath. Starch residues were centrifuged and washed with distilled water until the pH was neutral. The residues were then dehydrated with ethanol and dried in a hot air oven (50 ºC). These prehydrolyzed samples were named Ph-20, Ph-45, Ph-70, Ph-95 and Ph-120 min, respectively.

Differential scanning calorimetry (DSC): Calorimetric measurements (gelatinization, temperature and enthalpy) of the processed parkia starch were analyzed with the Pyris-1 differential scanning calorimeter (DSC) (Perkin Elmer, Mexico, US). A native starch sample and prehydrolyzed parkia starch (3.0-3.5 mg) were mixed with distilled water (1:3 w/w) and hermetically sealed in aluminum pans. After equilibrating for 24 h at room temperature, samples were scanned at a heating rate of 10 ºC/min from 20-120 ºC.

Scanning electron microscopy (SEM): Native starch with different degrees of hydrolysis were dehydrated and dried in a hot air oven (50 ºC, overnight) and then were mounted on aluminum stubs using double-sided tape and sputter coated with gold to a thickness of 10 nm. Digital images of starch granules were obtained using an Electro scan Quanta 200 environmental scanning electron microscope (Fei Company, Netherlands).

X-Ray powder starch diffraction: A Shimadzu Lab XRD-6000 was used for X-ray powder diffraction to examine the crystalline properties of starch samples (native starch and prehydrolyzed starches). The scanning region of the two (θ) angles was from 2-40º, which covers all the significant diffraction peaks of starch crystallites.

Chromatographic analysis: molecular weight distribution profiles: Starch samples were dissolved in 90 % dimethyl sulfoxide in a boiling water bath with continuous stirring for 1 h and the dissolved samples were stirred overnight at room temperature to completely disrupt the starch granules. Starch molecules were then precipitated with 80 % ethanol. After centrifugation to remove the supernatant, the precipitates were vacuum-dried and dissolved in distilled hot water at a concentration of 2 mg/mL and subjected to continuous heating for 0.5 h in a boiling water bath to completely dissolve the samples.

The dissolved samples, filtered through a 5 µm filter, were injected into a high-performance intermediate-pressure size exclusion chromatograph system with multi-laser scattering and refractive index detectors (HPSEC-MALLS-RI), a pump (model LC-10AT vp, Shimadzu Corp., Columbia, MD) and a model 7125 syringe sample loading injector (Rheodyne Inc., Catati, CA) with a 200 µL sample loop. A HR 16/50 column containing Sephacryl S-500 HR gel (Amersham Biosciences, Piscataway, NJ), a DAWN DSP-F laser photometer fitted with argon laser (λ = 488.0 nm) with a K-5-129 flow cell (Wyatt Technology, Santa Barbara, CA) and an Optilab 903 interferometric refractometer (Waytt Technology) were used.

Statistics: The test results were processed using one-way analysis of variance (ANOVA). Differences at $p < 0.05$ were considered to be significant. SAS software (version 8.1) was used for the analysis.

RESULTS AND DISCUSSION

The isolated parkia starch was characterized by high moisture, low ash, total protein and total fat content of 8.6, 0.14, 0.08 and 0.13 %, respectively. The total protein, ash and fat contents of parkia starch were very low, indicating that starches were extracted with high purity. The purity of the parkia starch was judged on the basis of the low protein content (0.08 %). The protein content of the native starch was lower than 0.08 %, making it useable for the production of syrups with high glucose content. The carbohydrate was 91.05 %; the total sugar was 18.07 %, including 8.06 % reducing sugar.

Fraction of (*Parkia biglobosa*) starch: Based on the Englyst test, the rapidly digestible starch, slowly digestible parkia starch and resistant starch contents of starch from *Parkia biglobosa* are shown in Fig. 2 with values of 14.30, 52.29 and 33.41 %, respectively that was significantly different ($p < 0.05$).

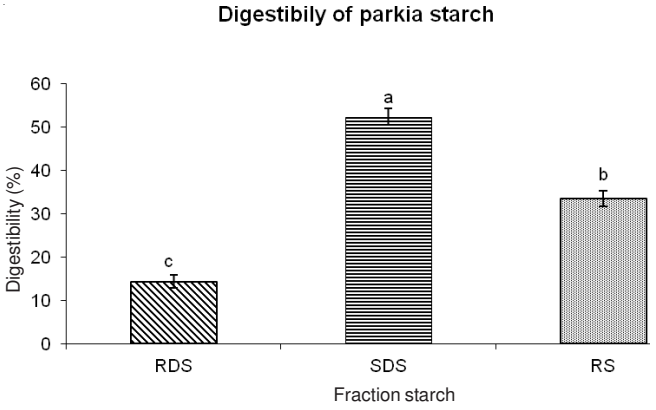

Fig. 2. Fraction of (*Parkia biglobosa*) starch RDS (rapidly digestible), SDS (slowly digestible) and (RS) resistant starch by Englyst test

Our results corroborate with the work of Zhang *et al.*[20,21] involving waxy maize, wheat, rice and maize. The rapidly digestible starch levels (14.30 %) in the parkia starches were generally similar than those reported for pea (18.2-23.8 %) and lentil (16.0-16.9 %). The slowly digestible parkia starch levels (52.29 %) were comparable to those of pea (53.7-59.0 %) and lentil (58.3-62.2 %). The resistant starch levels (33.41 %) were much higher than those reported by Chung *et al.*[22,23] for pea (8.1-12.6 %) and lentil (13.0-13.2 %). The rapidly digestible starch, slowly digestible parkia starch and resistant starch (Fig. 2) levels of the parkia starches cannot be compared with those reported for other legume starches because of differences in methodology, AACC17 *versus* Englyst *et al.*[2] and to different time periods of hydrolysis that have been defined for measurement of rapidly digestible starch, slowly digestible parkia starch and resistant starch levels. A moderate postprandial glycemic and insulinemic response of slowly digestible parkia starch implies that slowly digestible parkia starch-rich foods may provide a range of health benefits for preventing common chronic diseases such as obesity, diabetes and cardiovascular disease by reducing the stress on systems that regulate glucose homeostasis[11].

Slow digestion formation of parkia starch after prehydrolysis enzymatic: Normally, the digestion properties of rapidly digestible starch, slowly digestible parkia starch and resistant starch are maintained as consecutive fractions that are rapidly digestible, slowly digestible and resistant to digestion. However, further Englyst testing on the prehydrolyzed parkia starch residuals after different digestion times showed an almost constant amount of slowly digestible parkia starch (Table-1), although an increase of rapidly digestible starch accompanied a reduction of resistant starch with increasing prehydrolysis time. This observation further demonstrates that there is negligible resistant starch after prehydrolysis of parkia starch.

Our results are consistent with these studies and additionally show that the slow digestion property of native A-type cereal starches is sustainable beyond the 2 h limit used in the Englyst test, implying that there is negligible resistant starch in these starches[2,24].

The slow digestion property of native A-type parkia starch is likely controlled by the inherent structure of the starch and not granular size and morphology as the latter did not affect the amount of slowly digestible parkia starch; longer prehydrolysis time reduced the particle size and morphology without affecting the slowly digestible parkia starch content (Table-1). Both the Englyst test and the enzymatic hydrolysis kinetics showed that native parkia starch with an A-type semi crystalline structure belong to the category of slowly digestible starches and that the slow digestion property was sustained throughout the entire digestion process.

TABLE-1
ENGLYST TEST OF NATIVE AND PREHYDROLYZED (Ph) PARKIA STARCH FOR DIFFERENT TIMES

Samples	RDS	SDS	RS
Native (control)	14.30 ± 0.4^{d}	52.29 ± 0.3^{a}	33.41 ± 1.3^{a}
Ph-20 min	39.05 ± 0.9^{ab}	50.59 ± 1.2^{bc}	10.36 ± 0.6^{d}
Ph-45 min	39.53 ± 0.6^{a}	51.43 ± 1.2^{ab}	09.04 ± 0.4^{e}
Ph-70 min	38.58 ± 0.2^{b}	48.97 ± 0.6^{d}	12.45 ± 0.7^{c}
Ph-95 min	36.95 ± 0.4^{c}	49.03 ± 0.5^{d}	14.02 ± 0.9^{b}
Ph-120 min	36.50 ± 0.5^{c}	49.81 ± 0.9^{cd}	13.69 ± 0.8^{b}

Mean values with different letters within each column are significantly different, $p < 0.05$.

Apparent molecular size distribution of enzymatic parkia starch: Size-exclusion chromatographic analysis of native parkia starch hydrolyzed by amylolytic enzymes (α-amylase and amyloglucosidase) showed that amylopectin (AP, eluted from 20-120 min) was likely the major component digested (Fig. 3); the same amount of prehydrolyzed starch residuals was analyzed. As hydrolysis progressed, the content of the comparable amylopectin fraction is native starch at 70-120 min. Amylopectin was the major component of slowly digestible parkia starch as shown by the difference in chromatographic profiles between 20 and 45 min, which represents the major portion of slowly digestible parkia starch based on hydrolysis kinetics and molecular weight distribution analysis (Table-2) of the prehydrolyzed samples.

Amylose eluted from 75-120 min was also negligible during the slowly digestible parkia starch period (Fig. 3) in which the molecular weight of amylose decreased (chromatographic profiles) and the amylose peak narrowed. Thus, amylose also contributed quantitatively to slowly digestible parkia starch, although less than amylopectin. Fig. 3 and Table-2 show that amylose might has little impact on the inherent slow digestion property of native parkia starch, because parkia starch exhibited negligible amylose content after prehydrolysis.

TABLE-2
MOLECULAR WEIGHT DISTRIBUTION OF NATIVE AND PREHYDROLYZED (Ph) PARKIA STARCH FOR DIFFERENT TIME INTERVALS

Samples name	Polysaccharides amylose and amylopectin	Number average molecular weight (M_n)	Weight average molecular (M_w)	Peak molecular weight (M_P)	Area peak (Area)	Percentage peak area (% area)
Native (A)	Amylopectin	54582	239231	182538	2348325	78.55
	amylose	3905	4725	4042	641192	21.45
Ph-20min (B)	Amylopectin	71646	224570	174971	1181260	98.24
	amylose	3772	3929	3824	21212	1.76
Ph-45min (C)	Amylopectin	89090	260306	194455	1680711	100
	amylose	No	No	No	No	No
Ph-70min (D)	Amylopectin	87243	251919	212146	1771357	94.23
	amylose	6003	6629	6815	108416	5.77
Ph-95 min(E)	Amylopectin	89716	319311	250433	2414301	88.34
	Amylose	6295	7300	7252	318801	11.66
Ph-120min(F)	Amylopectin	81156	315778	235085	2785831	88.83
	amylose	5961	6946	7368	350198	11.17

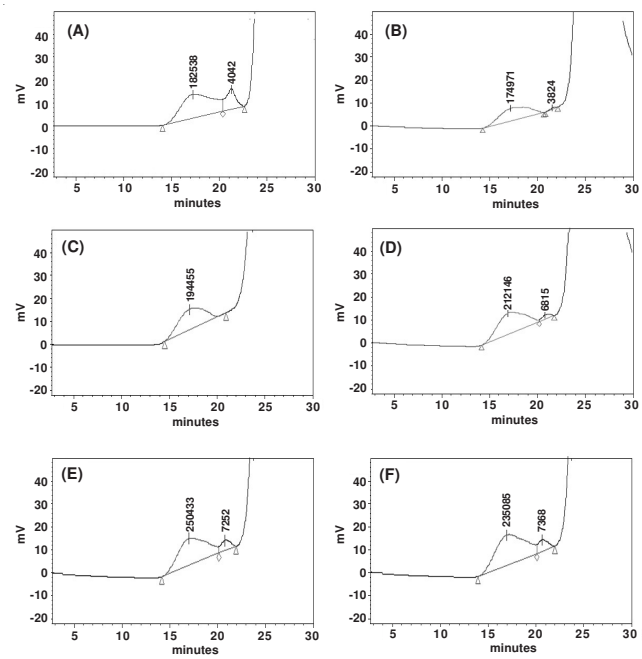

Fig. 3. HPSEC profiles of native and prehydrolyzed (Ph) parkia starch. (A) Native parkia starch, (B) Ph-20 min, (C) Ph-45 min, (D) Ph-70 min, (E) Ph -95 min and (F) Ph-120 min

X-Ray powder starch diffraction: The X-ray diffraction patterns of native starch and starches that were prehydrolyzed at different time intervals are shown in Fig. 4. There was no pronounced difference between the native and modified starches. The native and modified starches showed similar diffraction patterns with peaks at 15, 17.50, 22.50 and 25 (2θ), which are typical characteristics of A-type starch[25]. This result suggested that hydrolysis at different time intervals (20-120 min) did not dramatically alter the crystalline pattern of parkia starch. Since the side chains of amylose form the crystalline structure, it is expected that the crystallinity will be inversely related to the amylopectin content. This observation was after prehydrolysis of native parkia starch for 20-120 min and suggests that the hydrolysis mainly took place in the amorphous regions of starch granule and did not change the crystalline patterns of starches (Fig. 4). The crystalline order in starch granules is often the basic underlying factor influencing its functional properties. Starch granule size may affect its physico-chemical properties, such as gelatinization, enzyme susceptibility, crystallinity and amorphous regions[26].

Scanning electron microscopy (SEM): The slow digestion property of native A-type parkia starch is likely controlled by the inherent structure of the starch and not granular size and morphology as the latter did not affect the amount of slowly digestible parkia starch; longer prehydrolysis times (70, 95 and 120 min) reduced the particle size and morphology without affecting the slowly digestible parkia starch content (Fig. 5, Table-2). Both the Englyst test and the enzymatic hydrolysis kinetics showed that native parkia starch with an A-type semi crystalline structure belong to the category of slowly digestible starches and that the slow digestion property was sustained throughout the entire digestion process.

This is one reason B-type starches and some of the small starch granules with smooth surfaces in parkia starch (Fig. 5 D, E and F) are more resistant to digestion.

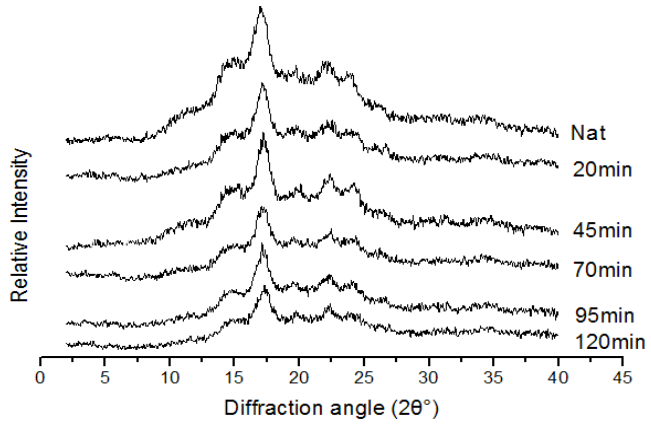

Fig. 4. X-Ray powder diffraction of native parkia starch residuals after pretreatment with enzymes (α-amylase and amyloglucosidase) for 20, 40, 60 and 120 min; control is native (Nat) parkia starch

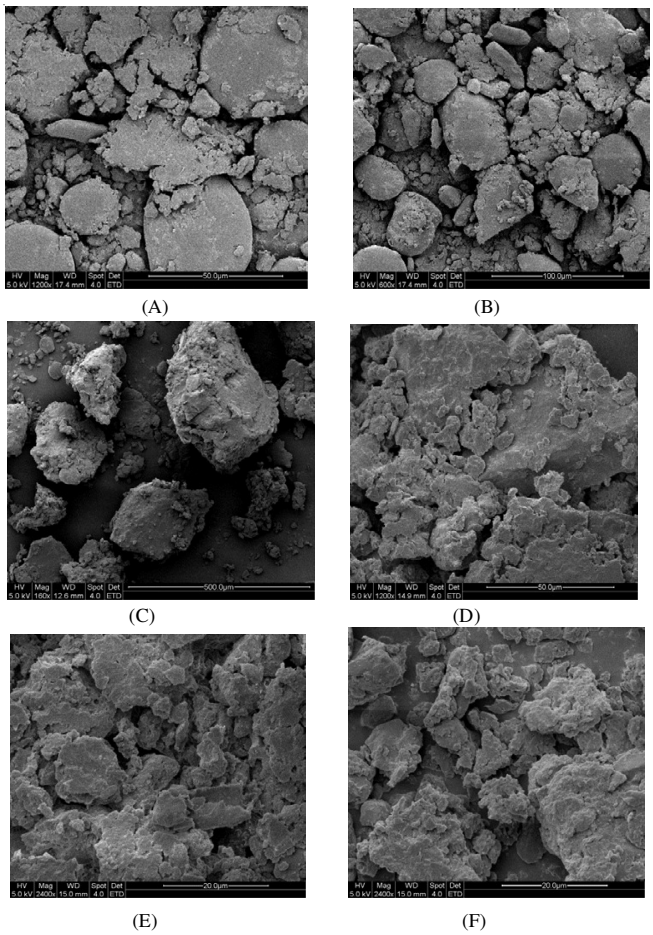

Fig. 5. SEM images of hydrolyzed native (A) parkia starch and starch prehydrolyzed for different times, 20 min (B), 45 min (C), 70 min (D), 95 min (E), and 120 min (F)

Amylopectin was the major component of slowly digestible parkia starch as shown by the difference in chromatographic profiles between Fig. 5 (B and C) that represents the major portion of slowly digestible parkia starch based on hydrolysis kinetics and molecular weight distribution analysis (Table-2) of the prehydrolyzed samples.

Thus, amylose also contributed quantitatively to the formation of slowly digestible parkia starch. Amylose might have little impact on the inherent slow digestion property of

TABLE-3
DSC PARAMETERS OF PARKIA STARCH RESIDUALS PREHYDROLYZED FOR DIFFERENT TIMES

Samples	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (J/g)
Control (native)	62.66 ± 0.4^{c}	89.58 ± 0.2^{e}	62.67 ± 1.3^{a}	13.92 ± 0.6^{c}
Ph-20 min	61.71 ± 0.5^{d}	89.01 ± 0.6^{f}	61.23 ± 0.8^{ab}	13.41 ± 0.6^{d}
Ph-45 min	62.90 ± 1.2^{b}	90.03 ± 1.1^{d}	60.29 ± 0.9^{ab}	13.11 ± 0.3^{e}
Ph-70 min	63.60 ± 0.8^{a}	91.20 ± 0.5^{c}	61.32 ± 0.6^{ab}	12.71 ± 0.5^{f}
Ph-95 min	63.61 ± 0.6^{a}	92.19 ± 0.4^{b}	62.76 ± 0.2^{a}	13.94 ± 0.7^{b}
Ph-120 min	63.60 ± 0.9^{a}	92.97 ± 0.3^{a}	63.92 ± 0.6^{a}	13.97 ± 0.8^{a}

Mean values with different letters within each column are significantly different, $p < 0.05$.

parkia starch, because parkia starch after analysis of the prehydrolyzed amylose content (1.76-11.66 %) showed a slow digestion property. Generally, it can be stated that parkia starch are more resistant against enzymatic hydrolysis, due to a higher granule surface, their surface properties and their supramolecular arrangement.

Differential scanning calorimetry (DSC): The even digestion of crystalline and amorphous regions is supported by DSC data of the starch residuals after different digestion periods that showed similar values for enthalpy and gelatinization temperature (Table-3), although there was a slight increase in T_p with increasing digestion times. Gelatinization involves melting and uncoiling of the external chains of amylopectin that are packed together as double helices in clusters. The enthalpy in DSC (ΔH) is mainly due to the disruption of the double helices rather than the long range disruption of crystallinity[27]. Furthermore, a high amylopectin content, such as in parkia starch or a high amount of branching favours slow digestion. To retain the slow digestion properties of native starches, the granular structure has to be protected in the food matrix, since it is lost by gelatinization. Hydrothermal treatment can increase the amount of slowly digestible parkia starch, depending on the botanical source and treatment conditions.

Conclusion

For native parkia starch granules, the layered structure of crystalline and amorphous regions is likely the fundamental structural basis for their slow digestion property. SEM observation did not reveal any enzyme-treated starch residuals with amorphous layers on the surface. The amorphous region was more susceptible to digestion than the crystalline regions, when the two regions were separate from each other. However, because they are contiguous, digestion proceeds evenly leading to a constant slow digestion profile. This study indicates that a slowly digestible parkia starch material can be made by encapsulating easily digestible material, for example, gelatinized starch, between round layers that are resistant to enzyme digestion and food products with multiple layers of this structure would likely have slow digestion properties.

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