

The polyphenol-rich baobab fruit (Adansonia digitata L.) reduces starch digestion and glycemic response in humans

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ABSTRACT

The baobab fruit (Adansonia digitata L.) is found throughout regions of Africa and is becoming increasingly recognized for its high nutrient and polyphenol content. Polyphenols have been beneficial for their effects on reducing the glycemic response (GR) and for improving various other metabolic parameters. Based on previous research, it was hypothesized that the baobab fruit extract would reduce starch digestion in vitro and would show potential for reducing the GR and for increasing satiety and diet-induced thermogenesis in humans. Six extracts of baobab from 6 different locations in Africa were measured for their antioxidant and polyphenol content using the ferric ion-reducing antioxidant power and the Folin-Ciocalteu methods, respectively. Baobab extract was baked into white bread at different doses to determine the optimal dose for reducing starch breakdown and sugar release from white bread after an in vitro digestion procedure. In vivo, baobab extract was consumed in solution at both a low-dose (18.5 g) and a high-dose (37 g) aqueous drink in 250 mL of water along with white bread, and resulting GR, satiety, and postprandial energy expenditure were measured. All extracts in this study were shown to be good sources of polyphenols. Baobab fruit extract added to white bread at 1.88 % significantly (P < .05) reduced rapidly digestible starch from white bread samples. In vivo, the baobab fruit extract at both low and high doses significantly (P < .05) reduced GR, although there was no significant effect on satiety or on energy expenditure.

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1. Introduction

Africa is abundant in novel plant species known to be rich in health-promoting compounds, many of which remain undiscovered or unused by western society [1]. The baobab tree, *Adansonia digitata* L., is widely distributed throughout sub-Saharan Africa and Western Madagascar and has many uses, including but not limited to its use in medicine, food, and beverages [2,3]. This fruit is of increasing nutritional interest because it may be a significant contributor to the daily intake of important nutrient and nonnutrient compounds [4]. Many studies have confirmed that baobab fruit pulp is rich in vitamins and minerals [3,5-7] and contains a high amount of both soluble and insoluble dietary fiber [3,5-9]. This fruit is also high in vitamin (vit) C, which contributes to its overall antioxidant capacity [1,10-13], and is a good

Abbreviations: ANOVA, analysis of variance; BG, blood glucose; CHO, carbohydrate; DIT, diet-induced thermogenesis; EE, energy expenditure; FAO/WHO, Food and Agricultural Organization/World Health Organization; FCR, Folin-Ciocalteu reagent; FRAP, ferric ion-reducing antioxidant power; GAE, gallic acid equivalents; GR, glycemic response; HD, high dose; IAUC, incremental area under the curve; LD, low dose; RDS, rapidly digestible starch; VAS, visual analog scales; vit, vitamin.

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source of polyphenols, including certain flavonoids [1,14] and tannins [15].

Polyphenols are thought to have an effect on starch breakdown in foods. Deshpande and Salunke [16] found that both tannic acid and catechins decreased the in vitro digestibility of different starch sources. Yoon et al [17] investigated the effect of phytic acid on starch digestibility in vitro and in vivo. When phytic acid was added to raw wheat starch, using an in vitro digestion model, it was found that the addition of phytic acid directly after the salivary phase decreased sugar liberation, yet little effect was seen throughout the rest of digestion. In addition, in vivo the higher the concentration of phytic acid added to a food in healthy people, the lower the glycemic index of the food being consumed.

Polyphenols may also reduce the glycemic response (GR) in vivo [18-21]. Fruit and fruit extracts are rich in polyphenols and have shown promise for improving markers of diabetes and increasing diet-induced thermogenesis (DIT) and satiety [22-26]. In a streptozocin-induced diabetes rat model, extract of baobab fruit caused a significant (P < .05) reduction in the GR compared with the control [23]. Fruit extracts are also rich in fiber; the baobab fruit extract powder contains approximately 30 g of insoluble fiber and 30 g of soluble fiber per 100-g extract. Foods rich in soluble fiber, such as β -glucan, have been shown to reduce the GR [27,28]. Therefore, polyphenols and/or the fiber in various fruit extracts may be responsible for a reduction in the GR postprandially.

Bolton et al [24] found that whole fruit produced a stronger satiety rating than fruit juice, and it was found that the return of appetite was also delayed with whole fruit consumption. This increase in fullness was thought to be due at least in part to the fiber content of the whole fruit. However, studies looking at the effect of different foods and food compounds on the association between satiety and DIT show conflicting results. In a study investigating the polyphenol and fiber-rich carob pulp, satiety postconsumption was increased compared with the control [25]. In the same study, postprandial energy expenditure (EE) was also found to be increased by 42.3%. In contrast, Raben et al [26] found a reduction in DIT and an increase in the feeling of fullness in subjects after they consumed a high-fiber meal when compared with a lowfiber meal. Therefore, polyphenols are thought to play a significant role in the increase of EE [29].

It is hypothesized that baobab fruit extract will reduce starch breakdown from white bread in vitro due to its high polyphenol and/or fiber content. It is further hypothesized that when tested in humans, baobab extract will reduce the GR postprandially and will increase the feeling of fullness and the amount of energy expended after consumption.

The specific aims of the present study were:

- 1. To analyze the antioxidant and polyphenol content and the polyphenol bioaccessibility of 6 baobab extracts.
- 2. To test baobab extract for its effect on starch breakdown and resulting sugar release from white bread samples at different doses.
- To determine the effect of a low-dose (LD) baobab drink and a high-dose (HD) baobab drink (sample 6, Baobab super fruit powder; Min Vita, London, UK) on postprandial GR, DIT, and satiety in humans.

2. Methods and materials

In vitro analysis.

2.1. Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Poole, UK). The extracts included 5 different extracts of baobab fruit donated from 5 different geographical locations in Africa and 1 commercially purchased baobab fruit extract (sample 6, Baobab super fruit powder; Min Vita, London, UK; Holland & Barrett, UK). A total of 6 extracts were selected to test for variability in polyphenol content and bioaccessibility between extract locations. The commercially available extract was the only extract used for the remaining in vitro sugar release and human part of this study and contained the following ingredients per 100g: 170 kcal, less than 1 g total fat, 78 g total carbohydrate, 1.8 g protein, 30 g soluble fiber, 30 g insoluble fiber, 14 g total sugars, 0.6 mg vit B1, 0.03 mg vit B2, 300 mg vit C, 2500 mg potassium, 350 mg calcium, 2 mg iron, 148 mg magnesium, and 0.5 mg sodium.

2.2. Study protocol

Each sample was weighed out at 500 mg and added to a 50-mL solution of distilled H_2O . All tests were carried out on a minimum of 3 separate occasions, and samples were analyzed in triplicate for each test.

2.3. Analysis of polyphenol and antioxidant content

2.3.1. Ferric ion-reducing antioxidant power

Samples from 6 baobab extracts were analyzed using the ferric ion–reducing antioxidant power (FRAP) method adapted from Benzie and Strain [30].

2.3.2. Folin-Ciocalteu

The polyphenol content of the 6 baobab extracts was analyzed using the Folin-Ciocalteu reagent (FCR) method [31]. An aliquot (200 μ L) of extract was added to 1.5-mL FCR, which had been diluted 1 in 10 with distilled H₂O. The mixture was allowed to equilibrate for 5 minutes and then mixed with 1.5 mL of sodium carbonate solution (60 g/L). After incubation at room temperature for 90 minutes, the absorbance of the samples was read at 725 nm using the respective solvent as a blank. The results were expressed as milligram gallic acid equivalents (GAE) per gram of sample.

2.4. Bioaccessibility of baobab polyphenols

Samples from 6 baobab fruit extracts were analyzed using an in vitro digestion model adapted from Ryan et al [32]. Samples were frozen until analysis.

2.5. Measurement of sugar release

The relative glycemic impact of baobab fruit extract was measured to assess the effect of the baobab polyphenols on the inhibition of starch breakdown. Bread was used as the starch source. This was achieved by performing a dose response using different concentrations of baobab fruit extract baked into bread at 1.25%, 1.88%, 2.50%, 3.13%, and 3.75% and then subjecting samples to an in vitro digestion procedure and measuring the resultant reducing sugars released.

2.5.1. Bread preparation

White bread dough was made to a recipe as in Table 1. The dough was then baked in a Russell Hobbs bread maker (model no: 18036; Manchester, UK) for a total of 3 hours and 20 minutes. Samples of the bread were then prepared by weighing 2.5 g and placing each into 60-mL specimen pots. The pots were inserted into an aluminum heating block and covered with an insulating sheet in readiness for testing.

When adding the baobab fruit extract powder (sample 6, Baobab super fruit powder; Min Vita) to the white breads, the flour and water content were altered to keep the overall weight of the loaf at 500 g. Percentages were calculated based on the entire 500-g loaf.

2.5.2. In vitro digestion

The in vitro digestion procedure consisted of a simulated gastric digestion phase followed by an ileal digestion phase with timed sampling at the end of the gastric phase and during the ileal phase [33]. Samples were then incubated for 120 minutes with constant slow mixing, and aliquots were taken at 20, 60, and 120 minutes during ileal digestion. The tubes were centrifuged ($1000 \times \text{rpm}$, 2 minutes) in a Biofuge Primo Centrifuge (Heraeus Instruments; Kendro Laboratory Products, Langenselbold, Germany), and an aliquot of the supernatant was removed for analysis of reducing sugars.

2.5.3. Analysis of reducing sugars released during digestion Sugar released from the bread during digestion was measured by a colorimetric method adapted from Englyst and Hudson [34] designed to measure monosaccharides after an amyloglucosidase secondary digestion to complete depolymerisation of starch fragments. Absorbance was read at 530 nm on a Shimadzu UV-1201 spectrophotometer (Shimadzu Corporation, Rydalmere, Australia), and sugar release was measured in milligrams per gram of bread sample. Slowly digestible starch

Table 1–Ingredients used to make white bread with added baobab						
Ingredients/ weights	Baobab % used per g bread (500 g)	Baobab % used per g CHO				
190 g warm tap water 1 tbsp virgin olive oil 1 tsp salt 1 tbsp sugar 1 tbsp dried milk powder 1 ½ tsp yeast 350 g strong	1.25% 1.88% 2.50% 3.13% 3.75%	2.70% 4.07% 5.46% 6.87% 8.30%				
white flour						

Baobab extract was added into breads and water and flour contents were altered to keep overall loaf weights at 500 g after baking. Values are expressed as percentage per 500 g loaf. Values are also expressed as baobab percentage per gram of available carbohydrate. was extrapolated by subtracting the rapidly digestible starch (RDS) measurement at 20 minutes from the reducing sugars measurement at 120 minutes during ileal digestion [33]. Previous studies have shown that the in vitro analysis of RDS can be correlated to the GR in vivo Englyst et al [35].

In vivo analysis.

2.6. Subjects

The current study was a randomized, single-blind, repeatedmeasures design with volunteers fed 3 different test meals on 3 different days. Ten subjects were originally recruited based on the guidelines on measuring the GR by the Food and Agricultural Organization/World Health Organization (FAO/ WHO) [36] to take into account individual variations, with this subject number being the same as that used by Sanaka et al [37]. However, 1 subject dropped out due to personal reasons, and therefore, 9 healthy female subjects (25.3 \pm 4.8 years; height 1.66 \pm 0.05 m; weight 61.2 \pm 8.0 kg; body mass index 22.3 \pm 2.6 kg/m²; body fat 25.4% \pm 7.7%; values are means \pm SD) were recruited for the study by means of advertisements and personal communications. Before inclusion in the study, potential participants were briefed on all aspects of the experiment and were given the opportunity to ask questions. This was followed by a health assessment, which included anthropometric measurements and a health questionnaire (giving details of food allergies/intolerances, metabolic diseases, special dietary needs, and smoking habits). Those who fulfilled all the acceptable criteria (age 18-60 years; body mass index <30 kg/m²; blood pressure between 110 and 120/75 and 85 mm Hg; fasting blood glucose [BG] <6mmol/L; not on prescription medication; no genetic or metabolic diseases) were included in the study. On the day before each test, subjects were asked to restrict their intake of alcohol and caffeinecontaining drinks and to refrain from strenuous physical activity. To avoid any influence of the menstrual cycle on the outcome of the study, all subjects were tested at the same stage of their menstrual cycle.

The study was conducted at the Functional Food Centre at Oxford Brookes University. All participants gave written informed consent before starting, and the study was initiated after the approval by the Oxford Brookes University Research Ethics Committee according to the guidelines laid down in the Declaration of Helsinki. On each test day, subjects arrived between 7 and 9 AM on the morning after an overnight fasting (10-12 hours before testing time).

2.7. Test meal

The 3 test meals were a control (C), an LD solution of baobab fruit extract (LD) in 250 mL of water and an HD solution of baobab fruit extract (HD) in 250 mL of water (sample 6; Baobab super fruit powder). The control consisted of 132 g of white bread (Sainsbury's White Sliced loaf, London, UK) and 250-mL still water. The LD consisted of 123 g of white bread and 250mL still water with 18.5 g of baobab fruit extract added to make a drink (37% baobab per g available carbohydrate [CHO]) and the HD consisted of 114 g of white bread and 250-mL still water with 37 g of baobab fruit extract (74% baobab per g available CHO). Each of the 3 meals contained 50 g of available CHO, which was calculated for each test meal using the procedure from FAO/WHO [36], according to the nutrition information available from the bread label and the baobab suppliers.

2.8. Study design

Volunteers participated in a randomized, balanced, controlled cross-over study where they consumed the C, LD, and HD on separate days in a random order. On the day before testing, volunteers were asked to record their food intake and repeat it before subsequent tests.

2.9. Energy expenditure

On arrival in the laboratory, volunteers were asked to rest for 30 minutes in a supine position on a bed before baseline measurements of resting metabolic rate were taken. Resting metabolic rate was determined in the morning between 7 and 9 AM. Resting metabolic rate was measured at 1-minute intervals for 30 minutes under the ventilated hood indirect calorimetry system (Deltatrac II Metabolic Monitor; Datex-Ohmeda, Inc, Helsinki, Finland). The analyzer was calibrated on each test day with standardized gases containing 5% CO₂ and 95% O₂.

Diet-induced thermogenesis was determined after the breakfast meal for 15 minutes in every 30 minutes until 180 minutes [38]. The first 5 minutes of every 15-minute period was discarded to allow for stabilization within the Deltatrac hood, and the average of the remaining 10 minutes was used. This period was recommended to be appropriate to measure the DIT [38]. Diet-induced thermogenesis was calculated as the increase in EE per minute above premeal values for 3 hours after meal intake. Energy expenditure and fat oxidation were calculated using the equations of Lusk [39].

2.10. Glycemic response

The protocol used to measure the BG response was adopted from that described by Brouns et al [40] and is in line with procedures recommended by FAO/WHO [36]. Blood was obtained by finger prick using the Unistick 3 single-use lancing device (Owen Mumford, Woodstock, UK). Before a finger prick, subjects were encouraged to warm their hand to increase blood flow. To minimize plasma dilution, fingertips were not squeezed to extract blood but were instead gently massaged starting from the base of the hand moving toward the tips. The first 2 drops of expressed blood were discarded, and the next drop was used for testing.

Blood glucose was measured using the HemoCue 201+ Glucose analyzer (HemoCue Ltd, Dronfield, UK). The HemoCue is a reliable method of BG analysis [41]. Fasting blood samples were taken at –5 and 0 minute, and the test food was consumed immediately afterwards within 15 minutes at a comfortable pace. Further blood samples were then taken at 15, 30, 45, 60, 90, 120, 150, and 180 minutes after consuming the test meal.

The change in GR was calculated by computing the difference between the BG concentration at a time point and mean baseline BG concentration (based on 2 baseline values taken 5 min apart). Because it represented the relative increment in the GR at any time point compared with the

baseline value, it was this change in GR that was used for all further analyses, including incremental area under the curve (IAUC) calculated using the trapezoidal rule [40,42], BG response curve construction, and statistics.

2.11. Satiety

One hundred-millimeter continuous line visual analog scales (VAS) were used to measure subjective feelings of hunger, fullness, desire to eat, and prospective food consumption. The volunteers provided VAS data at baseline (0 minute) and at 30, 60, 90, 120, 150, and 180 minutes after the commencement of eating the test food and after lunch. The specific questions asked were, "How hungry do you feel?", "How full do you feel?", "How strong is your desire to eat?", and "How much food do you think you can eat?"

2.12. Statistical analyses

The in vitro analysis experiments were carried out 3 times with a minimum of 3 replicates of each sample. Comparisons between samples were carried out by an analysis of variance (ANOVA) and Tukey's multiple comparison test, and statistical analysis was performed using the SPSS version 17 (SPSS, Chicago, IL). Results were expressed as the means \pm SEM, and significance was set at P < .05.

For in vivo testing, statistical analysis was performed using SPSS (version 20.0), and data and figures were processed in Microsoft Excel spread sheet (2006, Reading, UK). The IAUC was determined for BG, total DIT, and fat oxidation using the trapezoidal rule for values above the baseline. The relative increment in the GR and EE at any time point compared with the baseline value was used to assess the differences at each time. The differences were assessed using a 3-factor repeated-measures ANOVA with differences between the meal assessed using contrasts within the ANOVA. Significance was set at P < .05. Values are presented as means \pm SD.

3. Results

In vitro.

3.1. Antioxidant and polyphenol content

Baobab samples 3, 4, and 6 had a significantly (P < .05) greater antioxidant and polyphenol content than samples 1, 2, and 5, as measured by both FRAP and FCR (Table 2), respectively. The FRAP and FCR baseline values were shown to be positively correlated (P < .01).

3.2. Polyphenol bioaccessibility

Polyphenol content as measured by the FCR method significantly increased throughout digestion in all samples (P < .05; Table 3) compared with the baseline polyphenol content.

3.3. Sugar release

The addition of baobab to white bread at 1.88%, 3.13%, and 3.75% significantly (P < .05) reduced sugar release compared

Table 2 - FRAP and FCR values for baobab samples 1 to 6 at baseline, before in vitro digestion FRAP (µmol/L)^a Baobab FCR (mg GAE/g)^a 1 1755.07 ± 28.30 24.94 ± 0.32 2 1666.79 ± 23.51 21.85 ± 0.27 3 2093.76 ± 61.38 27.58 ± 0.20 4 2121.56 ± 44.80 26.95 ± 0.26* 5 1844.02 ± 43.69 24.68 ± 0.33 28.85 ± 0.47 * 6 2167.68 ± 52.89

Samples 1 to 5 were donated from different geographical locations in Africa. Baobab sample 6 is a commercially available extract (Baobab super fruit powder).

^a Values reported in micromoles per liter relative to ferrous sulphate (1000 μ mol/L) and GAE per gram of sample. Values represent means ± SEM of 3 independent experiments; samples 3, 4 and 6 were significantly higher in antioxidant content and polyphenols than samples 1, 2, and 5. * P < .05.

with the control white bread at 20 and 60 minutes into intestinal digestion (Fig. 1). There was no significant difference in the slowly digestible starch calculated values between any samples.

In vivo.

3.4. Glycemic response

The GR showed an initial rise in BG following the test meal to peak at 45 minutes for the 2 baobab doses and at 60 minutes for the control. All 3 tests reached their nadir at 180 minutes (Fig. 2).

The GR IAUC was significantly different between the 3 meals after 180, 120, and 60 minutes (P < .05), with the C meal having the greatest GR (Table 4). In the first 60 minutes, the HD had the lowest GR, whereas over the entire 180 minutes, LD attenuated the GR the most.

3.5. Energy expenditure

There were no significant differences in resting EE between the 3 test days (C, 0.880 ± 0.087 kcal/min; 18.5 g, 0.897 ± 0.066 kcal/min; 37 g, 0.872 ± 0.084 kcal/min) with all 3 days having similar baseline measurements.

Energy expenditure increased postprandially following each of the 3 test meals. There were no significant differences in EE following the 3 meals with total DIT being similar for the 3 meals (C, 13.75 ± 6.51 kcal; LD, 11.25 ± 5.91 kcal; HD, 13.42 ± 6.22 kcal).

3.6. Satiety

There was no significant difference between the visual analog scores for any of the satiety parameters (hunger: C, 567 ± 437 ; 18.5 g, 550 ± 411 ; 37 g, 650 ± 363 mm/min; fullness: C, 689 ± 404 ; 18.5 g, 750 ± 424 ; 37 g, 653 ± 362 mm/min; desire to eat: C, 430 ± 184 ; 18.5 g, 578 ± 413 ; 37 g, 670 ± 339 mm/min; prospective consumption: C, 409 ± 452 ; 18.5 g, 361 ± 345 ; 37 g, 567 ± 328 mm/min).

4. Discussion

4.1. Polyphenol content and bioaccessibility

Antioxidant content was measured using FRAP, and the high antioxidant potential was in agreement with previous studies, which used various methods of antioxidant detection [1,10-13]. All baobab fruit extracts were shown to be good sources of polyphenols, with the commercial extract (sample 6) being the most concentrated polyphenol extract out of the total 6 extracts. The donated extracts (1-5) were kept in clear plastic bags, which may have been subjected to more air and light oxidation than the commercial extract, which was in a solid dark container. Therefore, some of the polyphenols in the clear bags may have been destroyed before analysis. The antioxidant content of fruits and fruit extracts has been shown to be correlated with the amount of total polyphenols [43,44], and this was also found in the current study. It has previously been found that the bioaccessibility of phenolics increases throughout digestion [45,46]. The bioaccessibility of polyphenols in the current study increased throughout the digestive process, with approximately a 3-fold increase in polyphenol concentration between the baseline and duodenal phase.

4.2. Starch breakdown and sugar release from bread

In this study, there was a significant reduction in sugar release at 20 and 60 minutes into the duodenal phase of digestion in the 1.88% and also the higher concentrated baobab white breads (excluding 2.5%), compared with control white breads. Although polyphenols have been shown to reduce sugar release from starch-rich foods [16,17], other studies have shown an increase in starch digestibility with added polyphenols [47]. There are a few hypotheses that may account for the decrease observed in the baobab breads. Polymeric polyphenols are usually not absorbed to any significant extent,

Table 3 – Bioaccessibility of the polyphenol content after the gastric and duodenal phases of digestion				
Baobab	Gastric (%)	Duodenal (%)		
1	270.8	330.6		
2	293.0	361.2		
3	222.5	274.5		
4	239.7	292.0		
5	252.2	299.4		
6	223.9	269.1		

Values are expressed as percentage increase from baseline and were measured using an in vitro digestion procedure followed by the Folin-Ciocalteu method described in the "Methods and materials" section.

Samples 1 to 5 are baobab extract samples donated from different geographical locations in Africa. Baobab sample 6 is the purchased extract (Baobab super fruit powder).

Values are results from 3 independent experiments (n = 3). All samples showed a significant (P < .05) increase in bioaccessibility of polyphenols at the gastric and duodenal phase compared with the baseline.



Fig. 1 – Sugar release in milligrams per gram of bread sample for each bread containing different concentrations of baobab extract, expressed as percentages at each stage of digestion. Percentages of baobab were calculated with baobab extract added into white bread to make a total loaf weight of 500 g. Each colored bar represents different stages of digestion, with 20, 60, and 120 minutes representing the different intestinal stages of digestion. Twenty minutes into intestinal digestion is considered the RDS phase when there is a rapid increase in sugar release. Slowly digestible starch can be calculated from subtracting the sugar release at 20 minutes from the sugar release at 120 minutes and is considered the low glycemic sugar release. *P < .05 denotes significant decrease in sugar release compared with control white bread (no added baobab extract). Values represent means ± SEM of 3 independent experiments (n = 3).

and thus, they have potential to bind to starch molecules in foods, slowing the rate of starch breakdown [48]. Baobab is rich in compounds such as high-molecular-weight tannins [15], which may be interfering with starch degradation. The polyphenols in the extract may be inhibiting digestive enzymes such as α -amylase and α -glucosidase, thereby preventing the breakdown of starch [49,50]. However, the exact polyphenol composition of the baobab fruit is currently unknown, and further work is required to understand how these polyphenols may be exerting their effect.



Fig. 2 – The change in blood glucose response from baseline (expressed in millimoles per liter) following the consumption of white bread in conjunction with 250-mL water (C) or with 18.5-g baobab or 37-g baobab made up into solutions with 250 mL of water, to equal quantities (50 g) of available carbohydrate. Finger prick blood samples were taken at 15-minute intervals for the first hour and then every 30 minutes for the last 2 hours, following the consumption of the test meal. Data are given as means ± SD (n = 9).

Table 4–Glycemic response area under the curve after consuming 3 test meals					
GR (mmol/min/L)	Control	18.5 g baobab	37 g baobab		
GR AUC 60	86.1 ± 46.6	72.4 ± 44.4	59.6 ± 35.6 [*]		
GR AUC 120	175.3 ± 80.4	142.6 ± 76.7 *	135.8 ± 74.6 [*]		
GR AUC 180	237.4 ± 104.9	188.1 ± 114.4 *	193.1 ± 104.3		

Area under the curve at 60, 120, and 180 minutes for each test meal providing 50 g of available carbohydrate. This was a randomized cross-over design in 9 healthy females, with each participant serving as her own control (n = 9). The 3 test meals consisted of the control white bread and 250-mL water, white bread, and 18.5-g baobab (LD) in 250-mL water and white bread and 37 g baobab (HD) in 250-mL water.

* P < .05 denotes significant decrease in GR compared with control at each time point (an ANOVA was performed followed by a Tukey's test). Values represent means ± SD.

The soluble fiber in the extract may also be contributing to a reduction in sugar release, as fiber has continuously been shown to reduce postprandial glycemia [42,51-54]. In the current study, soluble fiber from baobab may have formed a viscous matrix reducing the rate of starch breakdown and sugar release into solution. However, Thondre et al [55] found that polyphenols, not soluble fiber, may be the main contributing factors to reduced GR in carbohydrate-rich foods such as oats. Oats were shown to have a significant amount of polyphenols, with a negative correlation between polyphenol content and RDS (-0.743). However, contrary to what was once thought, there was no correlation between fiber content and the digestibility of the starch in oats. This is a unique study in that it was the polyphenols, not the fiber content of the oats that reduced sugar release. The reduction in sugar release after consumption of baobab fruit extract in the current study may, therefore, be largely due to the polyphenols in the extract.

4.3. Glycemic response, satiety, and DIT

The consumption of the LD and HD baobab fruit extract drinks significantly reduced the GR to white bread. The HD baobab meal stabilized the BG peaks more over the 3-hour period compared with the other 2 meals. What may be more important than lowering overall IAUC may be preventing the large spikes in BG 30 to 45 minutes after a meal [56]. Both the fiber and the polyphenol content of the concentrated baobab extract may be contributing to the reduction in GR in vivo.

Satiety is dependent on many aspects of food intake, including the change in the GR, nutrient composition, hormonal changes, and gastric emptying rates. In the current study, baobab fruit extract had no effect on satiety measures. The glucostatic theory states that the change in BG levels between meals has an effect on hunger [57]. Therefore, any food that can stabilize BG levels, such as seen in the HD meal, may reduce hunger and thus prove beneficial in weight management. Taking this into account and also that the fiber content was increased in the HD baobab meal, it is of interest that there was no corresponding increase in satiety as shown in previous studies [25,26]. In the current study, there were the limitations of a low subject number for measuring satiety [58] and VAS were used as a measure of subjective

hunger and they may not always reflect actual subsequent food intake [59]. Therefore, an ad libitum test meal may have been a more accurate reflection of satiety; however, given the desire to measure DIT and GR for 180 minutes postprandially, this was not deemed possible as by 180 minutes any differences in satiety would not be visible.

There was no significant difference between the control meal and the LD or HD meal on postprandial EE. Macronutrients have a hierarchy for increasing postprandial EE, alcohol, and protein increasing DIT more than carbohydrate and fat [60]. In the current study, available CHO was kept constant between groups, and neither white bread nor baobab is a major source of protein and/or fat; therefore, macronutrient content between groups was not largely different. However, there was a large increase in the amount of fiber being consumed as baobab content increased. As mentioned, although fiber-rich foods have previously been shown to reduce the GR and increase satiety, the findings on DIT are more controversial with both increases and decreases in DIT demonstrated [25,26]. Polyphenols found in green tea, such as epigallocatechin-gallate, and other catechins have been shown to increase 24-hour EE [61]. However, Dulloo et al [61] analyzed polyphenols in isolation of other macronutrients, and therefore, more studies are needed to be done on whole polyphenol-rich extracts and total EE.

In conclusion, this is a novel study as no other study to date has looked at the effect of exogenously added baobab fruit extract on sugar release, satiety, or DIT. The baobab fruit is a rich source of bioaccessible polyphenols, and the current study shows the potential of baobab for reducing the GR to carbohydrate-rich foods both in vitro and in vivo. This is in good correlation with the results from previous studies. However, there was no effect of baobab fruit on satiety or on DIT in either the LD or HD meal. Apart from the use of VAS and the low subject number, there are some other limitations to the methods used in this study. Future studies will need to determine the individual polyphenols present in the extracts to identify which polyphenols or group of polyphenols may be eliciting the effects on GR. Furthermore, an enzyme inhibition assay should be performed to determine the effect of baobab fruit on digestive enzyme activity. Finally, further human studies on GR would be essential for determining the optimal dose of baobab fruit extract in reducing postprandial glycemia.

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REFERENCES

Lamien-Meda A, Lamien CE, Compaore MMY, Meda RNT, Kiendrebeogo M, Zeba B, et al. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 2008;13:581–94.

- [2] Diop AG, Sakho M, Dornier M, Cisse M, Reynes M. The African baobab (Adansonia digitata L.): key features and uses. Fruits 2006;61:55–69.
- [3] Gebauer J, El-Siddig K, Ebert G. Baobab (Adansonia digitata L.): a review on a multipurpose tree with a promising future in the Sudan. Gartenb 2002;67:155–60.
- [4] Nour AA, Magboul BI, Kheiri NH. Chemical composition of baobab fruit (Adansonia digitata L.). Trop Sci 1980;22:383–8.
- [5] De Caluwe E, Halamova K, Van Damme P. Adansonia digitata L. A review of traditional uses, phytochemistry and pharmacology. Afrika Foc 2010;23:11–51.
- [6] Osman MA. Chemical and nutrient analysis of baobab (Adansonia digitata) fruit and seed protein solubility. Plant Foods Hum Nutr 2004;59:29–33.
- [7] Oyelek GO, Salam MA, Adetoro RO. Some aspects of nutrient analysis of seed, pulp and oil of baobab (Adansonia digitata L.). J Env Sci Tox Food Tech 2012;1:32–5.
- [8] Arnold TH, Well MJ, Wehmeyer AS. Koisan food plants: taxa with potential for economic exploitation. In: Wickens GE, Goodin JR, Field DV, editors. Plants for Arid Lands. London: Allen & Unwin; 1985. p. 69–86.
- [9] Magaia T, Uamusse A, Sjohol I, Skog K. Dietary fiber, organic acids and minerals in selected wild edible fruits of Mozambique. Springer-Plus 2013;2:88.
- [10] Besco E, Braccioli E, Vertuani S, Ziosi P, Brazzo F, Bruni R, et al. The use of photochemiluminescence for the measurement of the integral antioxidant capacity of baobab products. Food Chem 2007;102:1352–6.
- [11] Blomhoff R, Carlsen M, Halvorsen B, Holte K, Bohn S, Dragland S, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutr J 2010;9:3.
- [12] Gruenwald J. Novel botanical ingredients for beverages. Clin Dermatol 2009;27:210–6.
- [13] Vertuani S, Braccioli E, Buzzoni V, Manfredini S. Antioxidant capacity of Adansonia digitata fruit pulp and leaves. Acta Phytoth 2002:5.
- [14] Mulaudzi RB, Ndhlala AR, Kulkarni MG, Finnie JF, Staden JV. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. J Ethnopharmacol 2011;135:330–7.
- [15] Ghani A, Agbejule AO. A pharmacognostic study of the fruits of Adansonia digitata L. The State of Medicinal Plant Research in Nigeria Ife. Nigeria: University of Ife; 1986. p. 181–5.
- [16] Deshpande SS, Salunke DK. Interactions of tannic acid and catechin with legume starches. J Food Sci 1982;47:2080–1.
- [17] Yoon JH, Thompson LU, Jenkins DJA. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. Am J Clin Nutr 1983;38:835–42.
- [18] Kim JH, Kang MJ, Choi HN, Jeong SM, Lee YM, Kim JI. Quercetin attenuates fasting and postprandial hyperglycemia in animal models of diabetes mellitus. Nutr Res Pract 2011;5:107–11.
- [19] Wedick NM, Pan A, Cassidy A, Rimm EB, Sampson L, Rosner B, et al. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. Am J Clin Nutr 2012;95:925–33.
- [20] Liu J, Wang M, Peng S, Zhang G. Effect of green tea catechins on the postprandial glycemic response to starches differing in amylose content. J Agric Food Chem 2011;59:4582–8.
- [21] Rojo LE, Ribnicky D, Logendra S, Poulev A, Rojas-Silva P, Kuhn P, et al. In vitro and in vivo anti-diabetic effects of anthocyanins from Maqui Berry (Aristotelia chilensis). Food Chem 2012;131:387–96.
- [22] Torronen R, McDougall GJ, Dobson G, Stewart D, Hellstrom J, Mattila P, et al. Fortification of blackcurrant juice with crowberry: impact on polyphenol composition, urinary phenolic metabolites, and postprandial glycemic response in healthy subjects. J Func Food 2012;4:746–56.
- [23] Tanko Y, Yerima M, Mahdi MA, Yaro AH, Musa KY, Mohammed A. Hypoglycemic activity of methanolic stem

bark of Adansonia digitata extract on blood glucose levels of streptozocin-induced diabetic Wistar rats. Inter J App Res Nat Prod 2008;1:32–6.

- [24] Bolton RP, Heaton KW, Burroughs LF. The role of dietary fiber in satiety, glucose, and insulin: studies with fruit and fruit juice. J Clin Nutr 1981;34:211–7.
- [25] Gruendel S, Garcia AL, Otto B, Mueller C, Steiniger J, Weickert MO, et al. Carob pulp preparation rich in insoluble dietary fiber and polyphenols enhances lipid oxidation and lowers postprandial acylated ghrelin in humans. J Nutr 2006;136: 1533–8.
- [26] Raben A, Christensen NJ, Madsen J, Holst JJ, Astrup A. Decreased postprandial thermogenesis and fat oxidation but increased fullness after a high fiber meal compared with a low fiber meal. Am J Clin Nutr 1994;59:136–9.
- [27] Cavallero A, Empilli S, Brighenti F, Stanca AM. High (1-3,1-4)-β-glucan barley fractions in bread making and their effects on human glycaemic response. J Cer Sci 2002;36:59–66.
- [28] Thondre PS, Henry CJK. High-molecular-weight barley βglucan in chapatis (unleavened Indian flatbread) lowers glycemic index. Nutr Res 2009;29:480–6.
- [29] Harada U, Chikama A, Saito S, Takase H, Nagao T, Hase T, et al. Effects of the long term ingestion of tea catechins on energy expenditure and dietary fat oxidation in healthy subjects. J Health Sci 2005;51:248–52.
- [30] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70–6.
- [31] Sharma P, Gujral HS. Antioxidant and polyphenol oxidase activity of germinated barley and its milling fractions. Food Chem 2010;120:673–8.
- [32] Ryan L, O'Connell O, O'Sullivan L, O'Brien NM. Micellarisation of carotenoids from raw and cooked vegetables. Plant Foods Hum Nutr 2008;63:127–33.
- [33] Mishra S, Monro JA, Hedderley D. Effect of processing on slowly digestible starch and resistant starch in potato. Starch 2008;60:500–7.
- [34] Englyst HN, Hudson GJ. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. Food Chem 1987;24:63–76.
- [35] Englyst HN, Veenstra J, Hudson GJ. Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. Br J Nutr 1996;75:327–37.
- [36] FAO/WHO. Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation. FAO Food Nutr Pap 1998;66:1–140.
- [37] Sanaka M, Yamamoto T, Anjiki H, Nagasawa K, Kuyama Y. Effects of agar and pectin on gastric emptying and postprandial glycaemic profiles in healthy human volunteers. Clin Exp Pharmacol Physiol 2007;34:1151–5.
- [38] Reed GW, Hill JO. Measuring the thermic effect of food. Am J Clin Nutr 1996;63:164–9.
- [39] Lusk G. The elements of the science of nutrition. WB Saunders Co; 1928.
- [40] Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, et al. Glycemic index methodology. Nutr Res Rev 2005;18:145–71.
- [41] Stork AD, Kemperman H, Erkelens DW, Veneman TF. Comparison of the accuracy of the HemoCue glucose analyser with the Yellow Springs Instrument glucose oxidase analyser, particularly in hypoglycemia. Eur J Endocrinol 2005;153: 275–81.
- [42] Wolever TMS. Glycaemic index and disease. Wallingford: CABI; 2006.
- [43] Soon YY, Barlow PJ. Antioxidant activity and phenolic content of selected fruit seeds. Food Chem 2004;88:411–7.
- [44] Tzulker R, Glazer I, Bar-Ilan I, Holland D, Avriam M, Amir R. Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates

prepared from 29 different pomegranate accessions. J Agric Food Chem 2007;55:9559–70.

- [45] Ryan L, Prescott SL. Stability of the antioxidant capacity of twenty five commercially available fruit juices subjected to an *in vitro* digestion. Inter J Food Sci Tech 2010;45:1191–7.
- [46] Wootton-Beard PC, Moran A, Ryan L. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. Food Res Int 2011;44:217–24.
- [47] Wu Y, Chen Z, Li X, Li M. Effect of tea polyphenols on the retrogradation of rice starch. Food Res Int 2009;42:221–5.
- [48] Gonthier MP, Donovan JL, Texier O, Felgines C, Remesy C, Scalbert A. Metabolism of dietary procyanidins in rats. Free Rad Bio Med 2003;35:837–44.
- [49] McDougall GJ, Stewart D. The inhibitory effects of berry polyphenols on digestive enzymes. Biofactors 2005;23: 189–95.
- [50] Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of α-glucosidase and α-amylase by flavonoids. J Nutr Sci Vitaminol 2006;52:149–53.
- [51] Brennan CS. Dietary fibre, glycaemic response, and diabetes. Mol Nutr Food Res 2005;49:560–70.
- [52] Slaughter SL, Ellis PR, Jackson EC, Butterworth PJ. The effect of guar galactomannan and water availability during hydrothermal processing on the hydrolysis of starch catalysed by pancreatic α -amylase. Biochimica Et Biophysica 2002;1571:55–63.

- [53] Kwong MG, Wolever TMS, Brummer Y, Tosh SM. Attenuation of glycemic responses by oat β-glucan solutions and viscoelastic gels is dependent on molecular weight distribution. Food & Func 2013;4:401–8.
- [54] Vosloo MC. Some factors affecting the digestion of glycemic carbohydrates and the blood glucose response. J Fam Ecol Cons Sci 2005;33:1–9.
- [55] Thondre PS, Ryan L, Henry CJK. Influence of polyphenols on in vitro starch digestibility of oat porridges. Proc Nutr Soc 2011:70.
- [56] Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes 2005;54:1–7.
- [57] Mayer J. Glucostatic mechanisms of regulation of food intake. New England Med Cent Bull 1953;14:43.
- [58] Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Eila M, et al. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Br J Nutr 2000;84: 405–15.
- [59] Ranawana V, Henry CJK. Liquid and solid carbohydrate foods: comparative effects on glycemic and insulin responses, and satiety. Int J Food Sci Nutr 2011;62:71–81.
- [60] Westerterp KR. Diet induced thermogenesis. Nutr Metab 2004:1.
- [61] Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, et al. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. Am J Clin Nutr 1999;70:1040–5.