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In Vitro Starch Hydrolysis and Prediction of Glycemic Index (PGI) in "Amala" and Plantain Based Baked Products

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Abstract

Various levels of bambara groundnut protein cocnentrate ranging from 0 to 15% were used in the formulation of plantain paste (Amala) and plantain baked products. 'Amala' and cookies were produced from 85% plantain flour and 15% bamabara groundnut protein concentrate, while cakes and bread were produced from 70% wheat flour, 20% plantain flour and 10% bambara groundnut protein concentrate. Starch fractions and *in vitro* starch hydrolysis of the products were determined. The lowest total starch value was found in plantain flour (51.51%) and highest in cakes (70.62%). There was no significant difference in resistant starch between plantain flour and 'amala' (5.22% and 4.99%, respectively). The lowest resistant starch was observed in bread (0.94%), while digestible starch was higher in bread and cakes compared to plantain flour. Higher total starch also resulted in higher digestible starch. The kinetic constant of plantain products showed very low values suggesting generally, higher resistance to enzymatic hydrolysis. The highest hydrolysis index (HI) of 74.85%, and 74.25% were observed in cakes and bread, respectively; which also resulted in higher predicted glycemic index (PGI) of 80.79% (Cakes) and 80.45% (Bread). These values were significantly different from that obtained for 'amala' with H1 of 56.40% with a corresponding PGI of 70.67% while cookies recorded HI value of 62.64% and PGI of 74.10%. The lowest HI (53.98%) and PGI (69.35%) was observed in plantain flour. This study showed that the more plantain flour in the product formualtions, the lower the hydrolysis index (HI) and the predicted glycemic index (PGI).

Keywords: starch hydrolysis, glycemic index, plantain products, bamara groundnut, protein concentrate

1. Introduction

In vitro starch hydrolysis has been identified as a simple inexpensive experimental method used in estimating glycemic response of carbohydrate meals (Jenkins et al., 1987; Araya et al., 2002; Dona et al., 2010). These findings helped in reducing the use of human beings and avoiding the complexities associated with human management involved in *in vivo* experimental designs. Various studies have demonstrated the influence of different nutrients in foods on starch hydrolysis. These nutrients included protein content (Anderson et al., 1981; Chung et al., 2008), moisture content (Lynch et al., 2007), phosphorus content (Noda et al., 2008; Absar et al., 2009) and resistant starch (Frei et al., 2003; Deepa et al., 2010). All these factors had been found to affect starch digestibility. When raw starch granules are gelatinized during heating, the disruption of starch sometimes increases its susceptibility to enzymatic degradation (Holm et al., 1988). In many starchy foods, a portion of residual starch is not fully gelatinized during processing, either due to limited water content or insufficient heating. According to Rashmi and Urooj (2003), such foods include breakfast cereals, flakes and some baked products (cookies, cakes and bread). The post-prandial responses of food containing raw or partially gelatinized starches have become the subject of increasing interest in recent years. Slowly digested carbohydrates are generally considered to be beneficial for the dietary management of metabolic disorders including diabetes and hyperlipidemia (Brand-Miller, 2003; Lehmann & Robin, 2007).

Different processing conditions affect the gelatinization of starch as measured by the enzymatic method. Englyst et al. (1992) had earlier classified starch based on its digestibility (measured by *in vitro* enzyme) as rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) all based on time of hydrolysis.

Rapidly digested starch is the starch fraction that causes a rapid increase in blood glucose level after ingestion while slowly digested starch is the starch fraction that is digested slowly but completely in the large intestine (Englyst et al., 1992). The potential health benefits of SDS include stable glucose metabolism, diabetes management, mental performance and satiety (Lehmann & Robin, 2007). Also, the health benefits of RS have been reported as prevention of colon cancer, hypoglycemic effects, substrate for growth of probiotics, reduction in gall stones formation, hypo-cholesterolemic effect, in-hibition of fat accumulation and increasing absorption of minerals (Sajilata et al., 2006). Long-term intake of low GI foods was reported to associate with the reduced incidence and prevalence of heart diseases, diabetes and also some form of cancer (Brand-Miller, 2007; Jenkins, 2007). Studies have shown that a low GI diet not only improves certain metabolic consequences of insulin resistance but also reduces it. Based on the above, the Food and Agriculture Organisation (1998) expert consultation on dietary carbohydrates strongly advocate the relevance of the GI concept. The therapeutic value of low GI diet in diabetes has been demonstrated in type 1 and type 2 patients (Brand-Miller, 1994).

In Nigeria, plantain (*Musa paradisaca*) is a popular food commodity and a cheap source of carbohydrate. Important attribute of unripe plantain fruit is its glycemic index (GI) which compares equal quantities of carbohydrates and provides a measure of carbohydrate quality and not quantity (Liu et al., 2000). Unripe plantain has also been reported to contain high slowly digestible total starch with a low glycemic index (GI); high content of resistant starch and dietary fibre (Okafor & Ugwu, 2013). The international table of glycemic index and load showed that unripe plantain has a glycemic index of 40±4. Since low GI foods release glucose at a slower rate compared to high GI foods, plantain flour has the potential to slow down the rate of starch hydrolysis in plantain-based products. This is expected to be useful in the formulation of diets for diabetic and obese individuals. Hence, the objective of this research was to formulate food products such as amala, cakes, cookies and bread from plantain flour enriched with bambara groundnut protein concentrate in order to increase the protein content of the products, to carry out the *in vitro* starch hydrolysis and prediction of glycemic index on the products.

2. Materials and Methods

2.1 Plantain and Bambara Groundnut Seeds

A local cultivar (agbagba) of plantain (*Musa paradisiaca*) was collected from the International Institute for Tropical Agriculture, (IITA) High Rainfall Station; Onne, agro-ecology, located at lat, 04° 431 N, long. 07° 01; E and l0m, near Port Harcourt, Rivers State and used for this study. Bambara groundnut (Vigna subterrenea L. verdc) seeds - (the cream coloured variety) was purchased from markets in Enugu, Enugu State, all in Nigeria. Pepsin (Cat. No P6887, 0.4 units/mg), and α-amylase (cat. No A 3176, 16 units/mg) enzymes were purchased from Sigma-Aldrich Chemcials Ltd, U.S.A. Other chemicals include potassium hydroxide, 3-5 dinitrosolicyclic acid, D-glucose, sodium hydroxide and phosphate buffer.

2.2 Preparation of Bambara Groundnut Flour

Bambara groundnut flour was prepared using the method described by Barimalaa et al., (1994). The beans were soaked for 24h in tap water and dehulled manually. The seeds were further boiled for 10 min, (1:4 bean to water ratio, w/v) in a stainless steel pot, drained and dried for 19h at 50°C in an air circulating oven. The dried samples were milled (FOSS, Cyclotec 1093, Sweden) and sieved into flour using 0.25mm sieves.

2.3 Preparation of Protein Concentrates

The protein concentrates from Bambara groundnut flour (BGFC) were prepared using the alkaline wet extraction process described reported by Giami and Isichei (1999), for fluted pumpkin seeds, and adopted for bambara groundnut protein concentrate as earlier reported by Kiin-Kabari and Giami (2015).

2.4 Product Formulations

2.4.1 Amala

"Amala" is a common plantain paste produced by stirring plantain flour enriched with 15% bambara groundnut protein concentrate in hot water (1:4, w/v) until a smooth paste was formed as reported by Kiin-Karbari et al. (2015).

2.4.2 Baked Products

2.4.2.1 Cookies

Circular cookies were produced from 85% plantain flour and 15% Bambara groundnut protein concentrate as described by Arisa et al. (2013) with modififcations as reported by Kiin-Kabari and Giami (2015).

2.4.2.2 Bread

The batter method described by Ogazi (1984) was used with modifications. Bread was produced using 70% wheat flour, 20% plantain flour and 10% bambara groundnut protein concentrate as recommended by Kiin-Kabari et al. (2015). After mixing, the resultant batter was scaled (500g), proofed for 20min and baked at 180^{o}C for 45min.

2.4.2.3 Cake

Queen cake was produced from the composite flour (70% wheat flour, 20% plantain flour and 10% bambara groundnut protein concentrate) using the creaming method of blending. Half of the composite flours were mixed with all the fat for 2 min to obtain a creamy dough before the remaining composite flour and other ingredients were added, more water was added gradually and mixing continued until the dough was soft and greasy. The dough was molded, shaped and baked in the oven at 200^{o}C for 15min.

2.5 Starch Hydrolysis

2.5.1 Total Starch

The total starch in the plantain products (amala, cakes, cookies, bread) developed was determined enzymatically according to the modified method of Goni et al. (1997). The samples were ground to pass through a 0.5mm sieve. Subsequently, 25mg of each sample was dissolved in 6ml of 2MKOH and shaken vigorously for 30min at room temperature. A stock solution of α-amylase was prepared by mixing 20mg of porcine pancreatic α-amylase with 50ml of 0.2m phosphate buffer (pH 6.9). One (1ml) of the α-amylase solution was added to the sample suspension and incubated at 37^{o}C for 45min in a shaking water bath. After incubation, 1ml of 3 – 5 dinitrosolicyclic acid was immediately added and the mixture was heated for 5 min to inactivate the enzymes. The solutions after cooling was centrifuged for 10min at 3000 xg, colour absorption of glucose concentration in the supernatant was determined using a speetrophotometer (Cecil 1021, U.K) at a wavelength of 450nm. A standard glucose graph ($Y = 18.719x$ and $R^2 = 0.9445$) of 0.02 mg/l to 0.1mg/l was also prepared and the glucose concentrations of each sample obtained. The results was converted to starch by multiplying the percentage glucose concentration by a factor 0.9.

2.5.2 Resistant Starch (RS)

This was also determined according to the method of Goni et al. (1997). One hundred (100mg) of each sample (residue from total starch) was incubated with pepsin solution containing 20mg of pepsin (Cat. No. P6887) for 60 min at 40^{o}C for protein removal. Then the starch was hydrohysed by adding the enzyme solution 40mg of α-amylase and incubated at 37^{o}C for 120min. After hydrolysis, samples were centrifuged and the supernatant discarded. The residue was analysed for starch as described for total starch.

Digestible starch (DS) content was taken to be the difference between the total starch (TS) and the resistant starch (RS) as reported by Frei et al. (2003), Capriles et al. (2008) and Kiin-Kabari and Giami (2015).

2.6 In-vitro Starch Digestion Rate and Prediction of Glycemic Index (PGI)

The starch digestion rate for plantain products (amala, cookies, cakes and bread) were expressed as the percentage of total starch hydrolysed over time intervals of 30min, 60min, 90min and 120min of incubation. The Hydrolysis Index (HI) was derived from the ratio between the areas under the hydrolysis curve of the various products developed and the reference sample (glucose). From the hydrolysis index obtained, the predicted glycemic index (GI) was calculated using the equation established by Goni et al. (1997); PGI = 39.7 + 0.548HI.

$$HI = \frac{\text{AUC of product}}{AUC\ of\ \text{glucose}} x100$$

Where AUC = Area under the curve

$$\text{AUC} = C\infty(t_f - to) - (C\infty/k)(1 - \exp) - K(t_f - to)$$

Where t_f = Final time, to = Initital time (min).

$C\infty$ = Equilibrium percentage of starch hydrolyzed after 120min, which is the glucose content after 120min divided by the total starch.

The kinetics of starch hydrolysis was calculated using a non-linear equation

$$C = C\infty(1 - e^{-kt}),$$

Where K = Kinetic constant. K was derived to be

$$K = -In\frac{(I - C/C\infty)}{t}$$

Where C = Percentage of starch hydrolysed at time t.

2.7 Statistical Analysis

Results were expressed as mean values and standard deviation of three (3) determinations. The obtained data were analysed using a one way Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20.0 software 2011 (Soft Inc. Tulsa, USA) to test the level of significance ($p<0.05$). Duncan New Multiple range Test was used to separate the means where significant differences existed. Correlation coefficients (r) were determined on all standard curves.

3. Results and Discussion

3.1 In-vitro Starch Hydrolysis

The results for total starch of plantain flour was low (51.51%), which also indicated a low digestible starch of 46.29% as shown in Table 1. This resulted in a high resistant starch value of 5.22%. High resistant starch value of 4.99% was also observed in 'amala'. These values are significantly different from the low resistant starch observed in cakes and bread. This observation may be attributed to the fact that the heat treatment applied in both cases may not be sufficient to fully gelatinize starch granules thus making it resistant for α-amylase digestion. This agreed with the work of Oboh and Erema (2010) which showed that processing of unripe plantain meals alters the physical form of the carbohydrates. Unripe plantain had also been reported to have small concentration of free sugars and rapidly digestible starch (Ramdath et al., 2004). Cookies had a resistant starch (RS) of 3.30% and a digestible starch fraction of 60.23% which in addiction may be related to the properties of the granules and its physical association with the plant cell wall (fibre), which could contribute to reducing total starch gelatinization. Plantain bread and cakes had the lowest resistant starch values of 0.94% and 2.95%, respectively which also resulted in higher values for digestible starch of 68.45% for cakes and 67.40% for bread. This observation may be due to product formulation and processing method which made the starch granules to gelatinize and more available to enzymatic digestion.

Table 1. Total Starch (TS), Resistant Starch (RS) and Digestible Starch (DS) of plantain products

Sample	Total starch (%)	Resistant starch (%)	Digestible starch (TS – RS) (%)
Plantain flour	51.51^{c}	5.22^{a}	46.29^{c}
Amala*	64.33^{b}	4.99^{a}	59.34^{b}
Cookies*	63.53^{b}	3.30^{b}	60.23^{b}
Bread+	68.34^{a}	0.94^{c}	67.40^{a}
Cakes+	70.62^{a}	2.95^{b}	68.45^{a}

^{a,b,c} Means bearing the same superscript within the same column do not differ significantly ($p<0.05$).

Key:

* = 85% plantain flour and 15% Bambara groundnut protein concentrate.

+ = 70% wheat flour, 20% plantain flour and 10% Bambara groundnut protein concentrate.

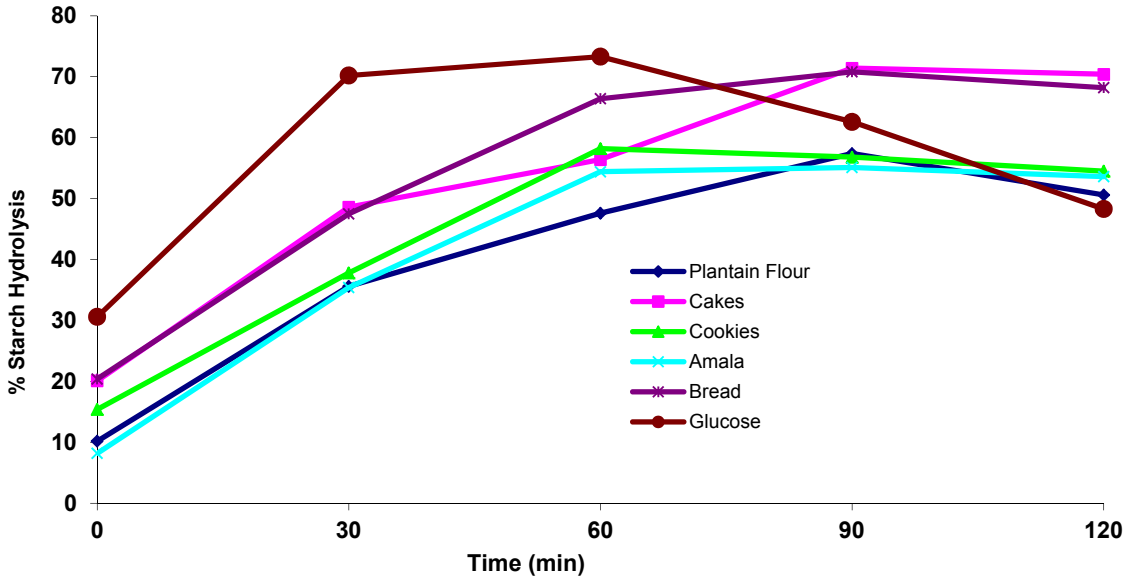

Figure 1. Rate of starch hydrolysis of plantain product

Key: Amala and Cookies = 85% plantain flour and 15% Bambara groundnut protein concentrate

Bread and Cake = 70% wheat flour, 20% plantain flour and 10% Bambara groundnut protein concentrate.

3.2 Equilibrium Concentration (C), Kinetic Constant (K), Hydrolysis Index (HI) and Predicted Glycemic Index (PGI) of Plantain Products

The equilibrium concentration (C) was higher in cakes (71.4%) and bread (70.8%) which indicated no significant difference ($p<0.05$) as presented in Table 2. However, when related to the rate of hydrolysis over time as shown in Figure 1. These plateaux were reached at 90min compared to glucose that attained its peak at 78.4% w-ithin 60min. Kinetic constant (K) of the various plantain products showed very low values between 0.0274 (amala) to 0.038 for bread and 0.028 for cake. These low values suggest generally higher resistance to enzymatic hydrolysis. This is in agreement with the report of Jaisut et al. (2009) that observed direct influence of both K and C parameters on the starch hydrolysis of brown rice.

The highest hydrolysis index (HI) of 74.85% were observed in cakes and 74.25% in bread which resulted in higher predicted glycemic index (PGI) of 80.79% for cakes and 80.45% for bread The values were significantly different from that obtained for 'amala' with HI of 56.40%, PGI 70.67% and cookies with HI value of 62.64% and PGI of 74.10%. The lowest HI (53.98%) and PG1 (69.35%) was recorded in plantain flour. The result showed that the more plantain flour in the product formulations, the lower the HI which subsequently led to the reduced predicted glycemic index (PGI). This may be that plantain contains carbohydrates whose bulk may consist of non-starch polysaccharides with a low GI. Studies have shown that a low GI diet not only improves certain metabolic consequence of insulin resistance but also reduces insulin resistances Perse (Del-Prato et al., 1994). In addition to improvement in glucose and lipid metabolism, there are indications of improvements in fibrinolytic activity suggesting beneficial roles in the management of diabetes and cardiovascular diseases (Brand-Miller et al., 1991; Jarvi et al., 1999). However, Oboh and Erema (2010) reported a glycemic index of plantain flour to be 65.05% which is lower than 69.35% recorded in this study. This difference may be attributed to factors such as processing methods, product compositions such as proteins (Mander et al., 2005) and fat (Collier et al., 1984). Although, predicted glycemic index for white bread had been reported to be 94.61% (Capriles et al., 2008) compared to 80.45% obtained for composite plantain bread in the study. Again, this difference may probably be due to the inclusion of plantain flour and protein concentrates in the formulation of the bread. Although, the addition of bambara groundnut protein concentrate did not affect enzymatic hydrolysis, it may contribute to the functionality of the baked products.

Table 2. Equilibrium Concentration (C), Kinetic Constant (K), Hydrolysis Index (HI) and Predicted Glycemic Index (PGI) of Plantain Products

Sample	Equilibrium concentration (%)	Kinetic constant (K)	Hydrolysis index (HI)	Predicted glycemic index (%)
Cakes +	71.4[b]	0.028[a]	74.85[a]	80.79[a]
Cookies *	66.8[b]	0.025[a]	62.64[c]	74.10[c]
Amala *	58.1[c]	0.024[b]	56.40[bc]	70.67[b]
Plantain flour	53.4[d]	0.028[a]	53.98[d]	69/35[c]
Bread +	70.8[b]	0.038[a]	74.25[a]	80.45[a]
Glucose	78.4[a]	0.023[b]	-	-

[a,b,c,d] Means bearing the same superscript within the same column do not differ significantly ($p<0.05$).

Key:

+ = 70% wheat flour, 20% plantain flour and 10% Bambara groundnut protein concentrate.

* = 85% plantain flourand and 15% Bambara groundnut protein concentration.

4. Conclusion

The results showed that plantain flour and 'amala' contained high values of resistant starch or slowly digestible starch. The addition of plantain flour in the baked products lowered the hydrolysis index and the corresponding predicted glycemic index. The low starch digestibility values of 60.23% and 59.34% for plantain cookies and amala are an indication that these products may be useful as functional food for diabetic and obese individuals.

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