

## ***In vitro* starch digestibility of plantain and cooking-banana at ripe and unripe stages**

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### **Article history**

Received: 12 June 2013

Received in revised form:  
29 July 2013

Accepted: 10 August 2013

### **Keywords**

Plantain

Cooking-banana

Unripe and ripe stages

*In vitro* starch digestibility

Resistant starch

### **Abstract**

This study aimed at assessing the starch fractions, kinetics of starch digestion and predicted glycemic index (pGI) of three plantain and two cooking-banana varieties at ripe and unripe stages. Starch digestibility was estimated by an *in vitro* enzymatic digestion of dried and uncooked samples. Results showed significantly higher resistant starch (from 7.5 to 17.9%) among unripe samples than ripe samples (from 4.7 - 14.0% dry matter). Kinetic parameters demonstrated higher extent of starch hydrolysis and rate of digestion among the ripe samples. The pGI values at both ripe and unripe stages of samples ranged between 44.9 to 51.4%, suggesting their classification within the low glycemic index food. The pGI of plantain samples were comparable to those of cooking-banana but cooking-banana had lower RS fraction than the plantain samples.

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### **Introduction**

Bananas and plantains belong to the genus *Musa*, known simply as the banana family. There is no formal botanical distinction between bananas and plantains. The efforts of research bodies like International Institute of tropical Agriculture (IITA) had led to availability of high yielding varieties of plantain and banana such as cooking-banana varieties (FAO, 2005). Nigeria is among the largest consumers and producers of plantain (FAOSTAT, 2006). Plantain is a rich source of carbohydrate and an important starchy staple in Nigeria (Onwuka and Onwuka, 2005; Ketiku, 1973). A study by Agunbiade *et al.* (2006) reported 72.1% of carbohydrate (dry matter) in banana samples and 81.6% in plantain (*Musa* specie). The concentration of carbohydrate varied between unripe (32.6%) and ripe (29.6%) plantain samples (Giami and Alu, 1994). A range between 21 and 26% starch content was reported in unripe plantain (Zakpaa *et al.*, 2010).

Cooking-banana and plantain are consumed both in their unripe and ripe stage unlike dessert bananas which are conventionally consumed in their ripe stages (Aurore *et al.*, 2009). Regular intake of plantain has been documented in several food consumption surveys in Nigeria (Odenigbo and Inya-Osuu, 2012; Okeke *et al.*, 2008; Ogechi *et al.*, 2007). Literature data indicated high intake of unripe plantain dishes among Nigerians suffering from diabetes mellitus (Ayodele and Erema, 2010;

Iya *et al.*, 2006). The increase consumption rate of plantain/banana was linked to traditional folklore and medical benefits including management of diabetes mellitus (Odenigbo *et al.*, 2013). Agama-Acevedo *et al.* (2012) related health benefit of unripe bananas to its resistant starch (RS) content.

During ripening stages of plantains, starch content is converted to sugar and there is decrease in RS content of plantain (Gnakri *et al.*, 1996). Resistant starch is the starch fraction, which escapes enzymatic digestion in the small intestine. The unavailability of RS to digestion in the small intestine reduces post-prandial response.

Glycemic index describes the rate of blood glucose absorption after food consumption (Wolever *et al.*, 1991). Several reports had associated high glycemic index food to metabolic disorders such as diabetes mellitus and cardiovascular disease (Jenkins *et al.*, 2002; Brand-Miller *et al.*, 2003; Sun *et al.*, 2010).

Numerous studies had demonstrated the increase in RS content of food products with unripe banana and plantain substitution (Rendon-Villalobos *et al.*, 2008; Hernández-Nava *et al.*, 2009; Ovando-Martinez *et al.*, 2009; Saifullah *et al.*, 2009). Also, Agama-Acevedo *et al.* (2012) found lower predicted glycemic index in cookies substituted with unripe banana flour. This study therefore, aimed at screening the *in vitro* starch digestibility, total starch, resistant and digestible starch fractions of commonly available plantains and cooking-bananas at ripe and unripe stages.

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## Materials and Methods

### Samples

Three plantain varieties; Ebegegghi-Ejima (P1), Ebegegghi Nwankiri (P2), Ebegegghi-Obulabo (P3) and two cooking-banana varieties; Unere-Nwankita (Cb1), Unere-Mkpumkpu (Cb2) were selected for this study. These samples were identified based on the focus-group discussion with members of the four communities in Ikwuano local government area of Abia state, Nigeria.

Samples of the identified species were collected from different markets and households within the studied communities at unripe stage (green). Samples were randomly collected from different heads of the bunch, and then separated into two portions; unripe (stage 1) and ripe (stage 5). According to United Fruit Sales Corporation, unripe stage 1 represented green peel, while ripe stage 5 represented more yellow colour than green peel (Fagbemi, 1999).

The ripe portions were samples from the respective unripe bunch, which were allowed to ripen by wrapping in a black cellophane bag for 3-6 days at room temperature until colour changes to more yellow than green (ripe stage 5). Samples were dried in Gallenkamp air oven at 60°C for 24 h, and then stored in polypropylene plastic bags at room temperature before transportation to McGill University, Canada for analysis.

### Sample preparation for analysis

Dried samples (Figure 1) were ground using a coffee grinder (SUMEET Multi Grind, India) and passed through a 0.5 mm sieve (CE Tyler, Ontario, Canada).

### Predicted glycemic index (pGI)

A modified *in vitro* method based on the procedure of Goni *et al.* (1997) was adopted. The ground samples as described above were incubated with 10 ml HCl-KCl buffer (pH 1.5) and 200 µl pepsin solution (100 mg/ml HCl-KCl buffer) at 40°C for 1 h with constant shaking. The pH was raised by addition of 200 µl pancreatic α-amylase solution (1.5 mg /10 ml phosphate buffer; pH 7.8) and incubated at 37°C for 45 min. Enzyme reaction was stopped with 70 µl Na<sub>2</sub>CO<sub>3</sub> solution and samples diluted to 25 ml with tris-maleate buffer (pH 6.9). Five (5) ml of pancreatic α-amylase solution (3 U/5 ml tris-maleate buffer) was thereafter added to the sample and incubated at 37°C with constant shaking. Aliquots (duplicate) of 1 ml were taken at 30, 90 and 120 min from the samples and placed into boiling water with vigorous shaking for 5 min to inactivate the enzyme reaction. Samples were kept in refrigerator (4°C) after each inactivation



Figure 1. Sliced and dried samples of plantain and cooking-banana varieties at ripe and unripe stages

until the end of incubation time (120 min).

All aliquots were treated with 3 ml of 0.4 M sodium acetate buffer (pH 4.75) and 60 µl of amyloglucosidase (3,300 U/ml) then incubated at 60°C for 45 min with constant shaking. After incubation, volume was adjusted to 10 ml with distilled water, mixed properly and centrifuged before transferring 0.1 ml aliquots (in duplicate) of the solution into glass test tubes for glucose measurement.

The glucose released was measured using a glucose oxidase-peroxidase (GOPOD) kit (K-GLOX, Megazyme Bray, Co. Wicklow, Ireland). Absorbance was measured at 510 nm wavelength against the reagent blank using a UV-vis spectrophotometer. Glucose was converted into starch by applying the factor of 0.9.

Rate of starch digestion was expressed as a percentage of the total starch hydrolysed at different times (30, 90, 120 min). A proposed equation by Goni *et al.* (1997) was applied in calculation of predicted glycemic index using the 90 min hydrolysis.

$$pGI = 39.21 + 0.803(H_{90})$$

### Starch fractions

The resistant starch (RS) and digestible starch (DS) were determined with Megazyme resistant starch kit (AOAC Method 2002.02; AACC Approved Method 32-40). The dried samples (100 ± 0.5 mg) as described above were incubated with 10 ml HCl-KCl

buffer (pH 1.5) and 200  $\mu$ l pepsin solution (100 mg/ml HCl-KCl buffer) at 40°C for 1 h with constant shaking. Afterwards, samples were incubated with pancreatic  $\alpha$ -amylase (10 mg/ml sodium maleate buffer; pH6.0) containing amyloglucosidase (3,300 U) for 16 h at 37°C with constant shaking. After hydrolysis, samples were washed thrice with ethanol. The separated pellet from supernatant was further digested with 2M KOH. Digested pellet and supernatant were separately incubated with amyloglucosidase. Glucose released was measured using a glucose oxidase-peroxidase (GOPOD) reagent kit (K-GLOX, Megazyme Bray, Co. Wicklow, Ireland) by absorbance at 510 nm against the reagent blank. The glucose content of the supernatant and digested pellets were used in calculation of digestible starch (DS) and resistant starch (RS) respectively by applying the factor of 0.9. Total starch (TS) was then derived as the sum of DS and RS (Goni *et al.*, 1997).

#### Statistical analysis

Data were expressed as mean values of four replicate measurements. Variation in levels of starch fractions among samples were determined by a one way analysis of variance (ANOVA) followed by Fisher's least significant-difference (LSD) test ( $P < 0.05$ ).

Statistical software used was SAS version 9.2 (SAS Institute Inc., 2010, Cary, NC, USA). The kinetic parameters were calculated by a nonlinear regression in MATLAB (Version 7.6.0.324 R2008a, The Mathworks, Inc., Natick, MA, USA).

## Result and Discussion

Table 1 presented a significant reduction in dry matter (DM) content at ripe stage among samples. Similar observation was reported previously on plantain and cooking banana hybrids (Sakyi-Dawson *et al.*, 2008). The study by Sakyi-Dawson *et al.* (2008) reported DM reduction from 260 to 190 g/kg among FHIA 03 cooking-banana hybrids.

Total starch content on dry matter basis, varied between 22.3 to 37.7% (% DM) among samples. Ripe samples contained more starch (from 23.6 to 37.7%) than unripe samples (22.3 to 27.5%). Except for Ebegegghi Obulabo (P3), the unripe samples showed significantly increase in total starch content with ripening process. This finding is contrary to previous reports that described reduction in starch content during ripening process (Ketiku, 1973; Gnakri *et al.*, 1996; Sakyi-Dawson *et al.*, 2008;). Ketiku (1996) found decreased starch concentration in plantain pulp from 83 to 66%. Lower starch content at ripe stage was explained by enzymatic breakdown of starch into

Table 1. Starch fractions of plantain and cooking-banana samples (% dry matter)

Samples	TS %	RS %	DS %	(% Dry matter)
P1 unripe	27.51 $\pm$ 7.21 <sup>bc</sup>	17.89 $\pm$ 5.32 <sup>a</sup>	9.62 $\pm$ 1.96 <sup>e</sup>	90.81 $\pm$ 0.03 <sup>c</sup>
P1 ripe	37.74 $\pm$ 5.36 <sup>a</sup>	13.96 $\pm$ 3.65 <sup>ab</sup>	23.78 $\pm$ 1.75 <sup>c</sup>	89.81 $\pm$ 0.06 <sup>e</sup>
P2 unripe	22.33 $\pm$ 7.53 <sup>c</sup>	12.18 $\pm$ 6.81 <sup>bc</sup>	10.15 $\pm$ 0.78 <sup>e</sup>	90.72 $\pm$ 0.10 <sup>c</sup>
P2 ripe	35.29 $\pm$ 2.39 <sup>a</sup>	8.21 $\pm$ 2.25 <sup>cd</sup>	27.09 $\pm$ 0.29 <sup>bc</sup>	87.11 $\pm$ 0.10 <sup>b</sup>
P3 unripe	22.26 $\pm$ 1.54 <sup>c</sup>	11.56 $\pm$ 0.31 <sup>bc</sup>	10.69 $\pm$ 1.31 <sup>e</sup>	89.36 $\pm$ 0.18 <sup>f</sup>
P3 ripe	23.60 $\pm$ 4.67 <sup>c</sup>	4.93 $\pm$ 0.43 <sup>d</sup>	18.67 $\pm$ 4.50 <sup>d</sup>	91.16 $\pm$ 0.23 <sup>b</sup>
Cb1 unripe	25.43 $\pm$ 1.94 <sup>c</sup>	7.89 $\pm$ 6.15 <sup>cd</sup>	17.54 $\pm$ 5.77 <sup>d</sup>	90.21 $\pm$ 0.02 <sup>d</sup>
Cb1 ripe	37.68 $\pm$ 7.59 <sup>a</sup>	5.41 $\pm$ 2.92 <sup>d</sup>	32.27 $\pm$ 5.51 <sup>a</sup>	89.62 $\pm$ 0.03 <sup>e</sup>
Cb2 unripe	21.85 $\pm$ 0.73 <sup>c</sup>	7.48 $\pm$ 3.39 <sup>cd</sup>	14.36 $\pm$ 3.53 <sup>de</sup>	91.53 $\pm$ 0.04 <sup>a</sup>
Cb2 ripe	34.07 $\pm$ 3.68 <sup>ab</sup>	4.65 $\pm$ 1.68 <sup>d</sup>	29.43 $\pm$ 3.45 <sup>ab</sup>	87.67 $\pm$ 0.10 <sup>e</sup>

Mean  $\pm$  SD values within same column followed by same letters are not significantly different ( $P > 0.05$ )

TS = total starch; DS = digestible starch; RS = resistant starch

P1 = Ebegegghi-Ejima (Plantain); P2 = Ebegegghi Nwankiri(Plantain);

P3 = Ebegegghi-Obulabo(Plantain); Cb1 = Unere-Nwankita(cooking-banana);

Cb2 = Unere-Mkpumpku(cooking-banana)

sugars (sucrose, glucose and fructose) during ripening process. The increase in TS at ripe stage observed in this present study could be explained by the analytical method which involved pepsin treatment. Goni *et al.* (1997) reported enhanced enzymatic accessibility in samples treated with pepsin for protein removal before starch hydrolysis.

However, this present study had limitations of not determining the glucose (sugars) in the ripe/unripe samples before *in vitro* digestion with enzymes since some of the glucose that are converted to starch by application of 0.9 factor may already have existed as free glucose in the sample and not as starch. This could have influenced the data for TS content particularly in the ripen stage. A significantly higher level of DS fraction was observed at ripe stage (18.67 -32.27%) than the unripe stage (9.62 -17.54%). RS fraction at unripe stage varied between 7.5-17.9% while the ripe stage had 4.7-14.0%. Similar result of higher RS content at unripe stage had been described in plantain (Gnakri *et al.*, 1996; Ketiku, 1973). P1 presented the highest RS content in both ripe and unripe stages while Cb2 had lowest RS content (ripe; 4.7% and unripe; 7.5%).

Generally, the cooking-banana indicated higher DS and lower RS fraction compared to plantain samples. This finding is consistent with the study by Sakyi-Dawson *et al.* (2008) which reported faster hydrolysis of starch to sugar during ripening in cooking-banana than in plantain.

The kinetics of *in vitro* starch digestibility was described by a non-linear model (Goni *et al.*, 1997) and is presented in Table 2. The extent of starch hydrolysis indicated by equilibrium concentration ( $C_{\infty}$ ) varied from 8.3 to 8.7% (unripe) and 13.89 to 14.3% (ripe) among cooking-banana samples. The plantain samples varied from 11.2 to 12.9% (unripe)

Table 2. Kinetics parameters and predicted glycemic index of plantain and cooking-banana samples

Samples	(C <sub>∞</sub> )* (%)	(k)* (min <sup>-1</sup> )	H <sub>90exp</sub> (%)	pGI	R <sup>2</sup>
P1 unripe	12.1	0.03	9.07±3.46 <sup>ab</sup>	46.50±2.78 <sup>ab</sup>	0.91
P1 ripe	14.62	0.92	13.37±4.36 <sup>ab</sup>	49.95±3.50 <sup>ab</sup>	0.98
P2 unripe	11.15	0.09	8.97±5.53 <sup>ab</sup>	46.41±4.44 <sup>ab</sup>	0.90
P2 ripe	13.02	0.89	12.25±4.41 <sup>ab</sup>	49.05±3.55 <sup>ab</sup>	0.97
P3 unripe	12.94	0.19	9.34±5.43 <sup>ab</sup>	46.71±4.36 <sup>ab</sup>	0.70
P3 ripe	12.32	0.98	10.18±3.63 <sup>ab</sup>	47.39±2.92 <sup>ab</sup>	0.94
Cb1 unripe	8.27	0.08	7.98±2.92 <sup>ab</sup>	45.61±2.35 <sup>ab</sup>	0.96
Cb1 ripe	14.33	0.09	15.01±4.95 <sup>a</sup>	51.26±3.97 <sup>a</sup>	0.99
Cb2 unripe	8.74	0.10	7.06±2.89 <sup>b</sup>	44.88±2.33 <sup>b</sup>	0.90
Cb2 ripe	13.98	0.43	15.17±6.62 <sup>a</sup>	51.39±5.31 <sup>a</sup>	0.98

Mean ± SD values within same column followed by same letters are not significantly different (P > 0.05).

\*Parameters of model equation  $C = C_{\infty}(1 - e^{-kt})$ ; C<sub>∞</sub> = equilibrium concentration; k = kinetic constant; pGI = predicted glycemic index;

H<sub>90exp</sub>; experimental values for total starch hydrolysis at 90 min

and 12.32 to 14.62% (ripe). Kinetic constant (k) parameter in both plantain and cooking-banana showed higher values among the ripe samples (0.09-0.98 min<sup>-1</sup>) compared to the unripe samples (0.03-0.19 min<sup>-1</sup>). This indicated higher resistance to enzymatic hydrolysis among the unripe samples. P3 (0.98 min<sup>-1</sup>) and Cb2 (0.43 min<sup>-1</sup>) had the highest k values among plantain and cooking-banana samples, respectively. This result indicated higher rate of starch digestion in P3 and Cb2.

The pGI values observed among samples ranged between 44.9 to 51.4%. These values fell within the low GI classification of food. According to Jenkins *et al.* (2002), foods with GI of ≤ 55, 56-69 and ≥70 are classified as low, medium, and high GI, respectively. The observation of low pGI in this study could be attributed to high fraction of RS among samples. RS is a source of indigestible carbohydrates and there is a considerable interest to improve control of diabetes mellitus by altering the glycemic impact of ingested carbohydrates (Agama-Acevedo *et al.*, 2012). Cooking-banana samples had comparable pGI values (44.9 - 51.4%) to plantain samples (46.4 - 50.0%). This indicated similarity in starch digestibility among varieties of plantain and cooking-banana. A previous study by Hettiaratchi *et al.* (2011) found similar low glycemic index among four different varieties of banana. As expected, the levels of pGI were higher in ripe samples (47.4 - 51.4%) than the unripe samples (44.9 - 46.7%) which could be explained by their higher values for kinetic parameters and lower RS content. However, the difference in pGI between ripe and respective unripe samples was not significant except for Cb2 (P < 0.05). The use of uncooked samples in this study

could have influenced the pGI result. The impact of cooking on improvement of starch digestibility in food material through gelatinization had been documented. Starch in uncooked food material is less susceptibility to pancreatic amylase during in vitro digestion (Menezes *et al.*, 2010). Rehman *et al.* (2001) reported poor starch digestibility in uncooked food sample as compared to cooked samples.

## Conclusions

This study demonstrated the influence of ripening on RS fraction, kinetic of starch hydrolysis and glycemic index of plantain and cooking-banana. The plantain and cooking-banana samples demonstrated similarity in pGI but cooking-banana presented lower RS fractions. The pGI values at both ripe and unripe stages fell within low glycemic index food classification. However, a further screening of these samples in cooked form is necessary.

## Acknowledgements

This study was supported by the STEP-B project through Michael Okpara University of Agriculture, Umudike research fundings.

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