Comparison of the apparent bioavailability of starch in raw, cooked and extruded matooke flours using weanling mice

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Abstract

The aim of the study was to compare the apparent bioavailability of starch in raw, cooked and extruded matooke flours using weanling mice. Two control and three test groups consisting of seven mice each (initial body weight of 21.32 ± 1.05 g) were fed diets incorporating soluble unavailable starch (Control I), soluble starch (Control II) and raw, cooked and extruded matooke flours as carbohydrate bases, for three weeks. The growth rate, food intake, adipose tissue size and 12 h fasting glucose levels were measured. The mean values of the growth parameters were separated by ANOVA using GENSTAT statistical package. There was a significant difference (P<0.001) in food intake between the control and test groups. The mice fed on solubilised matooke starches (cooked and extruded) exhibited significantly higher (P<0.001) growth rates than the ones fed on raw starch, showing a higher apparent bioavailability of the former flours. The mice fed on Control I appeared malnourished despite an excessively high food intake. The raw matooke group displayed less pronounced symptoms of malnourishment despite recording the highest weight loss. The fat pad sizes were in agreement with the growth rate data. The glucose levels, though on the lower side particularly in the Control I and raw matooke flour groups, were within the normal range. The results demonstrated that solubilised matooke starches adequately met the energy requirement of a growing animal. Nonetheless extrusion cooking appeared to confer a marginal advantage over the cooked flour, due to extruded flour's lower peak viscosity. This advantage would be enhanced in humans if the rations are taken as porridges.

Keywords: Starch-bioavailability, matooke, protein-energy malnutrition.

INTRODUCTION

Matooke, an East African Highlands cooking banana, is the lead staple food for the people in the great lakes region of Eastern Africa, especially Uganda. Muranga (1998) reported that the matooke varieties in Uganda belong to the triploid *acuminate* genome group (AAA-EA), (a group that also includes juice/beer banana types or "mbidde"). They are further classified into soft and hard cooking types. The latter have marginal market potential. Matooke are generally harvested between three- quarters to full maturity, peeled and boiled or steamed in banana leaves during which the color of the pulp changes from a creamy white to an almost golden yellow color depending on original maturity of the bunch. The extent of tenderness and yield to mash is, however, varietal-dependant and it is a key attribute of consumer acceptability (Sem-wanga,

1996). The cooked dough, popularly known as "emmere" literally meaning "the food" is virtually free from astringency (Semwanga, 1996; Sebasasigari, 1996). These varieties blossom from leaf green into a bright yel-low fruit on ripening and become quite succulent at maxi-mum ripeness when left to ripen.

Early researchers recorded matooke as virtually consis-ting of water and consequently associated it with the high prevalence of kwashiorkor, a form of Protein Energy Mal-nutrition (PEM), among the matooke diet-dependant po-pulations (Amann et al., 1972).

PEM is a consequence of poor or insufficient supply of protein and energy in the diet (Kikafunda-Kakuramatsi, 1996). National statistics put the prevalence of PEM in Uganda at 26% of children under 2 years of age (Amref, 2000). The key underlying factors for malnutrition in the

country include low energy density of weaning foods and lack of ready-to-serve formulations. Consequently, feeding is infrequent due to heavy workload of caregivers especially among the rural poor (Kikafunda-Kakuramatsi, 1996). On this background the HIV/AIDS pandemic has further exacerbated the malnutrition situation, particularly given that HIV/AIDS predisposes its victims to increased energy and protein requirement. For children in general and those with HIV/AIDS in particular, a low bulk food is desirable to ensure high energy density. The national children's rehabilitation centre (Mwanamugimu) at the main referral Hospital, Mulago in Kampala, Uganda, in response to the need for energy and protein dense foods, has developed a series of multi-mix recipes or "kitoobero" from locally available foods. The recipes incorporate local starchy staples. They are, however, prepared using laborious traditional methods and consequently, their rate of adoption by the caregivers, after the children leave the Unit, is limited. The main starchy staple in the formulations is matooke. The protein-energy efficiency ratio (PER) of matooke (and consequently raw matooke flour) is unfortunately rather low (Muranga, 1998) even in the presence of high protein mixes due to its excessive bulk. The physicochemical characteristics of matooke starches have been documented (Muranga, 1998). Of significance is the fact that the matooke contains low amylose starch (C 8%) and consequently, low retrogradation potential. Muranga (1998) further reported that matooke generally contained over 80% starch on dry basis, and that the reduction of bulk in matooke was feasible using two pre-gelatinisation (starch solubilisation) technologies; namely extrusion cooking of raw banana flour and cooking of the fresh fruit staple prior to drying.

Preliminary results indicate that the cooked flours have a superior color though with a lower protein efficiency ratio (PER) compared to the raw and extruded flours for the same protein fraction (Katebarirwe, 2004). The low PER was presumed to indicate lower bioavailability of the pre-gel starch.

The extruded flour's overall superiority, however, lies in its lack of apparent hot water viscosity. The overriding advantage of the technology for cooked flour is that it is an appropriate technology, which can be easily adopted by grassroots communities in the country. On the con-trary, extrusion cooking would only be industrial based and would therefore entail processing overhead costs. Nonetheless, similarity of the cooking technique to the traditional processing practice for matooke which has time and again been blamed for energy insufficiency due to bulk, calls for evaluation of the bioavailability of cooked matooke starch.

The objective of the study therefore was to establish whether the cooked and extruded matooke flours had significant advantage over raw matooke flour and each other with respect to bioavailability. We used two controls: a high amylodextrin starch (unavailable starch) and the highly available soluble cornstarch as references for impact

of maximal malabsorption and optimal bioavailability respectively. This research is particularly important in the wake of the search for sustainable food based strategies that would meet the increased energy requirement of malnourished children particularly those with HIV/ AIDS. Here we seek to demonstrate the impact of processing technique on bioavailability of a starchy staple.

METHODS

Preparation of matooke flours

The raw and cooked matooke flours was produced after the procedures outlined in patent No AP/P/2005/003308 and filed patent No UG/P/04/00010, respectively, whereas extruded matooke flour was produced by the processes reported by Muranga (1998).

Matooke flour physicochemical properties

The moisture contents of the matooke flours were determined by vacuum oven drying at 70ëC for 5 h (Marlett, 1992). Nitrogen was determined by a micro kjeldjahl method (AOAC, 1999). Crude protein was thereafter estimated as the Nitrogen content multiplied by 6.25. To determine ash, 400 mg dry aliquots were ashed at 450ëC for 24 h and then allowed to cool, wetted with concentrated nitric acid, returned to the muffle furnace overnight (16 h), and allowed to cool in a dessicator before weighing. The total dietary fiber con-tent was determined by an enzymic gravimetric procedure (method 985.29) (AOAC, 1999; Prosky et al., 1992) by a commercial laboratory. Starch was measured by an enzymic–calorimetric method (method 76-11) (AACC 1976) as reported by Murray et al. (2001).

Energy and peak viscosity (viscosity, at 95ëC) were determined on the matooke flours using bomb calorimetry [CBA301, UK] (AOAC, 1999) and RVA technique, respectively. The latter was done using the flocken programme of the Rapid Visco Analyser (RVA Flocken, Thermocline version 2.0 Newport Scientific PTY, Ltd 1992, NSW Australia). A 2.5 g sample, which had been ground to pass the 0.5 cm sieve of solids (14% moisture basis) and 25 ml distilled water were placed in aluminium can. It was mixed quickly and thoroughly prior to measurement. The test was programmed to last 18 min.

Animals and diets

B6 mice were purchased from Harlan-Teklad and housed in a pathogen free facility of the Department of Biochemistry (University of Wisconsin, Madison, USA) operating at room temperature in a 12 h light/dark cycle. The animals were weaned at 2 weeks on a commercial pelleted diet for one week after which they were split in five groups of seven. They were then weaned onto diets containing the following carbohydrate bases: soluble non-available starch (Control I), soluble cornstarch (Control II), raw matooke (RM), cooked matooke (CM) and extruded matooke (EM). The feeding was conducted for three weeks.

The diets were formulated to contain 90g of basal mix (30%) (purchased from Harlan Teklad, TD 02520), 195 g carbohydrate base (65%) and 15 g fat (5%) (0.95 g/ml of 18:1 Tri-olein purchased from sigma). Control I diet contained soluble starch (amylodextrin-purchased from Acros organics, New Jersey, USA) while Control II contained soluble cornstarch purchased from Sigma Chemical (St. Louis, MO).

Table 1. Physicochemical characteristics of raw and solubilised matooke flours.

| Matooke Flour Type | g/100g dry wt | | | | | | Peak Viscosity (RVU) | Energy (kcal/100g dry wt) |
	Moisture Content	Crude Protein	Crude Fat	Starch	Crude Fiber	Ash		
Raw	6.76±0.1[a]	4.04±0[b]	0.64±0.0[a]	77.29±0.6[c]	2.15±0.0[b]	3.22±0.0[b]	336±5.5[a]	367.62±0.0[a]
Cooked	6.44±0.0[b]	5.06±0.1[a]	0.64±0.0[a]	87.62±1[b]	3.57±1.5[a]	4.01±0.1[a]	82.5±0.5[b]	351.18±2.1[b]
Extruded	5.95±0.0[c]	5.14±0[a]	0.44±0.1[a]	89.76±0.1[a]	2.06±0.0[b]	3.20±0.0[b]	16.5±0.5[c]	369.30±26.1[a]
Potato				90.76±0.2[a]				
LSD	0.30	0.37	0.63	1.1	1.16	0.25	11.3	6.85
%CV	2.6	1.6	5.4	0.69	27.8	3.2	3.56	6.8

Data presented as Means ± Standard deviation; **LSD**: Least significant difference; **CV**: coefficient of variation; Superscripted values in the same column baring different letters were significantly different.
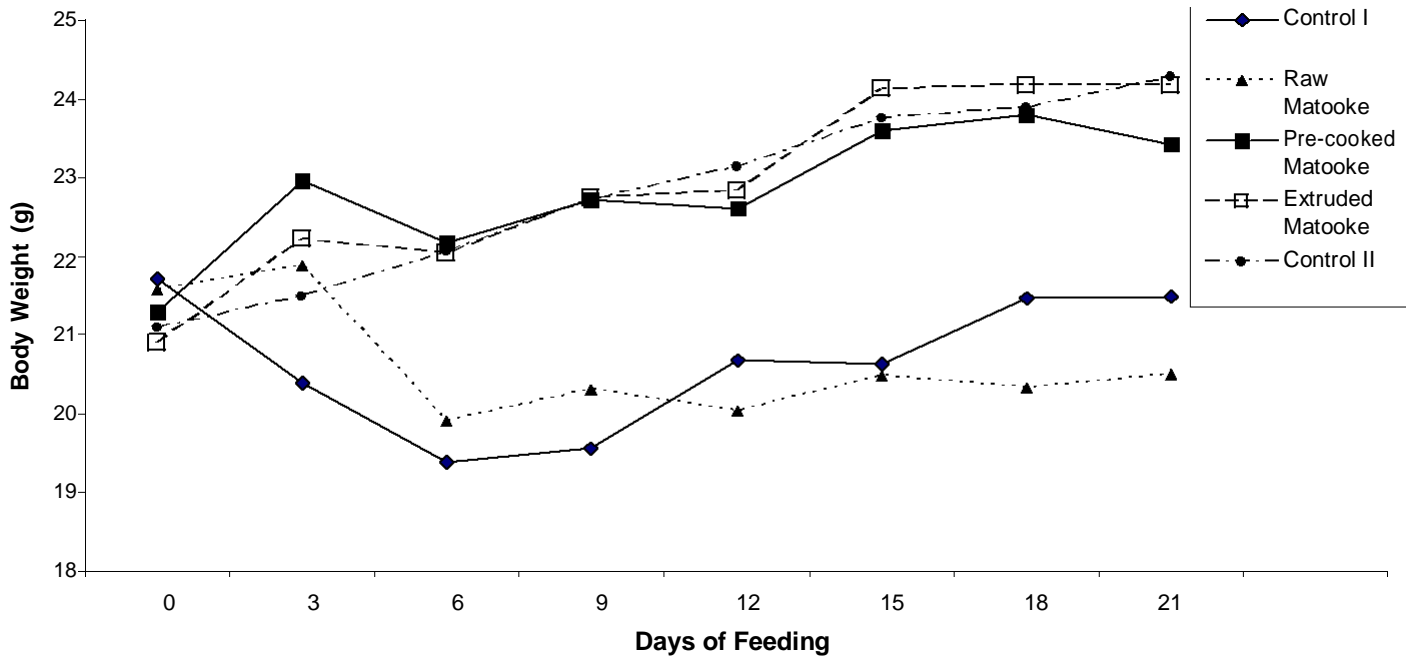

Figure 1. Change in body weight for mice on the different diets.

The initial body weight of weanling mice was 21.32 ± 1.05 g. The weight of each mouse within each group was measured at three day intervals while the food intake was calculated at seven day intervals. Fasting plasma glucose was measured using the glucose oxidase method (Rosevear et al., 1969) following a 12 h fast. Body fat mass was measured after sacrifice. All *in vivo* experiments involving the use of mice were approved by the animal care research committee of the University of Wisconsin-Madison.

Statistical analysis

Data presented as means were established using Analysis of Variance (ANOVA) (Genstat 5 Release 3.2, Second Edition 1996), means were separated using LSD, where as graphs and SDs were obtained using Ms Excel (Microsoft Corporation, 2003).

RESULTS

Physicochemical characteristics

Table 1 shows the physicochemical characteristics of the raw and the solubilised matooke flours. There were significant differences in the physicochemical characteristics of the flours, except for crude fat content. Extruded matooke flour had the highest starch content (P<0.001) followed by cooked and raw flours respectively. The starch content of extruded matooke flour was comparable to that of potato. Moisture content was highest (P<0.001) in raw matooke flour. Crude fat was significantly (P<0.001) higher in cooked and extruded flours than raw flour. Cooked matooke registered significantly (P<0.001) higher

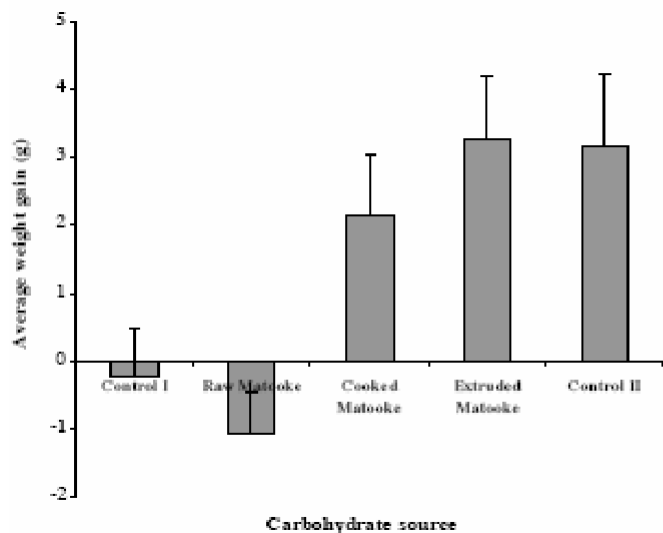**Figure 2.** Average weight gain for mice on different diets.
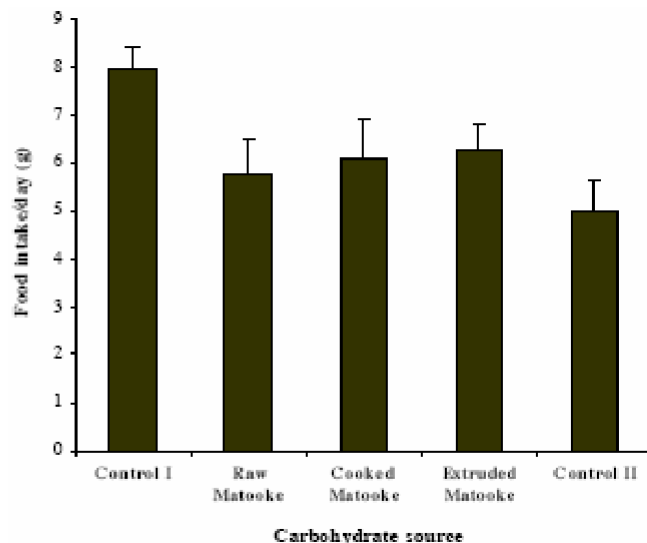

Figure 3. Average food intake in g/day for mice on different diets.

dietary fiber and ash contents than the other two flours; which were not significantly different. Raw matooke flour gave the highest peak viscosity (P<0.001) followed by cooked and extruded flours respectively. Gross energy was highest in raw and extruded matooke flours; which were significantly (P<0.001) different from cooked flour.

Food intake and body weight

Figures 1 and 2 shows the results of change in body weight with time and weight gain, respectively while the food intake levels are illustrated in Figure 3. The group of mice on the soluble non-available starch (Control I-SNS) diet showed a significantly higher (P<0.001) level of food intake in contrast to the other four groups (Figure 3). The differences in food intake for the groups on the matooke-based diets and control II were not significant despite their differences in carbohydrate source. In contrast, the average body weight gain of mice on the solubilised starch diets (Control II, EM and CM) was significantly higher (P<0.001) than that of the mice on raw starch diets (Control I and RM) (Figure 2). The mice on the matooke and soluble starch diets experienced a rapid weight gain in the stabilisation phase (1st 3 days) but thereafter the raw matooke diet mice fell to the same level as the soluble non-available starch mice group (Control I) (Figure 1). The groups on the raw starch diets (Control I and RM) also passed high amounts of bulky stool especially the control group (data not shown). The control I group experienced an initial traumatic loss of weight, which in spite of appreciable gains later, still made it impossible for rising beyond its weaning body weight. There was, however, no significant difference between the weight gain of animals on the extruded and cooked matooke starch diet.

Over the study period, the raw diet mice group registered the least food intake.

Body fat mass

Figure 4 shows the average fat pad weight for the mice on the different diets. On average, the control I mice group consumed over 20% more food than the mice on the other diets. However, these mice were emaciated and displayed other symptoms of malnutrition like hair standing upright in follicle and poor sheen. The control group (Control I) and the group on the raw matooke diet both accumulated less fat in their adipose tissue. The differences between adipose tissue mass for the mice on the raw starch diets (Control I and RM) and those on the solubilised starch diets (Control II, EM and PM) were significant (p<0.001). The Control II group overall had the highest amount of adipose tissue fat; however, it was not significantly higher than that of the cooked and extruded matooke groups.

Plasma fasting glucose levels

Figure 5 shows the results of the average fasting glucose levels for mice on different diets. The glucose levels after 12 h of fasting were within the normal plasma glucose levels for all groups of mice (130 - 200) but the values for the Control I mice were slightly lower. The latter is indicative of hypoglycemia and corresponds well to the very low levels of fat pads.
The glucose level of the cooked matooke mice group was; however, surprisingly lower than anticipated. Additionally, there was no significant difference in the fat pad weights of this group (cooked matooke group) and those
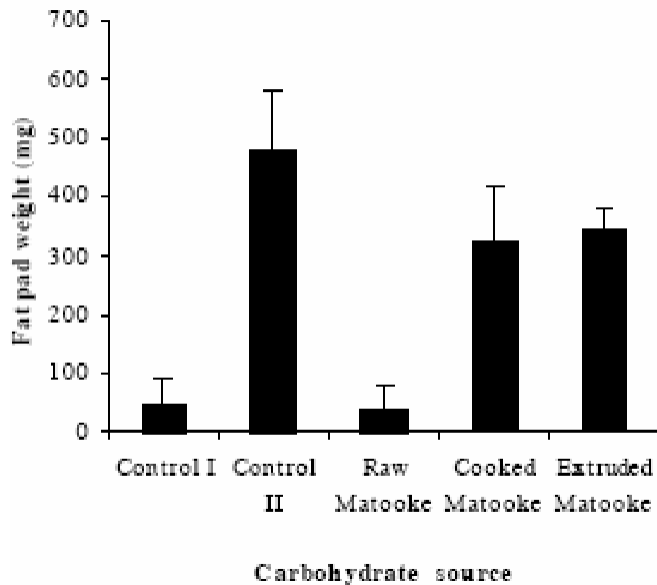
Figure 4. Average fat pad weight for mice on different diets.

Figure 5. Average fasting blood glucose levels for mice on different diets.

of the extruded matooke group**.**

DISCUSSION

The differences in chemical composition between the raw and cooked matooke flours are most likely due to the mode of peeling applied on to the matooke fingers (Katebarirwe, 2004). The raw flour, which was also the raw material for the extruded flour, was obtained through peeling of raw matooke with stainless steel knives following the customary peeling procedure of the Ganda people in which the matooke is cut at both ends and subsequently peeled longitudinally in a clockwise fashion. This technique separates the pulp from the peel but allows a significant amount of the outer layer of the pulp to go with the peel. On the contrary, the cooked pulp was obtained by peeling cooked fingers, which enabled very thin peeling with virtually no pulp loss. This difference appears to account for the difference in the proximate composition indicators; particularly protein, fiber and ash (Katebarirwe, 2004) . The starch difference may arise more from the fact that the enzymatic technique used in the analysis may have been more effective on the solubilized matooke starches (cooked and extruded). The latter may be consequent to the fact that native matooke starch is highly restricted in swelling especially under low starch: water ratios (below 8%) (Muranga, 1998) which if it were the case could compromise its enzymatic hydrolysis. The viscosity of the raw matooke flour is 4 times as high as that of the cooked and over 15 times that of the extruded flour, with the latter flour indicating virtual solubility. Therefore, for human intake as porridge, the difference in the amount of calorie intake at each serving for the three flo-
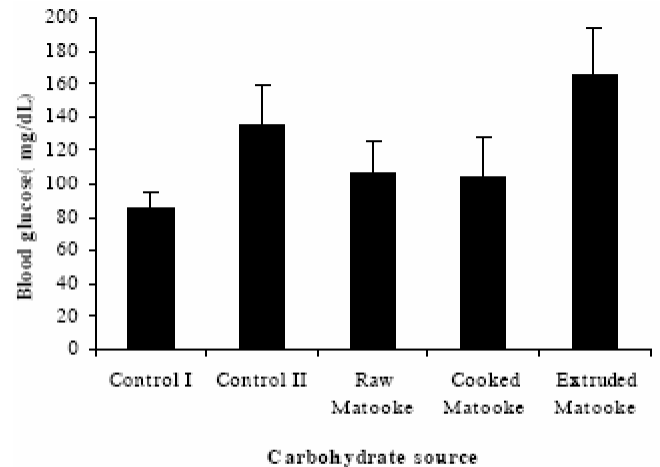
flours is highly significant (Katebarirwe, 2004). This gives the extruded flour unparalleled advantage as a weaning carbohydrate base for humans coupled with the fact that its energy output per 100 g dry wt was also higher than that of the cooked flour.

The results in this study also demonstrate the significance of available carbohydrate in the diet at the weaning stage of animals. Despite the fact that the mice on raw/unavailable starch diets (Control I and raw matooke flour) had all other nutrients required in the diet, they suffered significant set back in their growth typical of malnourished conditions. It is apparent that without the supply of sufficient energy the animals on the unavailable starch diets were unable to absorb the nutrients of the premix, which inevitably led to other forms of malnutrition. It is, however, also possible that the high turn over of stool that characterized these mice may have deprived them of these nutrients. The extruded matooke starch diet overall produced the best growth response against food intake which ascertained that this technology not only reduces the bulk in the food through enhancing starch solubility (Muranga, 1998) but also bioavailability. However, the fact that there was no significant difference for all the indicator parameters for the two solubilised test flours (Katebarirwe, 2004) confirms cooking prior to dehydration as an equally good technique for enhanced bioavailability which render the flour a credible base for ready to eat children's foods.

Summary

Our results demonstrated that solubilisation of matooke starch through heat processing improved its apparent bioavailability and thus ensured energy sufficiency for the weanling mice. We therefore recommend testing of the solubilised flours in combination with high protein mixes (e.g. soybean, sesame, milk) in malnourished children in

a clinical setting prior to adoption for home use as weaning foods.

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