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ORIGINAL ARTICLE

Effects of fermentation period and soybean flour supplementation on the glycemic indices and starch digestibility of biofortified provitamin A cassava meal

Anim Ekpo Ujong [1*], Olumide Oluwatoyosi Fashakin [2]

1. Department of Food Science and Technology, Rivers State University, Port Harcourt, Nkpolu, Oroworuwkwo. E-mail: animityekpo@gmail.com
2. Department of Food Science and Technology, Chiang Mai University, Thailand. E-mail: Olumideoluwatoyosi_f@cmu.ac.th

ABSTRACT

Background and aim: Across the globe, the prevalence of diabetes mellitus is rapidly increasing, even in populations with significant undernutrition. In this study, the effect of the fermentation period and soybean flour (SBF) supplementation on the glycemic indices and starch digestibility of biofortified provitamin A cassava meal was investigated. **Methods:** Biofortified provitamin A cassava (TMS 011368) was processed into cassava meal (*garri*) under two fermentation periods (24 h and 72 h) and supplemented with SBF at 0 – 80 % to produce different composite meals. These later were evaluated for their predicted glycemic index (pGI) and *in vitro* starch digestibility. **Results:** SBF supplementation significantly ($p<0.05$) decreased the hydrolysis index and pGI from 68.15 – 47.28 % and 73.20 – 44.56 % for composite meals fermented for 24 and 72 h, respectively. pGI, digestible starch, and resistant starch contents were significantly ($p < 0.05$) higher in composite meals fermented for 72 h than for 24 h. An increase in SBF supplementation significantly ($p < 0.05$) decreased the resistant starch, and digestible starch contents of the samples. **Conclusion**: Composite meals fermented for 24 h and supplemented with 40 – 80 % SBF had a reduced rate of in-vitro starch hydrolysis and glycemic indices, which may therefore be better suited for dietary prevention and management of diabetes mellitus.

ARTICLE INFORMATION

*Corresponding author: Ujong Anim Ekpo. E-mail: animityekpo@gmail.com

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1 Introduction

Cassava (*Manihot esculenta* Crantz) is one of the earliest stable foods and is eaten by more than 300 million people worldwide [1]. This food plant was first introduced to tropical Africa from America by the Portuguese in 1558. Presently, almost 50 % of the cassava production in the world comes from Africa, where it is used as a staple food due to food insecurity [2]. Cassava is rich in carbohydrates, so it is an important source of energy useful to maintain optimum functioning of the body's systems like digestion, breathing, heartbeat, regulation of blood sugar, etc. [3, 4]. Cassava-processed products contain starch and minerals [5] with an energy value of about 17 kJ/g (4 kcal/g).

Cassava is cultivated both as food and as industrial raw materials, where they are used for the production of starch, ethanol, biofuel, flour, biscuits, bread, etc. [6]. During the processing of cassava into its products, steps taken include peeling, drying, milling, roasting, sieving, pounding, etc. The combination of these steps leads to a reduction in the toxicity and improves the palatability of products that are acceptable to consumers [7]. In West Africa, examples of food products derived from cassava include *fufu, garri, cassava* flour, *eba*, etc. [8]. In rural areas, the roots are usually consumed boiled or baked, while people with more economical means tend to eat cassava roots as fried chips or snack food [9].

Glucose is frequently released into the bloodstream by amylase during the digestion of carbohydrate-rich foods, either rapidly or slowly depending on the type of food [10]. The release of glucose into the bloodstream causes an increase in insulin levels, which influences the satiety index and large intestine [11]. Biofortified provitamin A cassava is a type of cassava that is the result of cross-breeding. This type of cassava has a high level of carotene, is very resistant to disease, and can give children under 5 and pregnant women up to 40 % of the vitamin A they need every day [12, 13].

Glycemic index and glycemic load are utilized to monitor dietary control, especially in carbohydrate-containing diets. The glucose level is a factor of the glycemic index, and this phenomenon can be categorized between the scales of zero (very low glycemic index) and hundred (high glycemic index)[14]. According to Eli-Cophie et al. [15], there is a third type of glycemic index known as the medium glycemic index that ranges from 56 – 59. Cassava's glycemic index varies depending on the variety and environmental conditions under which it grows [16]. Health issues such as diabetes mellitus, obesity, and heart problems may be a result of an increase in glycemic index that is present in starch-containing foods such as cassava [7]. Given the global prevalence of diabetes mellitus (up to 537 million adults), the high consumption of cassava products and there diabetes mellitus implications have led to interventions to reduce its toxicity through processing and supplementation [17].

A number of factors, such as processing, cooking method, and the addition of dietary fiber, can reduce the glycemic index of a food product [18]. This leguminous vegetable, an indigestible carbohydrate of the dietary fiber type, possesses a low glycemic index and glycemic load, making it a potential supplement to reduce this index when added to another food product. The present research is therefore aimed at determining the effect of fermentation periods and soybean flour supplementation on the glycemic index and starch digestibility of biofortified provitamin A cassava meal.

2 Material and Methods

2.1 Source of experimental material

TMS 011368 (Biofortified Vit A cassava) was purchased from International Institute for Tropical Agriculture (IITA) Station, Onne, Rivers state. Soy flour was purchased from Spectra Company Ltd, Oko-Oba, Agege, Lagos state. α-amylase, pepsin and amyloglucosidase were purchased from Vantex Medical Consultancy Sapele Delta State, Nigeria. All chemicals used were of reagent grade and products of British Drug House (BDH), London.

2.2 Preparation of Biofortified Provitamin A cassava meal "*Garri*"

Cassava was processed into *garri* using the method of Ojo & Akande [19]. The cassava tubers were separately peeled, washed, grated and the mash bagged, dewatered and fermented for 3 days with subsequent dewatering. Cassava cakes was sieved and fried to obtain yellow cassava meal, then packaged and sealed in polythene bags.

2.3 Preparation of soybean flour

Soybean was cleaned, sorted, and toasted in clean potable water. After toasting, the soy beans were de-hulled, milled and then sieved to obtain soybean flour. The resulting soy flour was packaged and stored until further use.

2.4 Mixed meals (Combination Ratio)

Table 1 shows the meals made from fermented biofortified provitamin A cassava (TMS 011368) combined with soya flour. The different combinations were individually homogenized in a rotary mixer (Philips, model HR 1500/A, Holland) to generate a uniform flour, and then stored in airtight plastic containers until required for analyses. As a control, biofortified provitamin A cassava meal with no soybean flour replacement was used.

Table 1. Formulation of Biofortified provitamin A cassava meal/Soybean flour blends

Sample Code	Cassava meal	Soyabean Flour
A	100	00
B	80	20
C	60	40
D	40	60
E	20	80

2.5 Determination of total starch (TS) content, resistant starch (RS) and digestible starch

The Total Starch (TS) content was measured using the method described by Espinosa-Solis et al. [20]. To diffuse the starch, the samples (50 mg) were suspended in 2 M KOH and agitated at room temperature for 30 minutes. The samples were then hydrolyzed with amyloglucosidase (1 mL, 300 U/mL; Sigma A-7255) at 60 °C for 45 mins at pH 4.75. Glucose oxidase, peroxidase, and the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) test were used to assess free glucose. TS was determined as 0.9 multiplied by glucose. As a blank sample, wheat starch (Sigma S-1514) obtained from IITA, Nigeria, was used.

The content of resistant starch (RS) was determined using the method given by Moongngarm [21]. The samples were subjected to the following procedures: protein removal with pepsin (0.1 mL, 10 mg/mL), Sigma P-7012; hydrolysis of digestible starch with α-amylase (1 mL, 40 mg/mL), Sigma A-3176; residue treatment with 2M KOH for resistant starch solubilization, and hydrolysis of resistant starch solubilized with amyloglucosidase (80 mL, 140 U/mL). The difference between TS and RS was used to obtain the digestible starch (DS) value. The pepsin, α-amylase and amyloglucosidase used were obtained from Vantex Medical Consultancy, Nigeria.

2.6 *In-vitro* starch digestion rate and prediction of glycemic index (PGI)

The starch digestion rate for the samples was expressed as a percentage of total starch hydrolyzed after 30 minutes, 60 minutes, 90 minutes, and 120 minutes of incubation. The Hydrolysis Index (HI) was calculated as the ratio of the areas under the hydrolysis curves of the produced samples and the reference sample (glucose). The predicted Glycemic Index (GI) was computed from the hydrolysis index using the equation described by Kiin-Kabari and Yorte [22].

$$\text{HI} = \frac{\text{AUC of sample}}{\text{AUC of white bread}} \text{ X } \frac{100}{1}$$

Where AUC = Area under the curve
AUC = C ∞ (t f - to) - (C ∞ /k)(1 - exp) - K(t f - to).
Where tf = Final time, to = Initial time (min).
C ∞ = The equilibrium percentage of hydrolyzed starch after 120 minutes is the glucose content after 120 minutes divided by the total starch.
A non-linear equation was used to calculate the kinetics of starch hydrolysis.
C = C ∞ (1 - e-kt).

2.7 Statistical Analysis

Data was analyzed using analysis of variance (ANOVA). All means were separated using Fisher's test at 5% probability level ($p > 0.05$) using Minitab® version 16 software.

3 Results and Discussion

3.1 Predicted glycemic index of biofortified provitamin A cassava meal supplemented with soybean flour

The results of the Area Under Vurve (AUC), the Hydrolysis Index and the predicted glycemic index of biofortified provitamin A cassava/soybean composite meals at 24 and 72 h fermentation period are displayed in Table 2. AUC of the 24 h fermented composite meals ranged from 12.45 – 46.75 with sample E (20% biofortified provitamin A cassava meal) recording the lowest value (12.45) while sample A (100% biofortified provitamin A cassava meal) was the highest value (46.75). There was a significant ($p < 0.05$) difference in the AUC of the biofortified provitamin A cassava meals. AUC of 72 h fermented composite meals ranged from 6.48 – 55.06 with sample E recording the lowest value (6.48) and sample A as the highest value (55.06).

Hydrolysis index (HI) of the 24 h fermented composite meals ranged from 13.80 – 51.80 with sample E recording the lowest value (13.80) while sample A had the highest value (51.80). HI for 72 h fermented composite meals ranged from 8.64 – 61.01 with sample E recording the lowest value (8.64) while sample A had the highest value (61.01).

Predicted glycemic index of the 24 h fermented composite meals ranged from 47.28 – 68.15 % with sample E recording the lowest value (47.28 %) and sample A as the highest value (68.15 %). For 72 h fermented composite meals, predicted GI ranged from 44.56 % in sample E to 73.20 % in sample A.

Table 2. Area under curve (AUC), hydrolysis index and predicted glycemic index of biofortified provitamin A cassava meal supplemented with soybean flour

Sample	Area under curve (AUC) (%)	Hydrolysis index (HI) (%)	Predicted glycemic index (%)
	24 h Fermentation		
A	46.75 ± 0.27 ^{a}	51.80 ± 0.30 ^{a}	68.15 ± 0.16 ^{a}
B	24.30 ± 0.87 ^{b}	26.92 ± 0.96 ^{b}	54.49 ± 0.53 ^{b}
C	18.20 ± 0.11 ^{c}	26.16 ± 0.12 ^{c}	50.79 ± 0.07 ^{c}
D	17.09 ± 0.15 ^{d}	18.94 ± 0.16 ^{d}	50.11 ± 0.09 ^{d}
E	12.45 ± 0.04 ^{e}	13.80 ± 0.05 ^{e}	47.28 ± 0.03 ^{e}
L.S.D	**0.674**	**0.747**	**0.410**
	72 h Fermentation		
F	55.06 ± 0.54 ^{a}	61.01 ± 0.60 ^{a}	73.20 ± 0.33 ^{a}
G	43.64 ± 0.37 ^{b}	48.36 ± 0.41 ^{b}	66.26 ± 0.23 ^{b}
H	15.55 ± 1.29 ^{c}	17.24 ± 1.43 ^{c}	49.17 ±0.79 ^{c}
I	08.47 ± 1.86 ^{d}	09.38 ± 2.06 ^{d}	44.86 ± 1.13 ^{d}
J	06.48 ± 1.94 ^{d}	08.64 ± 3.64 ^{d}	44.56 ± 2.00 ^{d}
L.S.D	**2.218**	**3.260**	**0.674**

Values are means of duplicate determination ± SD. Means having the same letter within a column are not significantly different ($p < 0.05$).
Keys: A = 100 % biofortified provitamin A cassava meal fermented for 24 h; B= 80 % biofortified provitamin A cassava meal/20% soybean meal fermented for 24 h; C = 60 % biofortified provitamin A cassava meal/40 % soybean meal fermented for 24 h; D = 40 % biofortified provitamin A cassava meal/60 % soybean meal fermented for 24 h; E = 20 % biofortified provitamin A cassava meal/80 % soybean meal fermented for 24 h; F = 100 % biofortified provitamin A cassava meal fermented for 72 h; G = 80 % biofortified provitamin A cassava meal/20 % soybean meal fermented for 72 h; H = 60 % biofortified provitamin A cassava meal/40 % soybean meal fermented for 72 h; I = 40 % biofortified provitamin A cassava meal/60 % soybean meal fermented for 72 h; J = 20 % biofortified provitamin A cassava meal/80 % soybean meal fermented for 72 h.

3.2 Total Starch (TS), Resistant Starch (TS) and Digestible Starch (DS) of biofortified provitamin A cassava meal supplemented with soybean flour

The results of the total starch, resistant starch and digestible starch of provitamin A cassava meal supplemented with soybean flour are shown in Table 3. Total starch content of the composite meals fermented for 24 h ranged from 32.68 – 64.07% with sample E recording the lowest value (32.68%) while sample A had the highest value (64.07%). Total starch of the composite meals fermented for 72 h ranged from 45.49 % in sample E to 69.65 % in sample A. There was a significant ($p < 0.05$) difference in the total starch of the samples.

Resistant starch (RS) of the composite meals fermented for 24 h ranged from 1.22 – 3.47 % with sample E recording the lowest value (1.22 %) and sample A as the highest value (3.47 %). RS of the composite meals fermented for 72 h ranged from 1.56 % in sample E to 3.75 % in sample A.

DS of the composite meals fermented for 24 h ranged from 31.46 – 60.60 % with sample E recording the lowest value (31.46 %) and sample A having the highest value (60.60 %). DS of the composite meals fermented for 72 h ranged from 43.93 % in sample E to 65.90 % in sample A. There was a significant ($p < 0.05$) difference in the digestible starch of the samples.

Table 3. Total starch (TS), Resistant Starch (RS) and Digestible Starch (DS) of biofortified provitamin A cassava meal supplemented with soybean flour

Sample	Total Starch (%)	Resistant Starch (%	Digestible Starch (%)
24 h Fermentation			
A	64.07 ± 0.45[a]	3.47 ± 0.12[a]	60.60 ± 0.57[a]
B	53.96 ± 2.60[b]	2.77 ± 0.12[b]	51.19 ± 2.72[b]
C	50.34 ± 1.53[b]	2.38 ± 0.16[c]	47.97 ± 1.70[b]
D	43.48 ± 1.00[c]	1.92 ± 0.17[d]	41.56 ± 0.83[c]
E	32.68 ± 1.11[d]	1.22 ± 0.05[e]	31.46 ± 1.06[d]
L.S.D	**2.471**	**0.214**	**2.561**
72 h Fermentation			
F	69.65 ± 0.57[a]	3.75 ± 0.06[a]	65.90 ± 0.51[a]
G	64.14 ± 1.30[b]	3.16 ± 0.13[b]	60.99 ± 1.17[b]
H	58.50 ± 0.86[c]	2.65 ± 0.16[c]	55.86 ± 0.70[c]
I	51.78 ± 1.07[d]	2.33 ± 0.09[c]	49.45 ± 0.98[d]
J	45.49 ± 1.74[e]	1.56 ± 0.16[d]	43.93 ± 1.58[e]
L.S.D	**1.910**	**0.206**	**1.713**

Values are means of duplicate determination ± SD. Means having the same letter within a column are not significantly different ($p < 0.05$).

Keys: A= 100 % biofortified provitamin A cassava meal fermented for 24 h; B = 80 % biofortified provitamin A cassava meal/20 % soybean meal fermented for 24 h; C = 60 % biofortified provitamin A cassava meal/40 % soybean meal fermented for 24 h; D= 40 % biofortified provitamin A cassava meal/60 % soybean meal fermented for 24 h; E = 20 % biofortified provitamin A cassava meal/80 % soybean meal fermented for 24 h; F = 100 % biofortified provitamin A cassava meal fermented for 72 h; G = 80 % biofortified provitamin A cassava meal/20 % soybean meal fermented for 72 h; H = 60 % biofortified provitamin A cassava meal/40 % soybean meal fermented for 72 h; I = 40 % biofortified provitamin A cassava meal/60 % soybean meal fermented for 72 h; J= 20 % biofortified provitamin A cassava meal/80 % soybean meal fermented for 72 h.

4 Discussion

Composite meals fermented for 72 h had a higher predicted glycemic index (PGI) than the samples fermented for 24 h, except for samples supplemented with 40–80% soybean flour. A significant ($p < 0.05$) decrease in the predicted GI was also observed as the proportion of soybean flour increased. An increase in GI corresponds to an increase in digestion and absorption of food. The fermentation of the cassava for a period of 72 h will ultimately bring about the formation of more glucose and a subsequent increase in GI [23]. A similar increase (63.57 – 73.05 %) was reported by Ihediohanma [23] for cassava granules (*garri*) as fermentation time increased. GI gives an idea of how fast the body converts carbohydrates from food into glucose. This study's findings also revealed that a reduced HI resulted in a lower PGI. According to Ogbuj and David-Chukwu [7], cassava meals (*garri*) have a GI of 92.36. This value differs slightly from what was reported in the current study. This variation could be linked to several variables, such as processing techniques and product components such as proteins and fat [24]. The drop in the PGI of the composite meals as the proportion of soybean flour increased could be attributed to the fact that soybean flour contains carbohydrates, the majority of which may be non-starch polysaccharides with a low GI. Ogbuji and David-Chukwu [7] reported identical findings for wheat and processed tiger nut flour. A low GI meal enhances specific metabolic implications of insulin resistance [25].

In conjunction with improved glucose and lipid metabolism, there are indicators of improved fibrinolytic activity, implying favorable roles in the control of diabetes mellitus and cardiovascular disease [26]. The decrease and increase in the PGI of the samples as fermentation time increases shows the dual effect of fermentation on the GI, as reported by Ogbuji and David-Chukwu [7]. Ihediohanma [23] found a rise in the GI after consuming fermented foods, while Mlotha [27] found a reduction. The low PGI of some of the samples after 72 h of fermentation may be related to the short-chain organic acids generated during fermentation, such as lactic acid, acetic acid, and propionic acid [28], whereas the rise was possibly related to the ease of digestion and absorption of glucose as a result of fiber degradation by microorganisms during fermentation [7]. In line with the scale rating, the result shows that the composite meals are low glycemic foods, while biofortified provitamin A cassava meal fermented for 24 h is an intermediate glycemic food. The biofortified provitamin A cassava meal fermented for 72 h is a high glycemic food, implying that fermentation increases the GI of the cassava meal with time. The scale rating also shows that the 24 h fermented composite meal is a low glycemic food, while the 72 h fermented composite meal blend is an intermediate glycemic food. The 72 h fermented biofortified provitamin A cassava meal supplemented with 40–80 % soybean flour is a low-glycemic food, implying that an increase in soybean flour decreases the GI of the cassava meal.

When the fermentation time of the biofortified provitamin A cassava meals was increased, the overall starch content increased. Increases in the quantity of soybean flour, on the other hand, resulted in a significant ($p < 0.05$) drop in the starch content of the composite meals. Increasing soybean flour content resulted in a significant ($p < 0.05$) drop in resistant starch content, but increasing fermentation time for 72 h resulted in a rise in RS. The decrease in resistant starch of the biofortified provitamin A cassava meal after substitution with soybean flour could be attributed to soybean's poor resistant starch. Components in the food matrix such as protein, fat, dietary fiber, and minerals have been shown to influence the production of resistant starch in diets. The presence of lipids in soybeans causes the creation of starch-lipid complexes that are resistant to the enzymatic digestion of starch [29]. Furthermore, the presence of oil in the fermenting mash due to the inclusion of soybean flour may have harmed microbial activity, lowering the resistant starch concentration [30]. The resistant starch content of the composite meals in this study is comparable to the resistant starch content of white cassava meal (3.05 – 3.55 g/100 g) determined by Ogbo and Okafor [31].

The higher resistant starch values reported in biofortified provitamin A cassava meals fermented for 72 h compared to biofortified provitamin A cassava meals fermented for 24 h could be attributed to the prolonged fermentation time. Resistant starch is regarded as "the aggregate of starch and starch breakdown products that are not absorbed in the small intestine of healthy persons" [32]. Resistant starch in foods influences a variety of physiological activities and has been linked to a variety of health effects. Some established health benefits include lower energy content, hypocholesterolemic activities, and anti-colorectal cancer protection [33]. Studies on the influence of dietary resistive content on glucose response have yielded inconsistent results. Some studies have found a link between a higher amount of resistant starch and a lower response of glucose and insulin to food [34, 35], while others reported no effect on glucose levels [36].

Similarly, increasing the proportion of soybean flour (SBF) in the product formulation resulted in a significant ($p < 0.05$) decrease in digestible starch, while the digestible starch (DS) of samples fermented for 72 h was higher than the DS of samples fermented for 24 h. The lower the digestible starch level of food, the lower the risk that its consumption will result in diabetic diseases [31]. The decrease in digestible starch on substitution with soybean flour is attributable to the formation of carbohydrate-lipid complexes. Soong et al. [37] suggested that the digestibility of rice starch was reduced by the addition of saturated fatty acids, suggesting the formation of carbohydrate-lipid complexes. The difference in amylase to amylopectin content of the starches in the biofortified provitamin A cassava meals may also cause a difference in the digestibility of the starches [38]. The digestible starch of the composite meals from this study is slightly higher than the range of 39.80 – 40.50 g/100 g obtained by Ogbo and Okafor [31] for white *garri* from different cassava varieties. This difference may be attributed to the difference in processing methods. Composite cassava meals fermented for 72 h have higher digestible starch content than the composite meals fermented for 24 h indicating that the composite meals fermented for 72 h will be easily digested.

Increased digestibility implies greater susceptibility to pancreatic -amylase, implying that the highly ordered structure of carbohydrates in the food samples has been destroyed to varying degrees [38]. It has been suggested that starchy foods that are digested slowly and result in low blood glucose are more beneficial to health and the management of diabetes and hyperlipidemia [39] than are starchy foods that are digested rapidly. The inference is that the degradability of ingested starch is important, especially among diabetics and hyperlipidemic individuals [40]. Miao et al. [41] observed that starchy foods that are easily degraded tend to have a higher insulin demand than the slower-degrading starches. This can affect the sensitivity to insulin [42] and lead to or reduce the risk of developing type 2 diabetes [43], myocardial infarction in women, and high HDL cholesterol levels [40].

5 Conclusions

The results of this study revealed that the supplementation of biofortified provitamin A cassava meal with soybean flour resulted in a decrease in the predicted glycemic index, hydrolysis index, area under the curve, total starch, resistant starch, and digestible starch of the samples. The increase in fermentation time from 24 h to 72 h also led to an increase in the resistant starch, digestible starch, and glycemic indices of the samples. The current supplementation is suitable for people suffering from obesity and diabetes mellitus who want to lose weight. The rationale for this recommendation is that low-GI diets have been shown to reduce risk factors for type II diabetes and cardiovascular disease.

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