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Influence of Process Conditions on Digestibility of African Locust Bean (*Parkia biglobosa*) Starch

[1,2]Abdoulaye Sankhon, [1]Wei-Rong Yao, [1]Issoufou Amadou, [1]Heya Wang, [1]He Qian and [2]Moustapha Sangare

[1]School of Food Science and Technology, Jiangnan University, Lihu Road 1800, P.O. Box 214122, Wuxi, Jiangsu, Peoples' Republic of China

[2]Departement Chimie, Faculté des Sciences de la Nature, Université Julius Nyerere de Kankan, Guinée

Corresponding Author: Abdoulaye Sankhon, School of Food Science and Technology, Jiangnan University, Lihu Road 1800, P.O. Box 214122, Wuxi, Jiangsu, Peoples' Republic of China Tel: +86 510 85328726 Fax: +86 510 85329081

ABSTRACT

This study describes the isolation, digestibility and effect of process conditions on the *Parkia biglobosa* (African locust bean) starch digestibility. *Parkia* starch fractions are: Total Starch (TS), Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS). The results indicate that processing conditions can be changed to effectively control the relative content of SDS and RS in *Parkia* starch products. Amylose is the molecular basis of RS while amylopectin is the main constituent of SDS and plays a key role in the structure and digestibility of SDS. This methodology may enable process modifications to influence the functional digestibility properties of prepared *Parkia* starch products.

Key words: *Parkia biglobosa*, slow digestible starch, resistant starch, amylopectin, amylose

INTRODUCTION

Africa locust bean (*Parkia biglobosa*) is a perennial tree legume which belongs to the sub-family Mimosoideae and family Leguminosae. It grows in the savannah region of West Africa (Pelig-Ba, 2009; Ihegwuagu *et al*., 2009). Africa locust bean tree is an important food tree for both man and livestock such as husks and pods and plays a very vital role in the rural, corresponding author economics of West African countries; virtually every part of the species is of value as food or fodder (Teklehaimanot, 2004; Tee *et al*., 2009). Starch is the major storage carbohydrate in plants. It is produced as granules in most plants cells and is referred to as native in this state. Native starches from different botanical sources vary widely in structure and composition but all granules consist of two major molecular components, amylose (20-30%) and amylopectin (70-80%) (Sankhon *et al*., 2012). The physicochemical properties of starch and its use depend largely on its biological origin and source and the various sources include cereal, grain, nuts, seeds, leaves, tubers and root. Because starch finds application in various industries, the researches for new sources of starch, like *P. biglobosa,* becomes necessary. Although qualitative determination of the chemical and nutritional composition of *P. biglobosa* seeds revealed that it is rich in starch, lipids, protein, carbohydrates, soluble sugars and ascorbic acid (Pelig-Ba, 2009; Ihegwuagu *et al*., 2009). Studies indicate that the digestibility of starch-based products are not only affected by food type, degree of maturation, starch structure, starch content, food ingredients

and individual factors (FAO, 1998), processing and storage, pea starch is very susceptible to retrogradation and thus becomes rich in RS which is naturally indigestible (Skrabanja *et al*., 1999). The retrogradation rate and extent are mainly affected by the inherent starch properties, including molecular and crystalline structure and by storage conditions, such as temperature, time and water content (Gudmundsson, 1994; Liu and Thompson, 1998). Amylopectin contributes to the retrogradation occurring in long-term rheological and structural changes, whereas amylose is usually responsible for the short-term, rapid changes in food texture (Sathaporn and Jane, 2007). Starch often changes from an amorphous state to a crystalline state and thus this retrogradation process includes a recrystallization (Yuan *et al.,* 1993).

In this research, the *Parkia* starch powder was prepared to apply in the food industry via. a soaking and cooking process which differed from normal domestic preparation, therefore, the objectives of this study are to isolate and investigate how different process conditions affect on the digestibility of *Parkia biglobosa* starch and to reduce slow starch digestibility in favor of resistant starch.

MATERIALS AND METHODS

Africa locust bean (*Parkia biglobosa*) seeds were purchased from the local market in Madinah (Conakry, Guinea) in August, 2011 and shipped to Wuxi, China. Porcine (invertase, pancreatic α-amylase, amyloglucosidase) were purchased from Sigma Aldrich Co. Ltd. (Shanghai, China). The other chemicals, potassium hydroxide, sodium hypochlorite, ethanol, 3,5-dinitro salicylic acid were purchased from Sinopharm Chemical Reagent Co. Ltd. All other reagents used were of analytical grade.

The different samples ratio material/water (1:2, 1:3, 1:4, 1:5, 1:6 and 1:7 w/v) were prepared in a sterilization equipment chamber pot (YXQ-LS-SII shanghai Boxun, industry and trade Co. Ltd., medical equipment factory). The following conditions were applied: time of soaking (10 h) and cooking temperature (90°C) for 3 h. The samples were cooled at room temperature (28°C) and then placed in a refrigerator (4°C) for 72 h. Samples were dried in a hot air oven at 45°C until constant weight, after cooling in a desiccator the samples were ground and sieved using 60 mesh sieve and stored in sealed plastic bags until analysis for starch digestibility. The different samples ratio were taken one by one for the fraction of starch digestibility (RDS, SDS and RS). All samples were treated in triplicate.

This research was conducted in the School of Food Science and Technology Laboratory and State Key Laboratory of Jiangnan University, Wuxi from August 2011 to May 2012.

Starch isolation: The isolation of starch from *Parkia biglobosa* seed was performed according to the method of Sira and Amaiz (2004) with slight modification. Visible dirt and contaminants were removed from the dark-colored *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°C) for 3 h. The pH of the steep solution was elevated to 9 and the mixture was maintained at 100°C in a thermostat water bath for 3 h. Then, the solution was 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 4500 rpm for 10 min using a ZOPR-52D refrigerated centrifuge (Hitachi Koki Co. Ltd., Tokyo, Japan). The sedimented starch was re-suspended in water and the process was repeated six times. The resultant starch was dried at 60°C in a hot air oven, then grounded to powder using a mortar and pestle and stored in cellophane and wrapped before usage.

***In vitro* digestibility of *Parkia* starch:** Five hundred milligrams of starch fractions: Total Starch (TS), Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS) were measured. Samples were incubated with invertase, pancreatic α-amylase and amyloglucosidase at 37°C in capped tubes immersed in a water bath shaker according to the method of Englyst *et al.* (1992) and the supernatants were measured at 0, 20 and 120 min for glucose content. The glucose data obtained was used to calculate the content of various starch types using the following formulas:

$$\text{Total starch (TS)} = (\text{Total glucose (TG)-free glucose (FG)})\times 0.9$$

$$\text{Rapid digestible starch (RDS)} = (G20-FG)\times 0.9$$

$$\text{Slow digestible starch (SDS)} = (G120-G20)\times 0.9$$

$$\text{Resistant starch (RS)} = TS-(RDS+SDS)$$

where, FG, G20 and G120 (mg) represent the amount of glucose in the supernatant at 0, 20 and 120 min of hydrolysis, respectively. Total Glucose (TG) was measured with the 3,5-dinitrosalicylic acid according to method of Rose *et al.* (1991) after the starch was completely hydrolyzed into glucose by perchloric acid. The *in vitro* digestibility of starch was determined from the calibration curve equation:

$$y = 0.743x+0.0004 \text{ and } R^2 = 0.9958$$

Processing conditions of starch digestion: To improve the yield of modified starch from *Parkia* seeds, different parameters were taken in consideration according to the digestion method of Englyst *et al.* (1992). The sample/water ratio were fixed as (1:2, 1:3, 1:4, 1:5, 1:6 and 1:7) w/v, the soaking time (6, 8, 10, 12 and 24 h) and the cooking temperature (50,70, 90, 110 and 121°C) for 3 h. Then, the optimal operating conditions was determined by means of varying one parameter while keeping the others at a constant level. First the sample/water ratios were varied, after finding the best ratio; followed by cooking temperatures for 3 h and finally soaking times were varied to study the effects that affect on the digestibility of *Parkia biglobosa* starch.

Differential scanning calorimetry (DSC): Calorimetric measurements (gelatinization temperature and enthalpy) of the processed *Parkia* starch product with conditions of material/water ratio 1:4, soaking for 12 h, 110°C cooking for 3 h and the samples were cooled at room temperature (28°C) and then placed in a refrigerator (4°C) for 72 h were analyzed with the Pyris-1 Differential Scanning Calorimeter (DSC) (PE, USA). Samples (3 mg) were weighed directly into DSC aluminum pans and deionizer water, after sealing, the pans were left to equilibrate (24 h) at room

temperature and then heated from 30 to 120°C at 10°C/min. In all measurements an empty pan was used as reference. Onset (T_o), peak (T_p) and Endset temperature (T_e) of gelatinization as well as gelatinization enthalpy changes (DH) were determined.

Statistics: The test results were processed by using One-way Analysis of Variance (ANOVA) test using statistical software (SAS, version 8.1). Differences at $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

***In vitro* digestibility of *Parkia* starch:** Based on the Englyst test, percentages of RDS, SDS and RS in normal *Parkia* starch were 10.91, 52.29 and 33.41%, respectively exhibited significant difference ($p<0.05$).

Our results corroborate with the work of Zhang *et al.* (2006, 2008) in waxy maize, wheat, rice and maize. The RDS level (10.91%) in the *Parkia* starches were generally lower than those reported for pea (18.2-23.8%), lentil (16.0-16.9%) and cultivars of other chickpea (21.5-29.9%) starches (Chung *et al.*, 2008a). The SDS (52.29%) was comparable to those of pea (53.7-59.0%) lentil (58.3-62.2%) and other chickpea cultivars (45.7-57.7%). Whereas, RS (33.41%) were much higher than those reported by Chung *et al.* (2008a, b) for pea (8.1-12.6%), lentil (13.0-13.2%) and other chickpea cultivars (8.4-18.4%).

SDS content which is considered a desirable form of dietary starch, was 52.29%. This value are much higher than those reported by Zhang *et al.* (2006) for maize (53.0%), waxy maize (47.6%), wheat (50.0%), rice (43.8%) and potato (15.2%). RS content which is considered a desirable form of dietary starch, was 33.41%. This value is much higher than those reported by Pongjanta *et al.* (2009) of bean varieties AC Nautica (17.2%), Majesty (21.3%) and red Kanner (21.9%); although measured using the Englyst method. The RDS, SDS and RS (Fig. 1) levels of the *Parkia* starches cannot be compared with those reported for other legume starches, due to differences in methodology AACC (2000) vs. Englyst *et al.* (1992) and to different time periods of hydrolysis that have been defined for measurement of RDS, SDS and RS levels.

Generally, digestibility of native starch is influenced by starch source, granule size, amylose/amylopectin ratio, crystallinity and amylopectin molecular (Chung *et al.*, 2006; Singh and Ali, 2006). SDS which leads to a slower entry of glucose into the blood stream and a lower glycemic response, is digested completely in the small intestine at a lower rate as compared to RDS, while RS is the starch portion that cannot be digested in the small intestine but is fermented in the large intestine (Oates, 1997; Amadou *et al.*, 2011).

A moderate postprandial glycemic and insulinemic response of SDS implies that SDS rich foods may provide wide health benefits in reducing common chronic diseases such as

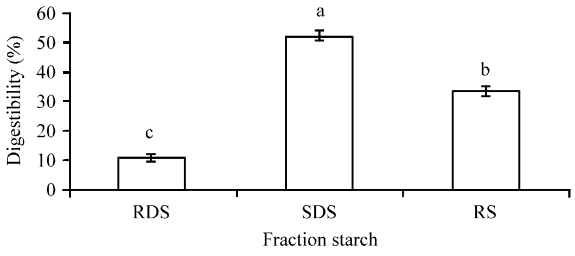

Fig. 1: Digestibility of *Parkia* starch by Englyst test

Table 1: Effect of the sample cooking (material/water ratio) on *in vitro* digestibility of *Parkia* starch soaked at 10 h, cooked at 90°C for 3 h and after storage at 4°C for 72 h

	Digestibility (%)		
Ratios	RDS	SDS	RS
Native	10.91±1.2^{b}	52.29±1.4^{a}	33.41±1.3^{e}
1:2	11.34±1.6^{ab}	51.76±1.2^{a}	36.51±1.4^{de}
1:3	11.43±1.3^{ab}	46.22±1.9^{b}	38.96±1.8^{cd}
1:4	12.09±1.8^{a}	39.76±1.1^{e}	44.76±1.2^{a}
1:5	11.56±1.6^{ab}	41.57±1.3^{de}	43.48±1.1^{ab}
1:6	11.45±1.3^{ab}	43.61±0.8^{cd}	41.55±1.8^{abc}
1:7	11.41±1.2^{ab}	44.92±1.1^{bc}	40.27±1.1^{bc}

Values (Mean±SD) in the same column with different letters are significantly different at $p<0.05$, n = 3

obesity, diabetes and cardiovascular disease through lessening the stress on regulatory systems related to glucose homeostasis (Ludwig, 2002).

Effect of sample/water ratio on the starch digestion: The results exhibited that ratio 1:4 (Table 1) as the best ratio for the digestion of *Parkia* starch. There are significant differences ($p<0.05$) among the values 12.09, 39.76 and 44.76%, respectively for RDS, SDS and RS. These results are comparable to those reported by Yao *et al.* (2010) and Zhang *et al.* (2008). Various studies have shown that several factors can contribute towards the differences in RS quantities in foods such as: botanical origin; nature of starch (amylose and amylopectin content and their ratio); food processing (degree of starch gelatinization and retrogradation); starch morphology (particle size and cellular structure), types of starch granules or their crystalline structure (such as A, B and C or V) and presence of other components (lipids, protein, dietary fiber, antinutrients and organic acids etc.) (Goni *et al.,* 1997). The material/water ratio might affect the space available for starch chain extension in the water/starch suspension. At high concentrations, starch chains may not be fully extended; and at low concentrations, extended linear and branched starch molecules have less probability to contact each other thus limiting the formation of organized molecular arrangements and crystals and subsequently forming high levels of RS (Yao *et al.*, 2010).

Previous research have demonstrated that in processed food RDS and glycemic index are highly correlated; while, SDS from the structural perspective show and prolonged release of glucose of native maize starch. Furthermore, RS is resistant to digestion and no glucose is available for glycemic response. However, the fermentation of RS in the large intestine generates short-chain fatty acids that are beneficial for colonic health. Both SDS and RS either alone or in combination, contribute to an improved nutritional quality of starch (Englyst *et al.*, 1999; Seal *et al.*, 2003; Zhang *et al.*, 2006; Topping and Clifton, 2001).

Effect of cooking temperature on starch digestion: After choosing the ratio (1:4), the temperatures were varied (50, 70, 90, 110 and 121°C) for 3 h and the soaking time was kept for 10 h and continued the process as in previous first case (material/water). The result showed in (Fig. 2) that the ratio (1:4), the temperature of 110°C was the best with RDS, SDS and RS, 12.97, 37.99 and 45.65%, respectively which are significantly different ($p<0.05$). The proportions of RS of cooked temperature (90, 110 and 121°C) starch samples are good results, exhibiting the best result of 45.65% digestibility. However, when compared to results following the proportion of SDS and RS fractions of pea starch (8.5 and 47.7%, respectively) (Yao *et al.*, 2010) which are slightly lower than our data in the same proportion of SDS and RS starches used.

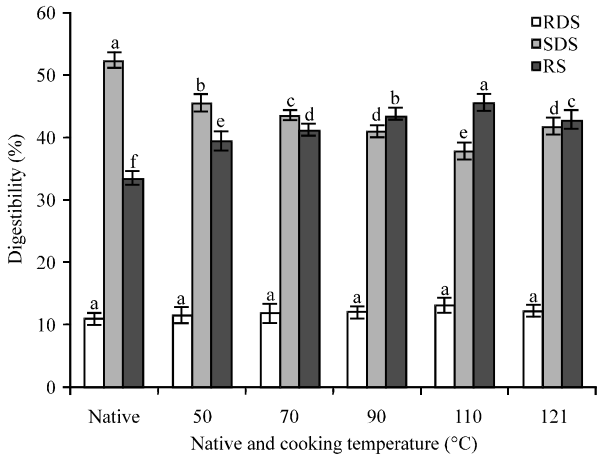

Fig. 2: Effect of the cooking temperature on the *in vitro* digestibility of *Parkia* starch, with material/water ratio; 1:4, soaking time; 10 h and cooking temperature; 50, 70, 90, 110 and 121°C for 3 h, after storage at 4°C for 72 h

Pongjanta *et al.* (2009) commented on the proportion of RDS, SDS and RS fractions in legume starches that it is difficult to make a meaningful comparison of the levels of RDS, SDS and RS proportion of legume starch since most of these studies used different digestibility methods varying in time of hydrolysis and enzyme sources. High temperature and high pressure treatment would lead to full gelatinization of starch granules and complete migration of amylose molecules. The more, cooking temperature the lower the viscosity which lead to more free starch. And when lower viscosity, free starch (such as amylose) molecules have easier access to each other and tend to form inter-molecular hydrogen bonds which is favorable to full association of amylose double helix molecules and the formation of RS. Too high a process temperature will give further decrease of starch molecules to provide low molecular weight hydrolyzates with limited potential for re-associations (Chinma and Igyor, 2008; Oates, 1997; Goni *et al.*, 1997). Chung *et al.* (2009) stated that the amylose-amylose interactions which are much stronger than those of amylose-amylopectin or amylopectin-amylopectin, may have continued to exist after gelatinization and thereby partly restricting accessibility of starch chains to the hydrolyzing enzymes.

Effect of soaking time on the digestion of *Parkia* starch: The results of RDS, SDS and RS for soaking time were 12.56, 37.82 and 46.22%, respectively. The best soaking time for the digestion of *Parkia* starch appear to be 12 h with significant ($p<0.05$) increase in starch digestibility as it is shown in Fig. 3. Similar phenomenon was observed in the work of Siddhuraju and Becker (2001) where soaked and boiled legumes had significant increase in digestibility.

Soaking affect the starch digestibility, involves entry of water into the legume kernels, wetting and dissolving soluble nutrients. The observed changes in RS and SDS level may be explained by the relatively slow cooling after gelatinization, starch molecules became less energy active and starch molecules of appropriate sizes rearranged to form orderly crystalline precipitation which made starch paste retrograde into a gel. Although starch is not generally soluble in cold water, our study showed that soaking significantly (in most cases) increased all starch fractions in all sample (Eyaru *et al.*, 2009). The soaking of seeds in plain or tempered water produces a swelling of the tissues and water uptake without cell separation; Bishnoi and Khetarpaul (1993) found that

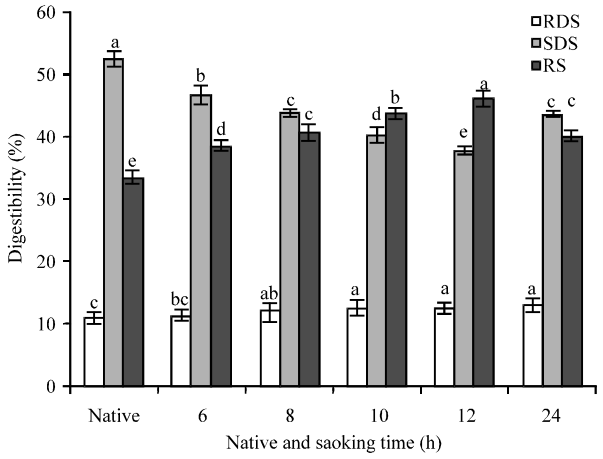

Fig. 3: Effect of the soaking time on the *in vitro* digestibility of *Parkia* starch with material/water ratio; 1:4, soaking time; 6, 8, 10, 12 and 24 h and cooking temperature; 90°C for 3 h, after storage at 4°C for 72 h

digestibility increased with soaking of all peas studied. The observed changes in RS and SDS level may be caused by the relatively slow cooling after gelatinization, starch molecules became less energy active and starch molecules of appropriate sizes rearranged to form orderly crystalline precipitation which made starch paste retrograde into a gel; as soaking time prolonged, the bundle structure formed between starch chains by hydrogen bonding may dissociate which is not conducive for the formation of RS but rather favors the formation of SDS. Conversely, the results of Eyaru *et al.* (2009) contributed to the different consequence of soaking and cooking. They soaked *Parkia* starch before cooking and this resulted in reduced starch fractions, possibly due to leaching of soluble fractions.

Differential scanning calorimetry (DSC): DSC is usually a tool used to investigate the phase transitions of the gelatinization process and it has been reported earlier that *Parkia* starch is a less stable one (Ihegwuagu *et al.*, 2009). The thermal properties of *Parkia* starches; onset, peak and endset temperatures (T_o, T_p and T_e, ΔH, respectively) are summarized in Table 2. This corroborates our earlier observation that to heat than corn starch (Ihegwuagu *et al.*, 2009).

In principle, fully gelatinized starch should produce a flat straight line with no absorption peak in DSC analysis. However, starch molecules rearrange and retrograde to form many crystal-like structures; breaking these crystal structures to re-solubilize starch molecules requires external energy. The gelatinization transition temperatures [onset temperature (T_o), peak temperature (T_p), Endset temperature (T_e) and the enthalpy of gelatinization (ΔH) of native and modified starches are presented in Table 2. Cooking starch influenced the gelatinization properties of all the samples. The gelatinization temperature of native *Parkia* starch ranged from 83.65-86.23°C which corroborate with those of other tuber starches, such as potato, cassava and new cocoyam (Collado *et al.*, 1999; Oladebeye *et al.*, 2009; Shi and Seib, 1992). The onset temperature for melting retrograded amylopectin is in the range of 63.74 to 66.30°C. In addition, Table 2 shows that after *Parkia* processing, its (ΔH) increased in parallel with its content of RS and SDS which led to decrease in digestibility of the final *Parkia* starch product, thus, this may be attributed to retrograded amylopectin.

Table 2: Differential scanning calorimetry (DSC) of *Parkia* native starch and cooked starch

Parameters	Native starch	Cooking starch
Onset temperature T_o (°C)	83.65±2.3^c	63.74±2.1^f
Peak temperature T_p (°C)	84.31±1.5^b	64.43±1.4^e
Endset temperature T_e (°C)	86.23±1.8^a	66.30±1.5^d
Enthalpy of gelatinization ΔH (J g^{-1})	6.59±1.4^h	9.68±1.6^g

Values (Mean±SD) in the same column with different letters are significantly different at $p<0.05$, n = 3

CONCLUSION

In conclusion, the study showed that the ratio 1:4; soaking time 12 h at 110°C for 3 h as the best conditions to produce the high *Parkia* starch digestion. However, cooking and processing can be carried out in various ways and under a range of condition, so that the products can vary widely in the degree of denaturation, gelatinization and retrogradation they have undergone.

Modifications in processing conditions for *Parkia biglobosa* starch product exhibited minimal impact on the content of RDS though had specific effect on the SDS and RS content. Results deduced that amylose is the molecular basis of RS and amylopectin plays a key role in the structure of SDS and is the main constituent of SDS. SDS and RS significantly indicated that various processing conditions promote the inter-conversion between them. Thus, processing conditions can be changed to effectively control the relative content of SDS and RS in *Parkia* starch products. This methodology may enable process modifications to influence the functional digestibility properties of prepared *Parkia* starch products.

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