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Impact of resistant starch in three plantain (Musa AAB) products on glycaemic response of healthy volunteers

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Abstract

Background and aim of the study: Plantains can be eaten in various forms providing a good opportunity to study the effect of starch type on glycaemic response, and so 3 products differing in their types of available carbohydrate and contents of resistant starch were tested. **Methods:** Boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC), ripe raw plantain (RRP) and white bread as reference (all 25 g available carbohydrate portion) were given to 10 pre-screened healthy individuals. Postprandial glycaemic responses and glycaemic indices (GI) were measured. **Results:** Peak blood glucose for BUP, BUPC and RRP was at 45, 45 and 30 min post-meal time respectively. The peak blood glucose concentrations for BUP, BUPC and RRP (1.8 ± 0.8 , 2.3 ± 0.8 , 1.9 ± 0.7 mmol/L, n = 10 respectively) reflected the in vitro quantities/types of rapidly available glucose (RAG) in the samples. On the other hand, **mean GI \pm SEM values obtained for the test products (BUP = 44.9 ± 3.6 , BUPC = 55.0 ± 4.2 , RRP = 38 ± 4.4 , n = 10) were neither significantly different nor directly correlated with RAG.** **Conclusions:** The results show a potential link between rapidly available glucose and glycaemic index, but the correlation is confounded by the presence of other constituents in the plantains.

Keywords

Plantain, glycaemic index, glycaemic response, resistant starch, available carbohydrates

Abbreviations

Boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC), ripe raw plantain (RRP)
glycaemic index (GI) rapidly available glucose (RAG)

Introduction

Diets consisting of low glycaemic index foods reduce the risk of diabetes and heart disease according to a meta-analysis of 45 publications [1]. A low glycaemic index may be due to low amounts of carbohydrate, the presence of other substances which inhibit carbohydrate digestion, or the presence of difficult to digest forms of carbohydrate such as resistant starch. Because of the high costs of conducting in vivo GI tests, in vitro tests to determine the amount of resistant starch and other dietary starch fractions have been carried out and used to estimate the glycaemic index (GI) of foods. However, in vitro starch digestion data are not always reliable in the accurate prediction of GI values of food [2].

GI is food specific because it compares equal weights of available carbohydrate and therefore is a unique measure of biological quality or property of the available carbohydrates in the food rather than a property of the food in itself [3, 4]. GI is based on glycaemic response which measures the net result of a sequence of chemical and biological processes between food ingestion and release of glucose into the peripheral blood stream [5]. Foods are generally classified as low (≤ 55), medium (56 - 69) and high GI (≥ 70) [6].

The basis for the determination of GI has been to compare the same quantity of available carbohydrates in a food to the reference food (usually glucose or white bread). This is because the

same quantity of available carbohydrates in different foods can produce a very different glycaemic response [5]. The argument by Monro et al [7] that GI is a function beyond just the quantity of the available carbohydrates is logical because the type of available carbohydrates is also very important. This is supported by an earlier report by Englyst et al [8] that GI is correlated with the quantity of rapidly available glucose (RAG), which precludes slowly digestible starch (SDS). The implication of this is that the GI of a food with higher quantity of slowly available glucose (SAG) in its available carbohydrates is likely to be lower than the one with a higher quantity of rapidly available glucose (RAG).

Digestible starch in food usually produces lower blood glucose response than an equivalent carbohydrate load of glucose, but major differences in blood glucose response are observed with starch in different foods leading to wide variation in GI values between foods and food types [9]. It is, however, still unclear why starch in some foods e.g. boiled potatoes causes a glycaemic response that is similar to that of glucose while starch in white bread with the same type of available carbohydrate (rapidly digestible starch) produces a lower glycaemic response than glucose. Many reasons have been suggested, such as the presence of fibre, resistant starch, enzyme inhibitors, lipids, etc [4]. It is easier to study the impact of some of these substances when they are added to foods, but when they are present within the food matrix it may be difficult to measure their impact if the foods are not fully characterised. Even in the case of well characterised products, when there are many interfering components, it may be difficult to measure the relative impact of each component as well as how component interactions affect the overall blood glucose response.

Starch fractions in foods have different impacts on blood glucose response and subsequently GI. Rapidly digestible starch is known to induce a rapid increase of blood glucose and insulin levels after food ingestion, whereas slowly digestible starch prolongs the release of glucose, consequently helping to lower GI. On the other hand, resistant starch reduces starch availability for digestion thus lowering blood glucose response [10, 11].

Green plantain is one of the few sources of granular resistant starch [12, 13] and has been reported as a low glycaemic index food [14, 15]; however, it is still unclear if the presence of resistant starch alone, or the type of resistant starch, is the reason for its low GI. In the present study, we have examined three different types of available carbohydrates in plantain foods and how the GI varies with respect to resistant starch components within the food matrix.

Materials and methods

Materials

Cultivars of ripe and unripe plantains from Columbia were purchased from a Leeds city market. Unripe plantains selected were full green (stage 2) while ripe plantains used were in the fully ripe stage (yellow) in colour (stage 6) on the plantain colour index scale prepared by Aurore et al [16].

Preparation test products

Unripe boiled plantain

One finger of unripe plantain was cut into five portions of ~ 300 g each and cooked in slightly salted and pre-boiled water for 20 min. The cooked plantain portions were allowed to cool down (for ~10 min) to room temperature before weighing and serving. The boiled unripe plantains were freshly cooked before each test to avoid starch retrogradation. The presence of retrograded resistant starch (RS3) was checked in freshly cooked plantain foods as outlined below.

Unripe boiled plantain crisps

Unripe plantain crisps were prepared by cooking unripe plantain as described above. After cooling to room temperature, they were cut into thin slices of about 2 mm thickness with a kitchen knife and subsequently freeze-dried.

Raw ripe plantain

Ripe plantain samples were peeled and served without cooking.

Reference food: white wheat bread

White bread used for the study was purchased from Morrisons supermarket, Leeds, UK, on the morning of each test day. The GI values obtained were however calibrated against glucose by dividing by a value of 1.4 [4, 17].

Chemical analysis and characterisation of test meals

Determination of available carbohydrates

Available carbohydrate was determined and calculated as available starch (RDS + SDS) + total free sugars (glucose + fructose + sucrose) using the method described by Englyst and Englyst [18]. Fructose, glucose and sucrose in the ripe and unripe samples were determined by extraction of sample in 80% ethanol at boiling and 50% ethanol at 80°C, respectively, and subsequent estimation using HPLC with Evaporative Light Scattering Detection.

Determination of resistant starch

Resistant starch types 1 and 2 (RS1 and RS2) were analysed in the test samples by the method of Englyst and Englyst [18]. For the determination of resistant starch type 3, one plantain finger was cut into four portions and cooked for 20 min. Retrograded starch was determined in cooked unripe plantain at time 0, 10, 20 and 30 min after cooking. Each portion was cut into thin slices, quenched in liquid nitrogen and subsequently freeze-dried. For each analysis tube, 15 ml of deionised water was added to 500 mg of freeze-dried sample. Starch digestion was carried out with pancreatin and amyloglucosidase using the method described by Englyst and Englyst [18] and glucose was subsequently measured using a glucose oxidase peroxidase kit (Megazyme International, Ireland). The quantity of retrograded starch (RS3) was estimated using the equation below.

$$RS3 = [G120_0 - G120_x] \times 0.9$$

Where $G120_0$ is the digestible starch for samples at time 0 and $G120_x$ is the digestible starch at time x (where x = 10, 20 and 30 min). Retrograded starch was also determined in freshly cooked plantain at 10, 20 and 30 min.

Total resistant starch content was subsequently estimated by addition of the three types of resistant starch determined.

Subjects

Ten pre-screened healthy subjects (using a pre-screening questionnaire and anthropometry measurements) were selected for the study. Inclusion criteria were: healthy male or female of any ethnicity, 20 to 40 years of age, body mass index (BMI) < 30 kg/m², not on medication, not diabetic, not pregnant or lactating mother, not allergic to any of the test products and/or their constituents. The ethics committee of the Faculty of Mathematics and Physical sciences, University of Leeds gave the approval for the study (MEEC 11-048) and all volunteers gave their written consent.

Study design

Subjects were allowed to eat their normal diet the day before participating in the test but were to avoid unusual and/or strenuous exercise, drinking alcohol and smoking [17]. They arrived at the School of Food Science & Nutrition, University of Leeds between 8 and 8.45 am on each test day. All meals were served as breakfast in random order after an overnight fast of 10-14 h with a two-day washout period between tests [17]. All subjects consumed all three test foods once and the reference food twice (25 g available carbohydrate portions). The meals were served with water (200 ml) and were consumed over a 10 min period [4]. A 25 g available carbohydrate portion was used for GI testing because initial tests carried out revealed that it is difficult for some participants to consume 50 g available carbohydrate portions of cooked unripe plantain foods within 15 min. This is due the texture and taste of boiled unripe plantain. Unripe plantain has a coarse dry texture and does not have sweet taste due to very low sugar content (Table 1). In a typical African meal, it is usually consumed with condiments to enhance palatability, but because our interest was in the GI of the characterised product, it was presented without condiments. Subjects remained seated throughout the experimental session. Capillary blood samples were collected during the fasting state and at 15, 30, 45, 60, 90 and 120 min after consumption of the test meal, for analysis of glucose.

Sampling and analysis

Capillary blood samples were taken using accu-chek fastclix finger pricker in the fasting state and at 15, 30, 45, 60, 90, and 120 min after each meal. One drop of blood was placed on a test strip for immediate analysis of glucose with an Accu-Chek® Aviva Nano Blood Glucose Meter for glucose measurement [19-21]. The incremental areas under the blood glucose curves were calculated geometrically using Microsoft excel. The GI was estimated by expressing the glycaemic response area for the test foods as a percentage of the mean response area of the reference food taken by the same subjects [22]. Information from the Accu-check aviva manual for blood glucose measurement used in our study reveals that the meter, which measures the glucose concentration in whole blood using a test strip containing glucose dehydrogenase, was calibrated using hexokinase giving a detection limit is 0.6 mmol/L and system measurement range is 0.6 - 33.3 mmol/L. A CV of 3.0% was obtained for repeatability (within series) while a CV of 1.8% was obtained for reproducibility (day-to-day).

Statistical analysis of data

The mean + standard deviation of each subject's repeated reference food was calculated. The area under the curve (AUC) for each food taken by each subject was expressed as a percentage of the mean AUC for the reference food (white bread) taken by the same subject. The mean of the resulting values (from individuals) divided by 1.4 was the food GI. The data obtained was subjected to the analysis of variance (ANOVA). The significant differences between food GI and the food blood glucose responses at time 15 - 120 min were determined on an SPSS statistics software using the Tukey's b post hoc multiple comparison test and further confirmation using Duncan's test.

Results

The composition of plantain products studied is shown in Tables 1- 4. Tables 2 - 4 present the RS, available carbohydrates and RAG composition in the food portion size tested (25g available carbohydrates) to give a clearer representation of their observed effects on GI and glycaemic response.

RS content was 2.0 ± 0.9 and 20.8 ± 1.9 for BUP and RRP respectively, no RS was detected in BUPC (Table 2). Mean glycaemic indices \pm SEM of the tested products for the healthy individuals were 44.9 ± 3.6 , 55.0 ± 4.2 , 38 ± 4.4 , for BUP, BUPC and RRP respectively and were not significantly different at 95% confidence level (Table 3). Table 4 indicates that although samples have the same quantity of available carbohydrates, the type/component analysis is different reveal different.

The blood glucose response curves after consumption of white bread (WB), boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP) are shown in Figure 1. There was a sharp rise in blood glucose concentration, rapid appearance (peak at 45 min post meal) and fast decline glucose for BUPC. Although BUPC has a postprandial peak of glucose at the same time as WB (45 min), after this time, blood glucose response on consumption of BUPC declines more rapidly than WB.

BUPC does not contain resistant starch (Table 2), and is the only sample with the same blood glucose response value and blood glucose response time as bread (Figure 1 and Table 3). Although BUP has the same peak time of 45 min, it elicits a lower blood glucose response than that of WB and BUPC (Figure 1 and Table 3). Ripe raw plantain (RRP) elicits a significantly lower glucose response between 45 and 120 min post meal time. At 15 min post-meal stage, RRP has the highest blood glucose response but this was not significantly higher ($p = 0.05$) than those of WB and BUPC.

In Figure 2, a positive correlation ($r^2 = 0.76$, $p \leq 0.5$) was obtained by plotting RAG values against GI values. Results presented in Table 3 indicate that RAG values for tested plantain products were

significantly different from each other ($p \leq 0.5$), however GI values obtained for BUP were not significantly different from that of BUPC and RRP (Table 3).

Discussion

The main purpose of the study was to test impact of resistant starch on the glycaemic response in healthy individuals using three commonly consumed plantain products with various levels of resistant starch. BUP is the most common form of consuming unripe plantain in Nigeria because of its claim to be effective for the management of type 2 diabetes, from anecdotal studies. BUPC was prepared to check the GI of plantain in the absence of all types of resistant starch in plantain i.e. RS1, RS2, and RS3. This product was prepared as a follow up to test product 1 which still contains some quantity of RS1 (see Table 2). No form of resistant starch was detected in the unripe plantain crisps we prepared for the study. RRP contains a significant quantity of resistant starch, even though its consumption is not as common as the consumption of the cooked form or the consumption of bananas. This is probably the reason why the GI of ripe plantain has been mostly unreported. We are therefore reporting for the first time the GI of completely gelatinised green plantain, boiled plantain crisps and ripe raw plantain.

The sharp rise in blood glucose concentration, the rapid appearance (peak at 45 min post meal) and fast decline glucose for BUPC appears to reflect a rapid breakdown of starch into glucose and fast absorption of the glucose released. Although BUPC has a postprandial peak of glucose at the same time as WB (45 min), after this time, blood glucose response on consumption of BUPC declines more rapidly than WB.

BUPC does not contain resistant starch (Table 2), and is the only sample with the same blood glucose response value and blood glucose response time as bread (Figure 1 and Table 3). Although BUP has the same peak time of 45 min, it elicits a lower blood glucose response than that of WB and BUPC (Figure 1 and Table 3). This can be attributed to a significantly lower quantity of rapidly available glucose (Table 3).

Although the available carbohydrates in WB, BUP and BUPC are composed mainly of digestible starch (Table 4), the presence of resistant starch (RS1) and slowly digestible starch in significantly higher quantities in BUP (Tables 2 and 3) appears to be responsible for its lower glucose response at all times when compared with WB and BUPC [23]. Slowly digestible starches and physically inaccessible resistant starch (RS1) are expected to produce lower glycaemic responses [11, 24 - 25] as we have observed here.

The shape of the glucose response curve for RRP is similar to that obtained for sucrose [4]. However, a lower glycaemic index (38.5 ± 4.4 , $n = 10$, mean \pm SEM) for RRP when compared to that of sucrose (~ 65) raises some questions. It appears that the presence of resistant starch type 2 (determined in vitro) in high quantity (10.7 ± 1.3 g/100 g WWB) and in an equivalent quantity to sucrose (10.8 ± 0.8 g/100 g WWB) in RRP is responsible for this observation. This observation indicates the presence of free sugars in high quantity constituting 97% of available carbohydrates in the sample (Table 4).

Englyst et al [24] had earlier proposed that the quantity of rapidly available glucose (RAG) was a good predictor of GI in a study involving twenty-three cereal products. The high positive correlation ($r^2 = 0.76$, $p \leq 0.5$) obtained by plotting RAG values against GI values is in accord with that reported by Englyst et al. In addition, peak glycaemic responses for test products were not significantly different from each other despite the significant variations in quantities of RAG. The type of RAG may therefore be more significant than the quantity of RAG in a food. Only RRP has a different type of RAG (mainly sucrose and glucose) and this was reflected in its earlier peak time of 30 min. It can therefore be concluded that the time of attaining the blood glucose response peak is a function of the type of RAG while the peak height is a function of quantity of the RAG.

The GI of ripe raw plantain has been mostly unreported while that of banana is common because plantain is preferentially eaten cooked because of its higher starch content. The GI of uncooked ripe plantain obtained here (38.5 ± 4.4 , $n = 10$, mean \pm SEM) is lower than an average GI of 52 ± 4 obtained from ten studies on banana [26]. A higher GI value obtained for banana may be due to higher quantity of sugar in banana than plantain. A high level of resistant starch in the RRP (20.8 ± 1.9 , $n = 3$, - Table 2) appears to be a possible reason for the observed low GI. Nevertheless, this is not

conclusive because of the presence of other food components in the food matrix such as fibre [27], polyphenols [28] and cell wall and/or components [29]. Further investigations on the possible reasons for the observed low GI of ripe raw plantain are therefore required.

The mechanism of enzyme resistance of each type of resistant starch is important in considering how glycaemic index and glucose metabolism are affected. The presence of RS1, which is a consequence of an “intact” form of food, is known to slow down the availability of starch to digestive enzymes, leading to a slow increase in postprandial blood glucose response as for SDS and may be more important in lowering the GI of a food than RS2 and RS3 [25, 11]. It is therefore necessary to try to establish the relationship, if any, between GI and each type of resistant starch (i.e. RS1, RS2 and RS3) rather than between GI and total RS.

CONCLUSION

Overall, amongst the samples studied, boiled unripe plantain (BUP) appears to be the best in terms of glycaemic control because it exhibits a slow and gradual rise in blood glucose response as well as a gradual decline (Figure 1). In addition, the GI values and blood glucose response curves of the three products reveal that a low GI food may not necessarily have an extended glucose profile or a sustained glucose response.

The presence of resistant starch and slowly digestible starch reduces plantain glycaemic index but the low values of plantain glycaemic index cannot be attributed solely to the contributions from resistant and/or slowly digestible starches. The equivalent quantity of available carbohydrates from the same source (as we observed for three different plantain products) may not elicit the same glycaemic index or glycaemic response and confirms the fact that glycaemic index is a property of the quality of available carbohydrates in the food. However, this may not be entirely true because glycaemic index is also dependent on the ability of the food to release the available carbohydrates within its matrix into circulation as glucose and this is can be affected by other food components. The contribution of other food components to the low glycaemic index of plantain therefore needs to be further investigated.

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Figure Captions

Fig 1 Blood glucose increments (responses) after ingestion of 25g available carbohydrate portion of white bread (WB), boiled unripe plantain (BUP), boiled unripe plantain crisps (BUPC) and ripe raw plantain (RRP)

Values plotted are means \pm SEMs of 10 subjects at time 0, 15, 30, 45, 60, 90 and 120 min. Significant differences (at 95% confidence levels) in blood glucose response values between samples for each time are indicated by letter annotations beside the data points

Fig 2 Correlation between glycaemic index and rapidly available glucose

Values plotted are means \pm SEMs of food GI obtained from 10 subjects and triplicate determinations of food RAG values in vitro

Table 1: Starch, sugar, moisture and available carbohydrate contents of plantain products and white bread

	BUP^a (g/100 g WWB)	BUPC^a (g/100 g DWB)	RRP^a (g/100 g WWB)	WB^a (g/100 g WWB)
Total starch	21.7±1.4	72.4±1.7	12.1±1.2	47.5±1.3
Total sugars	1.7±0.2	5.5±0.5	14.9±1.5	2.6±0.6
Glucose	0.1±0.0	0.3±0.1	2.0±0.1	ND
Fructose	0.1±0.2	0.4±0.1	1.9±0.1	ND
Sucrose	1.4±0.2	4.8±0.5	10.8±0.8	2.6±0.6
Total RS	1.6±0.8	ND	11.4±1.9	ND
Total CHO^a	23.4±1.2	78.2±1.3	27.0±1.3	49.8±1.6
Moisture	69.2±1.8	1.3±0.5	60.3±2.1	50.2±1.8
Available CHO	19.8±0.8	77.9±0.5	15.3±1.1	49.2±2.1
Food portion size (25 g AV CHO)	125±2.1	32 ±1.5	163±2.6	50.3±2.5

a. BUP = Boiled unripe plantain, BUPC = Boiled unripe plantain crisps, RRP = Ripe raw plantain, WB = white bread, ND = not detected, WWB = wet weight basis, DWB = dry weight basis, Total CHO = total carbohydrate determined i.e. starch and sugars. Results are expressed as mean ± standard deviation, n= 3

Table 2: Resistant starch content of plantain products (g/ 25g available carbohydrate food portion size)

	RS1	RS2	RS3	TOTAL RS
BUP	2.0±0.9	ND	ND	2.0±0.9
BUPC	ND	ND	ND	ND
RRP	3.4±1.2	17.4±2.3	0.0	20.8±1.9

Table gives breakdown of types and quantities of resistant starch present in the tested plantain products. ND = not detected, RS1 = inaccessible starch, RS2 = native resistant starch, RS3 = retrograded starch. Results represent quantities in 25 g available carbohydrate food portion sizes (Table 1) as served and are expressed as means ± standard deviations, n = 3

Table 3: Peak blood glucose response, peak time, rapidly available glucose and glycaemic index values for reference and test foods

	Peak blood glucose response (mmol/L)	Peak Time (min)	Rapidly available glucose (RAG) g/portion	Glycaemic Index
White bread (WB)	2.3±0.2 ^a	45	24.5±0.7 ^c	71.4 ^c
Boiled Unripe plantain (BUP)	1.8±0.3 ^a	45	15±1.1 ^b	44.9 ^{ab}
Boiled plantain crisps (BUPC)	2.3±0.3 ^a	45	24.9±0.2 ^c	55.0 ^b
Ripe raw plantain (RRP)	1.9 ±0.2 ^a	30	12.9±0.4 ^a	38.5 ^a

Peak blood glucose responses after ingestion 25g available carbohydrate portion of each test meal and reference are means ± SEMs of 10 subjects at time while RAG values are means ± standard deviations of triplicate in vitro determinations for each test food. Values with the same superscript in the same column are not significantly different at 95% confidence level.

Table 4: Available carbohydrate components of plantain products and white bread (g/ 25g available carbohydrate food portion size)

	BUP	BUPC	RRP	WB
Fructose	0.1±0.2 ^a	0.1±0.0 ^a	3.1±0.1 ^b	0.0±0.0 ^a
Glucose	0.1±0.0 ^a	0.1±0.1 ^a	3.3±0.1 ^b	0.0±0.0 ^a
Sucrose	1.9±0.2 ^a	1.5±0.2 ^a	17.6±1.2 ^b	1.2±0.4 ^a
RDS	12.9±1.5 ^b	23.2±0.5 ^c	0.8±0.3 ^a	23.9±0.8 ^c
SDS	9.8±0.7 ^b	0.0±0.1 ^a	0.2±0.3 ^a	0.1±0.2 ^a
Major AV CHO	RDS	RDS	sucrose	RDS
% of major AV CHO	52±3	93±1	71±1	96±2

Table gives a breakdown of the types and quantities of available carbohydrates (AV CHO) present in the tested plantain products. RDS= rapidly digestible starch, SDS = slowly digestible starch. Results represent quantities in 25 g available carbohydrate food portion sizes (Table 1) as served and are expressed as means ± standard deviations, n= 3. Values with the same superscript in the same row are not significantly different at 95% confidence level.

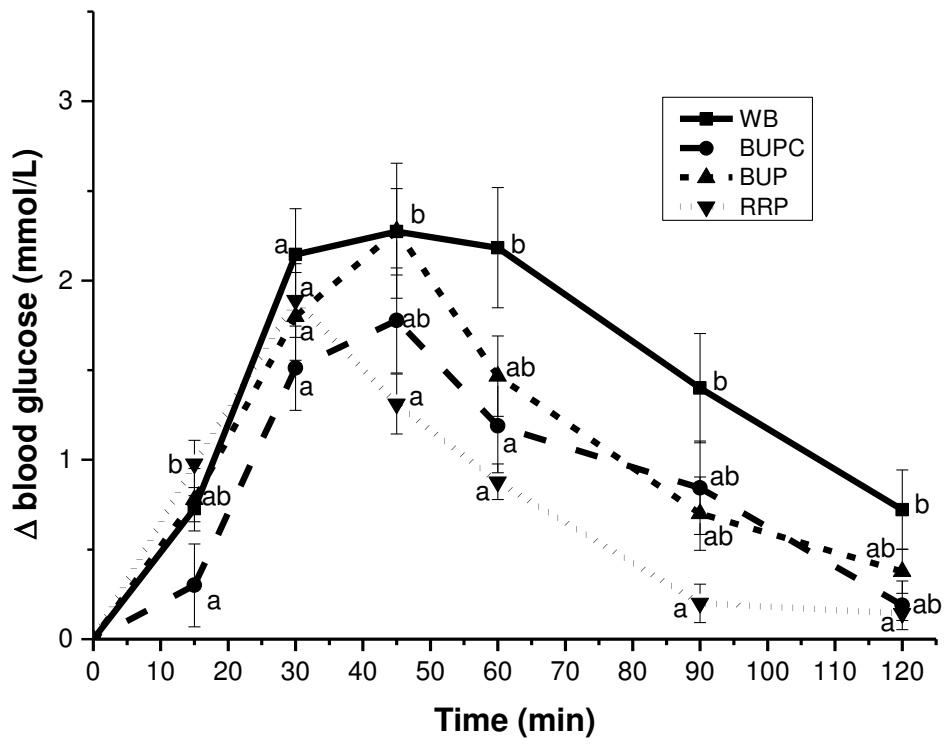


Fig 1

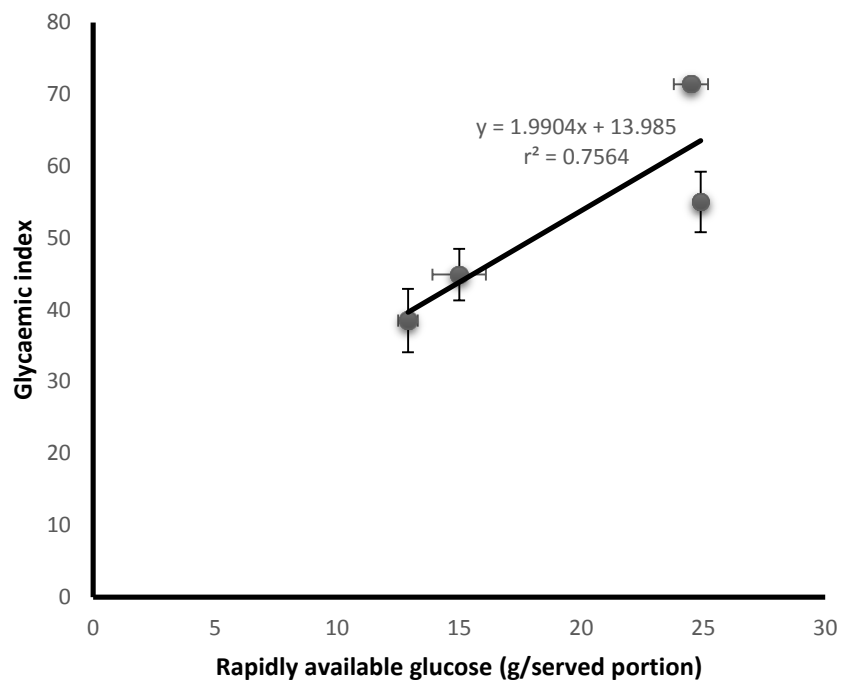


Fig 2