# WHEAT FLOUR BASED BISCUITS SUPPLEMENTED WITH MUSHROOM, PLANTAIN, SOY BEAN AND MAHOGANY BEAN MODULATED BLOOD GLUCOSE RESPONSE ON NORMOGLYCAEMIC ADULTS

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## ABSTRACT

**Background:** Readily available high-fiber, low glycaemic index local foods like mushrooms could help in reducing the global burden of diabetes.

**Objective:** This study compared the blood glucose response of normoglycaemic adults fed formulated wheat flour based biscuits supplemented separately with four common food samples.

**Materials and Methods:** Using traditional methods, the food items**:** mushroom (*Pleurotus tuber regium* fruiting body), plantain (*Musa paradisiaca*), soyabean (*Glycine max*), and mahogany bean (*Afzelia africana*) used to supplement wheat in this study were processed and blended into composite flour (containing 50g available carbohydrate): Mushroom and wheat flour (MW), plantain and wheat flour (PW), soybean and wheat flour (SW), and mahogany bean and wheat flour (MBW). Date palm (*Phoenix dactylifera*) was used as a sweetener. Proximate analysis of the formulated biscuits was done using standard method. Twenty and twelve healthy adult human subjects participated in the organoleptic and blood glucose response study respectively. Statistical analysis was done using analyses of variance (ANOVA) at $p<0.05$ and Duncans new multiple range test.

**Results**: Compared to other three biscuits, the biscuit MW had the highest crude fiber and the least carbohydrate and fat contents. Of the four biscuits, PW had the best organoleptic acceptability. The MW biscuit significantly lowered the blood glucose when compared to SW ($P<0.05$, 0.0054) and MBW ($p<0.05$, 0.002) after 120 minutes. However there was no significant difference in the blood glucose response between MW and PW ($p>0.05$, 0.0677). The mean AUC was lowest for MW and PW.

**Conclusion:** The incorporation of mushroom flour to wheat flour in this study, improved the nutrient content of the biscuits without significantly increasing the blood glucose response. This makes it suitable for diabetic patients. Also the addition of mushroom in biscuit has a greater potential in overcoming protein-calorie malnutrition.

**Keywords:** Biscuits, supplementation, mushroom, blood glucose

## INTRODUCTION

Considerable interest has been raised on the effects of various carbohydrate containing foods on postprandial blood glucose response. Processed, easily digestible, and quickly absorbable high caloric meals can lead to exaggerated postprandial elevations in blood glucose and triglycerides and can increase severity of type 2 diabetes mellitus (1,2). Low glycaemic diets have been associated with decreased risk of cardiovascular disease, type 2 diabetes, metabolic syndrome, stroke, depression, chronic kidney disease, formation of gall stones, neural tube defects, formation of uterine fibroids, and cancers of the breast, colon, prostate, and pancreas (3). Snacks like biscuits, usually produced from cereal flours (mainly wheat) are consumed extensively all over the world for nutrient availability, palatability, compactness and convenience. In Nigeria like many other developing countries, increasing urbanization, and the attendant rise in the numbers of working mothers, has contributed greatly to the consumption of snack foods such as biscuits. Wheat flour constitutes the basic ingredient for biscuit production because of its gluten proteins which are not present in flours of other cereals (4). At present the consumption of biscuit is very high in Nigeria and thus necessitate the need for the use of composite wheat flour supplementation for baking. *Detarium microcarpium* supplemented bread fed to non- insulin dependent diabetes mellitus (NIDDM) human subjects (5), unripe plantain incorporated feeds fed to diabetic rats (6) and diet supplementation with *Glycine max* (soy bean) (7,8) have been shown to have beneficial effect on the improvement of blood glucose control. The aqueous extract of bark of *Afzelia africana* commonly called Mahogany bean, possesses hypolycemic and haematopoietic properties

(9). Dates fruits are used in great variety of ways on cereal pudding, bread, cakes, cookies, ice cream or candy bars, juice, vine-gar, wine, beer, syrup, honey, pickle, paste, and food flavor (10). Date fruit pulp aside being high in dietary fiber (6.4 to 11.5%) (11) is being used as a sugar source on breakfast cereal and baked goods (12).Its reported low glycemic index (13), may be of benefit in glycemic control in non diabetics. Edible mushrooms have been valued throughout the world as both food and medicine for thousands of years. They have good taste, appetizing aroma, and nutrient contents which is low in calories, high in minerals, essential amino acids, vitamins and fibers (14). It has been reported that for centuries, mushrooms have been prescribed for treatment of diseases such as gastro –intestinal disorder, bleeding, high blood pressure and various bacterial infections (15). Common mushrooms belong to the following genera *:Lentinus, Termitomyces* and *Pleurotus* (16). The aim of this study is to produce biscuit from composite wheat flour supplemented with various blends of plantain, soybean, mahogany beans, and mushroom flour using date palm flour as sweetener and to determine their proximate composition and sensory properties and blood glucose response on normoglycemic adult subjects.

## MATERIALS AND METHOD

### Samples collection

The specie *Pleurotus tuber regium* fruiting body cultivated mushroom was collected from Faculty of Agriculture farm, University of Port Harcourt, Rivers state. Plantain *(Musa paradisiaca)*, soy bean *(Glycine max)*, mahogany beans (*Afzelia africa na*) and wheat flour was purchased at Ogbete market, Enugu, Nigeria. Date palm (*Phoenix dactylifera*) was procured at Modo Lawal market Bauchi, Nigeria.

### Processing of mushroom (*Pleurotus tuber regium* fruiting body) flour

The mushroom was sorted, destalked, washed and dried in a hot air oven at 55ºC - 60ºC for 24 hours and ground into mushroom flour with milling machine and sieved (0.25mm) (17). Flour was packaged in air tight container and kept in refrigerator for later use.

### Processing of Plantain into flour

Mature unripe plantain fingers were washed, peeled, sliced and dried in an oven at 60°C for 24 hours. Dried plantain slices was milled into flour using hammer mill, sieved (0.25mm) and packed in a polythene bag (18).

### Processing of Soy bean into flour

Soy bean flour was processed according to the methods of Oluwamukomi *et al*,(19). Soy bean (*Glycine max*) was sorted, washed and boiled in water at 100ºC for 30 minutes. It was dehulled manually, water drained off, oven dried at 100ºC - 120ºC for 3 – 4 hours, milled in a disc attrition mill and sieved (0.25mm), the flour was obtained and then kept in an airtight container until ready for use.

### Processing of Mahogany beans into flour

The seeds of *Afzelia africana* (Mahogany bean) was sorted, decapped, and cleaned to dislodge dust, foreign matter and stones. The seeds were roasted for 3-5minutes to aid dehulling. The dehulled seeds were soaked for 24 hours to ferment, water drained off, oven dried at 60ºC for 4 hours and milled into flours, sieved (1.0mm) and packaged (20).

### Processing of Date palm

The seeds of the date palm fruits was removed and discarded, sorted, washed and the pulp (pericarp) was oven dried at 45°C for 8 hours and subsequently milled, sieved and packaged (11).

### Proximate analysis of formulated biscuits

The crude protein, fat, moisture, ash and crude fibre contents of the formulated biscuits were determined according to (21).

### Determination of carbohydrate

The total carbohydrate was determined by subtracting the percentage (%) of fat (F), % crude protein (Cp), % moisture (M) and % ash content (A) from 100 (22), % Total carbohydrates =100- (F+Cp+M+A)%.

### Determination of crude protein

This was determined by a modification of the Kjeidahl gunnings procedure for organic nitrogen. The nitrogen of protein and other compounds were converted to ammonium sulphate by acid digestion with boiling sulphuric acid. A known weight of sample was placed in Kjeldahl flask and about 200 milligram of catalyst mixture was added. 10.0cm$^2$ of concentrated sulphuric acid was added to the content of the flask. Heat was applied gently for few minutes until frothing ceases and the heat was increased to digest for 3 hours. It was allowed to cool and made to a known volume with distilled water (100cm$^3$). 10.0cm$^3$ aliquot of the dilute solution of the digest was distilled by pipetting the volume into distillation chamber of micro Kjeldhal distillation apparatus. 10.0cm$^3$ of 40% sodium hydroxide solution was added and steam distilled into 10.0cm$^3$ of 2% boric acid containing mixed indicator (note colour from red-green) titrate with standard 0.01N or 0.2N hydrochloric acid to grey end point.

$$\% N = \frac{(a-b) \ x\, 0.01\, x\, 14.0057\, x\, c}{d\ x\ e} x\, 100$$

a = titre value for the sample
b = titre value for the blank
c = Volume to which digest is made up with distilled water
d = Aliquot taken for distillation
c = Weight of dried sample (mg)

Convertion to % crude protein, was multiplied by necessary conversion factor (6.25).

**Determination of fat**

The method used was that of exhaustive soxhlet extraction using the non-polar organic solvent-petroleum ether (B.p = 40°C to 60°C). A soxhlet extractor with reflux condenser and a small flask which has been previously dried in the oven and weighed were fitted, 2gm of sample was weighed and transferred to a fat-free extraction thimble, it was plugged lightly with cotton wool, the thimble was placed in the extractor and about $150cm^3$ of petroleum ether (B.P. $60\text{-}80^0C$) was added into the flask until it siphons over once. More ether was added until the barrel of the 100ml extractor was half full, the condenser was replaced, the joints were tight and placed on the water bath or electrothermal heating mantle. The source of heat was adjusted so that the ether boils gently, it was left to siphon over for at least 8 hours, until the ether was just short of siphoning over, the flask was detached and the contents of the barrel of the extractor was siphoned into the ether stock bottle. It was well drained, and the thimble removed and dried in the oven. The condenser was replaced and distilling the ether continued until the flask was practically dried. The flask was detached (which now contains all the oil), the exterior was cleaned and dried in the oven to constant weight. The extracted residue was kept for the "fibre" determination.

$$Ether\ extracts = \frac{Wt\ of\ oil}{Wt\ of\ bio\log ical\ material}\ x\ 100$$

**Moisture**

The water content was determined by weighing out 2.5g into silica dish, which has been previously dried and weighed. The dish including the sample inside it was placed in hot air oven for 24 hours at $60^0\text{-}70^0C$ (drying at high temperature may result in losses of heat labile or volatile component). It was finally dried to constant weight, cooled for ten minutes in a dessicator each time before weighing.

$$\%\ Moisture = \frac{W_1 - W_2}{W_1}\ x\,100$$

$W_1$ = Weight of biological material before drying

$W_2$ = Weight of biological material after drying

**Ash determination**

This was done using the muffle furnace method in accordance with (21). Porcelain crucibles with lids was dried for 15 minutes in a hot air oven at 105°C, cooled in a desiccator and weighed. Two grams of each sample was separately weighed into the appropriately labeled crucible and weighed again. Crucibles and contents were ignited in muffle furnace at 550°C for 10 hours to light gray ash. Thereafter, sample was removed and placed immediately in a dessicator to cool and the weight was taken. The difference in mass or loss in mass of the crucible and samples before ashing is the organic matter content of each sample. The difference between the mass of the crucibles alone and crucible plus ash gave the mass of ash of each sample. Values for ash was calculated and expressed in percentages.

% ash = <u>Weight of ash x 100</u>

Weight of samples

**Determination of crude fibre**

Fiber content of the sample was measured using the enzyme modified, neutral detergent fiber (NDF) method. Dried samples whose fat content was extracted using Soxhlet extraction approach was treated with standard NDF procedures up to the point that fiber containing residues was filtered and washed with water. The filtered residues were incubated with a porcine *á* – amylase solution at 37°C overnight. The residues were filtered after incubation, washed very well and dried. The NDF was calculated as filtered residual:

Fibre = residual weight – (weight of protein + ash)

**Available carbohydrate determination**

Available carbohydrate was determined using AOAC (23) method.

**Metabolic study**

Twelve volunteers were recruited through announcements in Nutrition and Dietetics Department University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu after prior approval by the Ethics and Research committee, University of Nigeria Teaching Hospital, Ituku / Ozalla, Enugu, Nigeria. Exclusion criteria included any known disease, abnormal glucose tolerance (fasting plasma glucose of 7.8 mmol/l or higher or a random plasma glucose concentration of 11.1 mmol/l or higher and/or > 11.1 mmol/l after 2 hours or more on oral glucose tolerance testing (24), the use of any medication that may affect blood glucose tolerance and a body mass index greater than 28 $kg/m^2$ (25). Subjects were requested to maintain their usual daily food intake and activity throughout the study period. Two groups (A and B) consisting of 6 healthy volunteers each were used in this study. After consent was given by the subjects, a random finger prick blood glucose test was taken to ensure normal glucose tolerance. The anthropometric measurements: weight (kg) and height (m) of each subjects were taken and the body mass index ($kg/m^{2)}$ was determined.

**Weight measurement**

The weight of the participants was measured with a Hanson bathroom scale graduated in kilograms with the capacity of 120kg. Measurements were done in minimum indoor clothing. Participants were asked to remove their heavy outer garments (jacket and coat) and shoes, and were made to empty their pockets. The participant stood in the centre of the platform with both arms at the sides, head back and knees erect, weight distributed evenly to both feet. The weight was recorded to the nearest kilogram.

**Height measurement**

Participants were asked to remove their shoes, heavy outer garments, and hair ornaments. The participant was asked to stand with his/her back to the height rule. The back of the head, back, buttocks, calves and heels were touching the upright, feet together. The participants were asked to look straight. The head piece of the stadiometer or the sliding part of the measuring rod was lowered so that the hair (if present) is pressed flat. Height was recorded to the nearest centimeter.

**Formulation of blends**

Biscuits were developed from four blends of composite flours of mushroom and wheat flour; plantain and wheat flour; soy bean and wheat flour and mahogany beans and wheat flour as indicated in Table 1. The four blends of composite flour contained 50 g available carbohydrate per serving from appropriate weight containing available carbohydrate equivalent of 30 g from wheat flour, 10 g from each of the four selected foods (mushroom, plantain, soy bean and mahogany bean) and 10 g from date palm flour used as sweetener. The weight of food item containing the required available carbohydrates contribution for each of the test foods based on 50 g available carbohydrate per meal was calculated as follows:

Weight of food item containing available carbohydrate equivalent $= \frac{100A}{B}$

Where:

A = Required available carbohydrate contribution by food item to food item-wheat composite flour

B=available carbohydrate content of food item as determined

The sequential steps in the preparation of the mushroom biscuits was the same for the four blends as outlined below:

1. Weigh all ingredients.
2. Sieve flour.
3. Vegetable fat and date palm were mixed together then other dry ingredients added.
4. Water was added gradually.
5. Knead into stiff meal dough.
6. Roll out on a rolling board and cut to desired shape using biscuit cutter.
7. Cuts was placed in greased pans.
8. Baked for about 20 minutes at oven temperature of 180°C

**Table 1: Flour blends and ingredients for biscuit production**

| Blend | Wheat Flour(g) | Food Item(g) | Date palm Flour(g) | Water(Ml) | Fat(g) | Baking powder(g) | Salt(g) | Weight of dough(g) | Weight of dough(g) |
|---|---|---|---|---|---|---|---|---|---|
| MW | 87 | 31 | 30 | 50 | 20 | 2 | 0.1 | 196 | 162 |
| PW | 87 | 30 | 30 | 56 | 20 | 2 | 0.1 | 186 | 168 |
| SW | 87 | 36 | 30 | 46 | 20 | 2 | 0.1 | 188 | 171 |
| MBW | 87 | 31 | 30 | 45 | 20 | 2 | 0.1 | 173 | 169 |

Key: MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat
SW = Biscuit based on soybean and wheat, MBW = Biscuit based on mahogany bean and wheat

**Sensory evaluation of food products**

Sensory evaluation of the four different biscuit products (MW, PW, SW and MBW) was done as reported by Giwa, *et al* (26). A 9 point hedonic scale was used in scoring the products where: 9 represented like extremely, 8 represented like very much, 7 represented like moderately, 6 represented like slightly, 5 represented neither like nor dislike, 4 represented dislike slightly, 3 represented dislike moderately, 2 represented dislike very much and 1 represented dislike extremely. The evaluated sensory properties included: colour, texture, taste, aroma and overall acceptability (27).

**Study design**

The study took place over 2 week period. Every morning, Tuesday to Friday, six subjects (per day) were studied. Each subject participated in the study twice a week for a two week period.

**Test foods**

The foods that were tested included four biscuits with the code MW (mushroom and wheat), PW (plantain and wheat) SW (soy bean and wheat) and MBW (mahogany bean and wheat). The test meals were prepared in the Department of Nutrition and Dietetics, University of Nigeria, Teaching Hospital Ituku / Ozalla, Enugu. Each test meal contained 50g available carbohydrate (CHO) and were consumed with 250 ml water. The 50g available carbohydrate content of each test meal was calculated from the result of available carbohydrate determination of the selected food samples.

**Procedures of blood sampling**

Samples were collected for two days per week, six of the twelve subjects per day participated in the study on that specific day. All subjects' hands were washed with soap and water before testing. Fasting blood sample was taken by finger pricking on separate occasions in the morning at time 0 (8.00am) after 10-12 hours overnight fast and the finger was gently squeezed and drop of blood was applied to the test strip on the blood glucose meter (25,28). The subjects were requested to consume the test foods (MW, PW, SW and MBW) containing 50g of available carbohydrate with 250ml plain water within 10 - 15 minutes after the fasting blood sample. Further blood samples were taken at 30, 60, 90 and 120 minutes after initial intake (29). The blood samples obtained were analysed using EZ Smart 608 blood glucose meter, (Tyson Bioresearch, Incorporated, Taiwan). The area under the curve (AUC) for a given time period for the subject was calculated using the blood glucose response after consumption of the formulated biscuits as described below in accordance with the trapezoid method recommended by Wolever (30).

**Table2: Protocol for the Ingestion of test meals**

| | Week1 | | | Week2 | | |
|---|---|---|---|---|---|---|
| Days | Group | Test meal | Number of subjects | Group | Test meal | Number of subjects |
| 1 | A | MW | 6 | A | SW | 6 |
| 2 | B | MW | 6 | B | SW | 6 |
| 3 | A | PW | 6 | A | MBW | 6 |
| 4 | B | PW | 6 | B | MBW | 6 |

Key: MW = Mushroom and wheat, PW = Plantain and wheat, SW = Soy bean and wheat, MBW = Mahogany bean and wheat

**Calculation of the area under the curve (AUC) for subject 1 using the blood glucose response after consumption of the formulated biscuit MW.**

Area under the curve (AUC) for a given time period can be calculated as described below in accordance with the method recommended by Wolever (30):

**Table 3: Sample blood glucose responses of subject 1 after consumption of the formulated biscuit MW**

| **Time(minute)** | 0 | 30 | 60 | 90 | 120 |
|---|---|---|---|---|---|
| **Blood glucose(mmol/l)** | 4.8 | 6.6 | 7.4 | 6.1 | 6.1 |

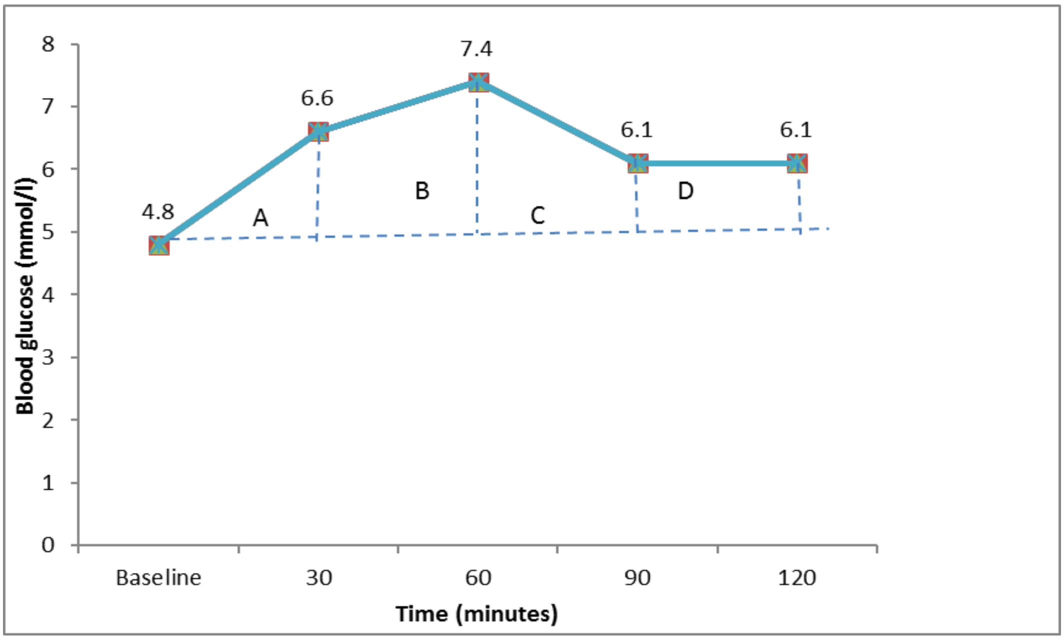


**Figure.1**: Blood glucose responses of subject 1 after consumption of formulated biscuit MW

The total AUC for subject 1 for the above example will be the sum of periods A+B+C+D
Triangle A = (start concentration + end concentration) x ½ time (minute) – baseline area
= (4.8+6.6)x15-(4.8x30)
= 27 mmol.min/l
Trapezoid B = (6.6+7.4)x15-(4.8x30)
= 66 mmol.min/l
Trapezoid C = (7.4+6.1)x15-(4.8x30)
= 58.5 mmol.min/l
Trapezoid D = (6.1+6.1)x15-(4.8x30)
= 39 mmol.min/l
Therefore, the AUC for subject 1 for the 2-hour period after consumption of the biscuit MW =
27 + 66 + 58.5 + 39 = 190.5 mmol.min/l

**Statistical analysis**
Data was analysed using statistical product and service solution (SPSS) version 21 for means, standard deviation and SEM. Analysis of variance (ANOVA) was used to compare the means, Duncan's new multiple range test was used to separate the means. $P < 0.05$ was set to be significant.

**RESULTS**
Table 4 shows the available carbohydrate content of selected food samples. The table showed that mushroom flour (MF) had 32.26 g available carbohydrate, plantain flour (PF) had 32.97 g, soy bean flour (SW) had 27.98 g, mahogany bean flour (MBF) had 32.55 g, wheat flour (WF) had 34.62 g and date palm flour (DF) had 33.04 g.

**Table 4: Available carbohydrate content of selected food samples per 100g**

| **Food samples:** | **MF** | **PF** | **SF** | **MBF** | **WF** | **DF** |
|---|---|---|---|---|---|---|
| **Mean*** | 32.2 | 32.97 | 27.98 | 32.55 | 34.62 | 33.04 |
| **Standard deviation*** | 0.42 | 0.16 | 0.76 | 0.28 | 0.42 | 0.38 |

*Mean and Standard deviation of three determinations
Key: MF = Mushroom flour; PF = Plantain flour, SF = Soy bean flour, MBF = Mahogany bean flour, WF = Wheat flour, DF = Date palm flour

Table 5 shows the proximate composition of formulated biscuits. The moisture content of the biscuits ranged from 5.89±0.38 to 11.22±0.19. MBW (11.22±0.19) was significantly (P≤0.05) higher compared to MW (6.33±0.29), PW (5.89±0.38) and SW (9.22±0.79). The fat content ranged from 12.11±0.19 to 13.78±0.19. SW (13.78±0.19) and MBW (13.44±0.20) were significantly (P≤0.05) higher compared to PW (12.89±0.19) and MW (12.11±0.19). Protein content ranged from 9.65±0.25 to 11.64±0.97. SW (11.64±0.97) was significantly (P≤0.05) higher compared to PW (10.38±0.38), MW (10.21±0.24), and MBW(9.65±0.25). The ash content of the biscuits was similar, MW (1.83±0.29) > PW (1.56±0.20) > SW (1.44±0.20) >MBW (1.35±0.04) which is not significantly different (P>0.05). Crude fibre content of the biscuits ranged from 6.57±0.20 to 16.67±0.34. MW (16.67±0.34) and PW (16.22±0.39) were significantly (P≤0.05) higher compared to SW (7.22±0.19) and MBW (6.57±0.20). The carbohydrate content ranged from 52.85±0.75 to 57.78±0.63. MBW (57.78±0.63) and SW (56.70±1.14) were significantly (P≤0.05) higher compared to PW (53.06±1.35) and MW (52.85±0.75).

**Table 5: Proximate composition of formulated biscuits per 100g**

| **Nutrients** | **MW** | **PW** | **SW** | **MBW** |
|---|---|---|---|---|
| Moisture (g) | 6.33±0.29 | 5.89±0.38 | 9.22±0.79 | 11.22±0.19 |
| Fat (g) | 12.11±0.19 | 12.89±0.19 | 13.78±0.19 | 13.44±0.20 |
| Protein (g) | 10.21±0.24 | 10.38±0.38 | 11.64±0.97 | 9.65±0.25 |
| Ash (g) | 1.83±0.89 | 1.56±0.20 | 1.44±0.20 | 1.34±0.04 |
| Crude fibre (g) | 16.67±0.34 | 16.22±0.39 | 7.22±0.19 | 6.57±0.20 |
| Carbohydrate (g) | 52.85±0.75 | 53.06±1.35 | 56.70±1.14 | 57.78±0.63 |

Mean ± Standard deviation of three determinations
Key: MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat
SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

Table 6 shows the sensory properties of the formulated biscuits. The colour of the biscuits differed. It ranged from 5.70±1.90 to 5.95±1.73. The biscuit SW (5.95±1.73) was preferred compared to MW (5.70±2.00), MBW (5.70±1.90) and PW (5.80±1.67). The biscuit produced from plantain and wheat (6.55±1.28) had higher values for aroma and was significantly (P≤0.05) different from that produced from MBW (4.70±1.81) and SW (5.45±2.01). However, there was no significant (P≥0.05) difference compared to MW (5.95±1.61). The taste of the biscuit ranged from 4.40±2.14 to 7.00±1.65. PW (7.00±1.65) was significantly (P≤0.05) higher in taste compared to MW (5.55±1.39), SW (4.90±2.73) and MBW (4.40±2.14). The texture of the biscuit ranged from 4.80±2.73 to 6.80±1.82. SW (6.80±1.82) has the highest value compared to MBW (4.80±2.73), MW (6.35±1.93) and PW (6.70±2.27) and was significantly (P≤0.05) higher than MBW. However, there was no significant (P≥0.05) difference compared to SW, MW and PW. The general acceptability of PW (6.95±1.54) was higher than that of MW, SW and MBW which had 6.15±1.46, 5.95±1.82 and 4.95±2.16 respectively. However, there was no significant (P≥ 0.05) difference compared to PW, MW, and SW.

**Table 6: Sensory properties of the formulated biscuits (n=20)**

| Formulated Biscuits | MW | PW | SW | MBW |
|---|---|---|---|---|
| Colour | 5.70 ±1.90$^{a}$ | 5.80 ±1.67$^{a}$ | 5.95 ±1.73$^{a}$ | 5.70±2.00$^{a}$ |
| Aroma | 5.95 ±1.61$^{b}$ | 6.55 ±1.28$^{b}$ | 5.45 ±2.01$^{ab}$ | 4.70±1.81$^{a}$ |
| Taste | 5.55 ±1.39$^{a}$ | 7.00±1.65$^{b}$ | 4.90 ±2.73$^{a}$ | 4.40±2.14$^{a}$ |
| Texture | 6.35±1.93$^{b}$ | 6.70±2.27$^{b}$ | 6.80±1.82$^{b}$ | 4.80±2.73$^{a}$ |
| General acceptability | 6.15±1.46$^{b}$ | 6.95±1.54$^{b}$ | 5.95±1.82$^{ab}$ | 4.95±2.16$^{a}$ |

Mean values of different superscripts in the same row are significantly different (P≤0.05) while those with the same superscripts are not significantly different (P≥0.05) even though there may be variations in the numbers.

**Key**: MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat, SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

Table 7 summarizes the mean characteristics of twelve (Male-5, Female-7) subjects given each test food. The mean age of participants included in this study was 28years (range: 25-34years). The mean BMI was 21.83kg/m$^2$ (range: 19.2-24.80Kg/M$^2$). The mean random blood glucose was 5.15Mmol/l. No medication was used during the duration of the study.

**Table 7: Mean characteristics of human subjects given each test food (n=12).**

| Characteristics | Mean | Standard deviation |
|---|---|---|
| Age (years) | 28 | 3.08 |
| BMI (Kg/M$^2$) | 21.83 | 1.81 |
| Random glucose (mmol/l) | 5.15 | 0.85 |

**Key**:BMI = Body mass index

Table 8 shows the amount of test food consumed. The table shows the amount of test food containing 50g available carbohydrate consumed by each subjects.

**Table 8: Amount (g) of test food consumed containing 50g available carbohydrate**

| Test food | Amount consumed (50g available CHO) |
|---|---|
| MW | 162 |
| PW | 168 |
| SW | 171 |
| MBW | 169 |

**Key**: CHO = Carbohydrate, MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat, SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

Table 9 shows the mean blood glucose values of human subjects. The mean blood glucose measured at different time periods (0, 30, 60,90 and 120 minutes) for the four formulated biscuits, showed that there was a significant increase in fasting blood glucose after ingestion of all test foods (P≤0.05). The test food MW (6.76mmol/l) reached peak blood glucose values at 30 minutes while the rest of the test foods (PW6.75mmol/l, SW6.61mmol/l and MBW6.64mmol/l) peaked at 60 minutes. There was significance (P≤0.05) difference between these blood glucose responses of the four formulated biscuits at 90 minutes and the biscuit MW(5.99mmol/l) significantly(P≤0.05) reduced the postprandial blood glucose more than the other biscuits (PW6.50mmol/l, SW6.30mmol/l and MBW6.52mmol/l).The blood glucose ranged from 6.04 to 6.34mmol/l at 120 minutes. MW(6.04mmol/l) and PW(6.07mmol/l) significantly (P≤0.05) lowered the blood glucose more than SW(6.34mmol/l) and MBW(6.30mmol/l).

**Table 9: The mean blood glucose (mmol/l) values of human subjects given each test food (n=12).**

| Time intervals (Minute) | MW | PW | SW | MBW |
|---|---|---|---|---|
| 0 | $4.82^{b}$ | $4.65^{a}$ | $4.72^{b}$ | $4.46^{a}$ |
| 30 | $6.76^{b}$ | $6.57^{a}$ | $6.60^{a}$ | $6.53^{a}$ |
| 60 | $6.60^{a}$ | $6.75^{b}$ | $6.61^{a}$ | $6.64^{a}$ |
| 90 | $5.99^{a}$ | $6.50^{d}$ | $6.30^{b}$ | $6.52^{c}$ |
| 120 | $6.04^{a}$ | $6.07^{a}$ | $6.34^{b}$ | $6.30^{b}$ |

Mean values of different superscripts in the same rows are significantly different (P≤0.05) while those with the same superscripts are not significantly different (P≥0.05) even though there may be variations in the numbers.

Key: MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat, SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

Table 10 shows the mean incremental blood glucose responses (mmol/l) of human subjects given each test food. The mean incremental glucose at 30 minutes showed that SW (1.89mmol/l) and PW (1.92mmol/l) significantly (P≤0.05) lowered the blood glucose levels compared to MW(1.95mmol/l) and MBW(2.07mmol/l) effect, however, the mean incremental blood glucose at 60 and 90 minutes showed that the formulated biscuits were significantly (P≤0.05) different from one another. MW significantly (P≤0.05) lowered the blood glucose level compared to PW, SW and MBW. At 120 minutes, MW significantly (P≤0.05) lowered the blood glucose level compared to SW and MBW, however there was no significant (p≥0.05) difference in the blood glucose response between MW and PW . The incremental values at 60 minutes were 1.78mmol/l, 1.90mmol/l, 2.1mmol/l and 2.18mmol/l for MW, SW, PW and MBW respectively, at 90 minutes, it was 1.17mmol/l, 1.58mmol/l, 1.85mmol/l and 2.06mmol/l for MW, SW,PW and MBW respectively, at 120 minutes, it was 1.22mmol/l, 1.42mmol/l, 1.62mmmol/l and 1.84mmol/l for MW, PW, SW and MBW respectively.

**Table 10: The mean incremental blood glucose (mmol/l) of human subjects given each test food (n=12).**

| Time intervals (Minutes) | MW | PW | SW | MBW |
|---|---|---|---|---|
| 30 | $1.95^{b}$ | $1.92^{a}$ | $1.89^{a}$ | $2.07^{c}$ |
| 60 | $1.78^{a}$ | $2.1^{c}$ | $1.90^{b}$ | $2.18^{d}$ |
| 90 | $1.17^{a}$ | $1.85^{c}$ | $1.58^{b}$ | $2.06^{d}$ |
| 120 | $1.22^{a}$ | $1.42^{a}$ | $1.62^{c}$ | $1.84^{d}$ |

Mean values of different superscripts in the same rows are significantly different (P≤0.05) while those with the same superscripts are not significantly different (P≥0.05) even though there may be variations in the numbers.

**Key**: MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat, SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

Table 11 shows the mean area under the blood glucose response (AUC) curve of human subjects given each test foods. The mean area under the blood glucose curve (AUC) ranged between 166.5±59.28 and 217.5±94.43. Among the test food, the mean AUC was highest for MBW (217.5±94.43) while the lowest was MW (166.5±59.28). This shows that the biscuit MW lowered the blood glucose more than PW, SW and MBW.

**Table 11: The mean area under the blood glucose (AUC) (mmol.min/l) response curve of human subjects given each test food (n=12).**

| Formulated biscuits: | MW | PW | SW | MBW |
|---|---|---|---|---|
| **Mean** | 166.5 | 195.36 | 181.38 | 217.5 |
| **Standard deviation** | 59.28 | 73.76 | 60.02 | 94.43 |

**Key**: MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat, SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

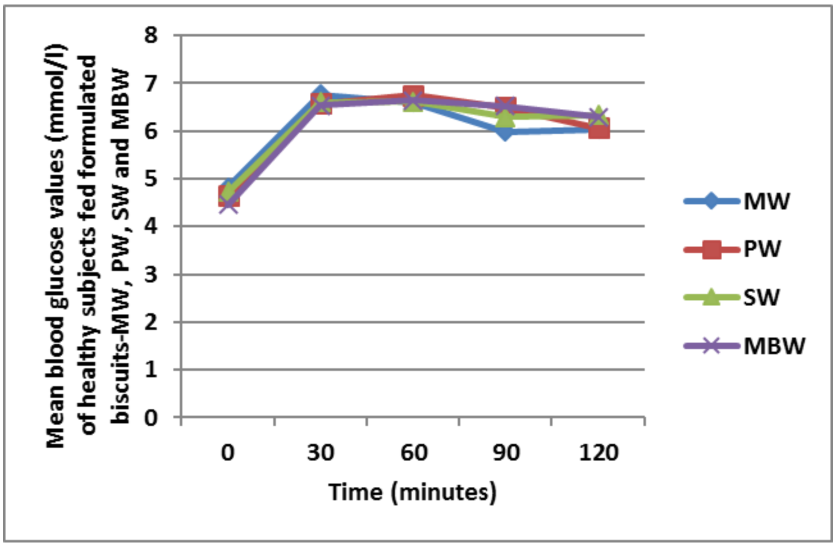


**Figure 2**: Mean blood glucose responses of healthy subjects at 2 hours time point elicited by the formulated biscuits

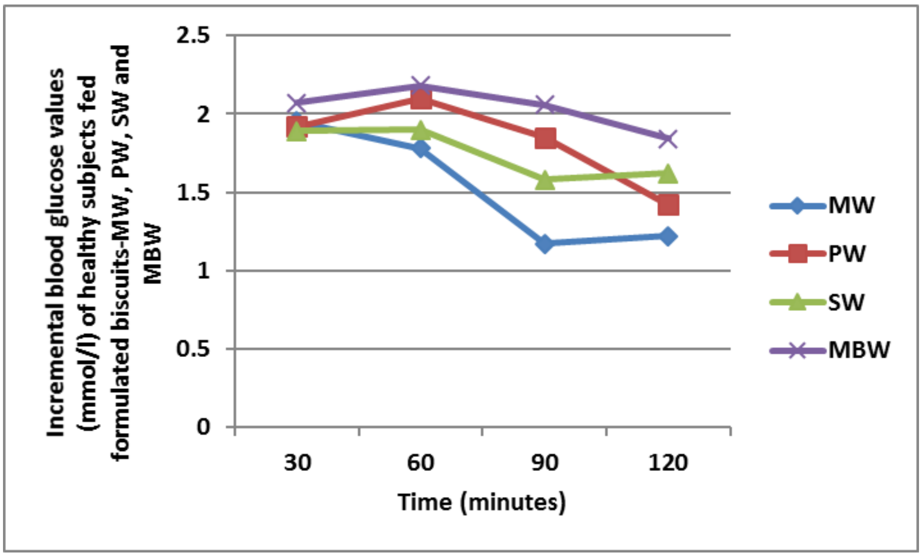


**Figure 3**: Incremental blood glucose responses of healthy subjects at 2 hours' time point elicited by the formulated biscuits

Key to Figures 2 and 3: MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat, SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

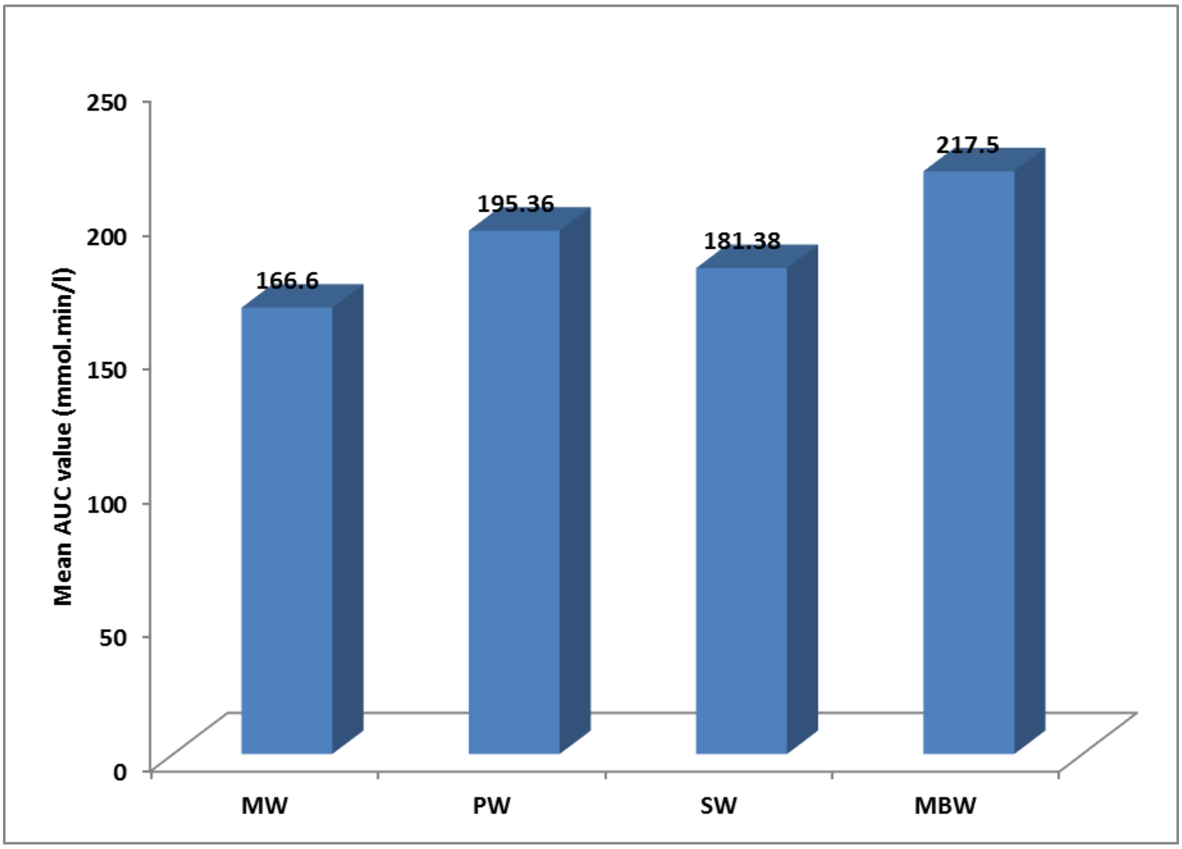


**Figure 4**: The mean area under the blood glucose response curve (AUC) for healthy subjects at 2 hours elicited by the formulated biscuits

Key Figure 4:MW = Biscuit based on mushroom and wheat, PW = Biscuit based on plantain and wheat
SW = Biscuit based on soy bean and wheat, MBW = Biscuit based on mahogany bean and wheat

## DISCUSSION

**Proximate analysis of the biscuits:** In the present study, the moisture content was found to be the highest in MBW (11.2±0.19) and SW (9.22±0.19) biscuit compared to other 2 biscuits. The moisture contents obtained in these biscuits is not in agreement with the research findings of Tasnim and Suman (31) and Paula *et al.*,(32). The result of their study showed that the biscuit with mushroom and wheat recorded 3.97g and 14.00g respectively. The baking temperature (180°C) and baking time (<25 min) used in this study led to a loss of appreciable amounts of moisture compared to the raw samples. However, different food substances have different capacities for absorbing and retaining moisture, leading to occluded or absorbed water according to Eddy *et al.* (33), therefore, it can be deduced that, even at the high baking temperature used in this study, some moisture will still be found in some samples.

The highest fat content was found in SW (13.78±0.19) biscuit and lowest was recorded for MW biscuit. This increase in fat could be due to high content of fat in soy bean flour in the flour blend as soy bean was high in fat and is globally considered as the number one edible oil source, containing a higher percentage of fat (20 t0 24%) than wheat flour (0.9 to 1.1%) (28), and most of which are unsaturated in nature.

The protein content of the biscuit SW (11.64±0.97) was significantly (P≤0.05) high compared to other 3 biscuits. The high protein value of the biscuit SW could be attributed to the formulation with Soy bean flour which is high in protein since wheat was common in the other 3 biscuits and addition of soy flour inevitably increased the protein content in the biscuit. This is in line with work done previously which showed that soy bean had higher protein (49.3g) than Mushroom (31.8g) (31), plantain (6.04g)(34) and Mahogany bean (22.43g) (35). This increase in protein content in the SW biscuit as compared to other 3 biscuits was supported by Ayo *et al*, (36).

The highest ash content was found in MW (1.83±0.29) biscuit and lowest value was recorded in MBW (1.35±0.04) biscuit. The result as shown is opposed by a previous study conducted by Tasnim and Suman (31) but in agreement with the findings of Ayo *et al.,* (36) on the supplementation of soy flour for the preparation of biscuits. The highest crude fiber content was found in MW (16.67±0.34) biscuit and lowest was recorded for MBW (6.57±0.20) biscuit. Similar findings in increase in crude fiber content were also reported by Tasnim and Suman (31). The high crude fiber content of MW and PW biscuit could be due to the high fibre content of mushroom and plantain flour also the decrease in moisture contents of the biscuits explains the more reason for increase fibre contents of the biscuits as supported by a study by (37). According to Eleazu, Iroaganachi and Eleazu (38), Crude fiber, which consists of cellulose and lignin, is used as an indicator of dietary fiber content, dietary fiber is the indigestible/unavailable carbohydrate present in the diet. Diets rich in dietary fiber decrease the re-absorption of bile acids, thus reducing circulating cholesterol levels and increasing glucose tolerance. According to well documented studies, dietary fiber plays a significant role in the prevention of several diseases such as; cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer, and diabetes (39), as a result, this mushroom enriched biscuit may be helpful in preventing these cases.

The result showed that the carbohydrate content of the biscuit MW (52.85±0.75) was low compared to other 3 biscuits. This makes it a good snack for diabetics. Okafor *et al.,* (40) observed that the supplementation of wheat flour with 10% mushroom powder showed that the carbohydrate content in the wheat flour (75.94%) decreased in the wheat flour with 10% mushroom powder (71.07%). This result is similar to this present study. The sugar (sucrose) content of the biscuit samples, were substituted with date palm *(Phoenix dactylifera)* fruit pulp. The sugars are easily digested and can immediately be moved to the blood after consumption and can quickly be metabolized to release energy for various cell activities (41). The benefit of carbohydrate restriction in diabetes are immediate and well documented, Richard, Wendy, Arne and Richard (42) has reported the use of low carbohydrate diets as the first approach to treating type 2 diabetes, low carbohydrate diets have been shown to lower postprandial glucose levels (43).

**Organoleptic Study:** In terms of colour and texture, the biscuit SW (5.95±1.73) was preferred compared to 3 other biscuits samples. There was no significant ($p \geq 0.05$) difference between the biscuits in terms of colour and no significant ($p \geq 0.05$) difference between SW, PW and MW in terms of texture, not withstanding, MBW was less accepted and significantly ($p \leq 0.05$) different compared to 3 other biscuits in terms of texture. On the basis of aroma, taste and general acceptability, PW was preferred and was significantly ($p \leq 0.05$) different compared to the 3 other biscuits in terms of taste. There was no statistical difference between PW, MW and SW in terms of aroma and general acceptability, however, MBW was less liked. This is similar to the findings of Horsfall *et al.,* (18) that biscuit from composite of plantain and wheat flour supplementation was acceptable, also when wheat was substituted with 10% soy flour, sensory evaluation indicated that there were no significant differences in taste, aroma and overall acceptability of the soy enriched biscuits with the unenriched. This is also similar to the findings of oluwumokomi *et al.,* (44), who studied the physicochemical and sensory properties of wheat cassava composite biscuit enriched with soy flour, they observed that there were no significant differences in colour, texture, flavour, taste and overall acceptability of soy enriched flour blend biscuits. However, the colour of the soy enriched biscuits was significantly ($p \geq 0.05$) different from the other biscuits.

**Blood glucose response study:** The blood glucose response after consuming the test foods at 30 minutes showed that SW and PW produced lower postprandial blood glucose when compared to MW and MBW. MW at 60 and 90 minutes significantly lowered the postprandial blood glucose when compared to SW PW and MBW ($P \leq 0.05$). MW at 120 minutes significantly lowered the postprandial blood glucose when compared to SW and MBW ($P \leq 0.05$), however there was no significant ($p \geq 0.05$) difference in the blood glucose response between MW and PW. The result demonstrated that the test foods gave varying effects on blood glucose responses. FAO (45) noted that there are several factors that may affect the digestion and absorption of carbohydrate foods and thus the blood glucose response, factors such as nature of the monosaccharide components, cooking or food processing, fat, protein, dietary fibre and antinutrient contents of food have contributed to the response of glucose level. In this study, MBW had the highest increment of blood glucose response at each level while SW showed the lowest rise at 30 minutes and MW showed the lowest rise in blood glucose response at 60, 90 and 120 minutes (Figure 3), the low blood glucose response of the subjects to MW test food is due to the fibre content which was more than twice the amount in other tested foods SW and MBW. This result is in line with that reported by Stevens, Ahn, Juhaeri, Houstan, Steffan and Couper (46), that fibre decreases postprandial blood glucose. Robert, Arch, Dan, and Kit (47), reported that fibre supplementation for type 2 diabetes mellitus can reduce fasting blood glucose. The result of the mean AUC revealed that MW showed the lowest in postprandial blood glucose response when compared to PW, SW and MBW while MBW showed the highest response (Figure 4). This means that the biscuit based on MW lowered the blood glucose more than PW, SW and MBW (Figure 4).

**Conclusion**

The results of this study, showed that the incorporation of mushroom flour to wheat flour improved the nutrient content of the biscuits with moderate amount of carbohydrate, fat and fibre suitable for diabetics, also, there is a difference in blood glucose response among foods tested with SW and PW being more effective in lowering the postprandial blood glucose of the healthy adults at 30 minutes, however, MW at 60, 90 and 120 minutes significantly had more lowering effect on the postprandial blood glucose levels of the subjects. Based on these results, the most suitable biscuit to be recommended for diabetic patients without significantly increasing the blood glucose response is MW.

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