

Nutritional Composition of Crackers Produced from Blend of Sprouted Pigeon Pea (*Cajanus cajan*), Unripe Plantain (*Musa parasidiaca*) and Brewers' Spent Grain Flour and Blood Glucose Level of Diabetic Rats Fed the Biscuit

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Abstract—The nutritional composition and hypoglycaemic effect of crackers produced from blend of sprouted pigeon pea, unripe plantain and brewers' spent grain and fed to Alloxan induced diabetic rat was investigated. Crackers were produced from different blends of sprouted pigeon pea, unripe plantain and brewers' spent grain. The crackers were evaluated for proximate composition, amino acid profile and antinutritional factors. Blood glucose levels of normal and diabetic rats fed with the control sample and different formulations of cracker were measured. The protein content of the samples were significantly different ($p < 0.05$) from each other with sample A having the lowest value and sample B with the highest value. The values obtained showed that the samples contained most of the amino acids that are found in plant proteins. The levels of antinutritional factor determined were generally low. Administration of the formulated cracker meals led to a significant reduction in the fasting blood glucose level in the diabetic rats. The present study concluded that consumption of crackers produced from this composite flour could be recommended for the diabetics and those who are sceptical about the disease.

Keywords—Crackers, diabetics rat, sprouted pigeon pea, unripe plantain and brewers' spent grain.

I. INTRODUCTION

CRACKERS are usually defined as biscuits, which are all more or less unsweetened, salty, thin and crisp and refers to products with very low sugar and fat content. In Nigeria, biscuits constitute a popular cereal food consumed by the young and the old. Some of the reasons for such wide popularity are low cost compared with other processed foods, good nutritional quality and availability in different forms, varied taste and longer self-life [1]. They are ready to eat, convenient and inexpensive food products, containing digestive and dietary principles of vital importance [2]. Diabetes remains one of the leading causes of death and disability in many countries in the world, including Nigeria. Several multiple risk factors act both independently and

jointly. Among dietary factors, amount of starchy or sugary food and type of fat intake in the dietary play important roles in determining risk of diabetes [3]. It was recommended that low carbohydrate diet should be taken by people suffering from diabetes. Type 2 "Adult Type" (Non-insulin dependent diabetes mellitus), which occurs in elderly people is treated by controlling the diet and oral hypoglycaemic drugs [4].

Addition of legumes such as germinated pigeon pea in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus and coronary heart disease [5]. Plantain (*Musa paradisiacal*) is an important staple food in Central and West Africa. According to [6], over 2.11 million metric tons of plantain is produced in Nigeria annually. However, about 35 – 60% post-harvest losses had been reported and attributed to lack of storage facilities and inappropriate technologies for food processing [7]. The use of plantain flour for production of baked goods if feasible would help to lessen our total dependence on imported wheat.

Brewers' Spent Grain (BSG) is the major by-product of the brewing industry, representing around 85% of the total by-products generated [8]. It has received little attention as a marketable commodity, and its disposal is often an environmental problem. Nevertheless, due to its relatively low cost and high content of protein and fibre (around 20 and 70% dry basis, respectively), it can be of value as a raw material for manufacture of flakes, whole wheat bread and biscuits [9]. BSG is too granular for direct addition in food and must first be converted to flour [10], [11].

The aim of this study was to determine the nutritional composition of crackers biscuits produced from blend of sprouted pigeon pea, unripe plantain and brewers' spent grain and assess its hypoglycaemic effect in vivo, so as to estimate the feasibility of applying these crackers as a healthy diet for preventing and managing diabetes.

II. MATERIALS AND METHODS

Dried Red variety of pigeon pea (*Cajanus cajans*) and matured green plantain fruits (*Musa parasidiaca*) were purchased from Ogbete Main Market in Enugu, Nigeria.

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Brewers' spent grain was purchased from Nigerian Breweries Plc 9th Mile Enugu, Nigeria.

A. Preparation of Germinated Pigeon Pea

Pigeon pea seeds were sterilized by soaking in 1% sodium hypochloride for 20 min prior to steeping. The grains were thoroughly washed and steeped in water for 6 hours. The steeped grains were spread on wet jute bags, covered with a cotton cloth and left to sprout at room temperature (28°C) for 72 hours.

Germinated seeds were dried in a Gallenkamp oven (BS model OV-160, Manchester, UK) at 50°C for 24 hours. Rootlets and shoots of the grains were separated from the grain (kernels) by rubbing off the germinated grains before milling and sieving through 100µm mesh sieve. The flour was kept in an airtight container at 4°C prior to use.

B. Preparation of Plantain Flour

The mature green plantain fruits bunch was cut into individual fruits, washed, peeled and cut to approximately 2mm thick using stainless steel knife. It was then soaked in 0.03% sodium metabisulphite solution for 20 min, dried at 65°C for 48 hours before milling using disc attrition mill, and sieved through 100µm mesh sieve. The flour was kept in an airtight container at 4°C prior to use.

C. Treatment of Spent Grain

The spent grains were treated to remove residual sugar and alcohol using the method of [12] with slight modification. 150g of brewers' spent grain (BSG) was made into a suspension by dissolving in 400ml of water and fermented with yeast *Saccharomyces cerevisiae* (0.8g) for 1 hour to convert its residual sugar to alcohol. The fermented suspension was subjected to distillation to remove residual alcohol. The pH of the distilled suspension was adjusted to about 6.0 – 7.0 by adding 5% (w/v) sodium hydroxide solution in a solid: liquid ratio of 1.2:1, and dried to a moisture content of 5% and milled into flour without sieving. The flour was kept in an airtight container at 4°C prior to use.

D. Composite Flour Formulation

Three composite flour samples containing sprouted pigeon pea, unripe plantain and BSG were formulated. 100% wheat served as control as shown in Table I.

TABLE I
PERCENTAGE COMPOSITION OF WHEAT FLOUR (WF), SPROUTED PIGEON PEA FLOUR (SPPF), UNRIPE PLANTAIN FLOUR (UPF) AND BREWERS' SPENT GRAIN COMPOSITE FLOURS BLENDS

Samples	Wheat flour	Sprouted pigeon pea flour	Unripe plantain flour	Brewers' spent grain
A	100	-	-	-
B	-	60	20	20
C	-	40	40	20
D	-	40	20	40
E	-	20	40	40

E. Production of Crackers from the Composite Flour Blends

The crackers were prepared according to the modified method of [13]. Formulation of the crackers was stated in Table II. Yeast was mixed with water (25°C) to form a suspension, to which the other ingredient and the composite flour were then added and kneaded to form smooth dough. The dough was proofed for 2 hours in a proofer (Bakbar E 81, New Zealand), followed by sheeting to 1.0mm thickness using a dough sheeter (Esmach, Italy). The dough was then cut into squares measuring 3cm x 3cm and 'docked' prior to baking at 160°C for 20 min. Crackers made from 100% wheat served as the control.

TABLE II
FORMULATION OF THE CRACKERS

Ingredients	Amount (g)
Composite flour	100.00
Shortening	9.64
Salt	2.02
Sodium bicarbonate	0.16
Yeast	3.48
Water	50.00

F. Determination of Proximate Composition

The moisture content, crude protein, fat, crude fiber and carbohydrate content of the crackers were determined as described by [14].

G. Determination Amino Acid Profile

The amino acid profile of the crackers was determined using modified method of [15].

H. Determination of Antinutritional Factor

The Tannin, Oxalate, trypsin inhibitor, Saponins and Flavonoid were determined as described by [16].

I. Animal and Diet

Adult wistar-albino rats weighing between 150g – 200g purchased from the Department of Animal Science, University of Nigeria, Nsukka, Nigeria were used in the experiments. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions, in their individually and partly restricted metabolic cages. (16 hours before the administration of the crackers, they were fasted overnight, but allowed free access to water. Five rats were used for each group of study.

J. Induction of Diabetes

Diabetes mellitus was induced by a single interperitoneal injection of ice-cold alloxan monohydrate freshly dissolved in normal saline (2%) at a dose of 180mg/kg body weight [17]. Single intraperitoneal injection of normal saline was given to animal in the control group. After 7 days, the fasting blood glucose (FBG) level of test animal was measured and only rat with FBG level more than 220mg/dl were used for the study.

K. Experimental Design

Thirty rats were divided into six groups, each consisting of five rats.

Group 1. Normal control, rats were non-diabetes induced, fed with normal feed rat/ control sample A

Group 2. Rats were diabetes induced, fed with normal feed rat/ control sample A

Group 3. Rats were diabetes induced, fed with sample B

Group 4. Rats were diabetes induced, fed with Sample C

Group 5. Rats were diabetes induced, fed with sample D

Group 6. Rats were diabetes induced, fed with sample E

After four weeks of feeding, the blood was collected and the animal decapitated. The study was performed in accordance with the International Guideline regarding animal experiment.

L. Determination of Blood Glucose Levels

Blood glucose concentration (mg/100ml) were determined using a glucometer – elite commercial test (Bayer), based on the glucose oxidase method. Blood samples were collected from the tip of tail after 16 hours fasting with free access to water. The blood sugar level was determined on the 1st, 2nd, 3rd and 4th week of the experiment.

M. Statistical Analysis

Data was subjected to analysis of variance using the statistical package for social science (SPSS), version 15.0. Results were presented as mean \pm Standard Deviations. One-way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $p < 0.05$ using the Duncan multiple range test. Values are average of triplicate experiment \pm standard deviation.

III. RESULTS AND DISCUSSION

A. Proximate Composition

The result of proximate composite of sprouted pigeon pea, unripe plantain and brewers' spent grain crackers obtained from different proportion of composition flour blends are presented in Table III. The moisture content of the crackers samples ranged between 3.14% and 4.99% with Sample A having the highest value. The low moisture content of crackers suggests that they will be generally free from microbial spoilage thereby ensuring longer shelf life.

TABLE III
PERCENTAGE PROXIMATE COMPOSITION OF CRACKERS PRODUCED FROM BLEND OF GERMINATED PIGEON PEA, UNRIPE PLANTAIN AND BREWERS' SPENT GRAIN FLOUR

Samples	Moisture	Protein	Fat	Ash	Fibre	CHO
Control A	4.99 \pm 0.11 ^a	10.12 \pm 0.04 ^a	3.84 \pm 0.00 ^a	2.01 \pm 0.03 ^a	2.11 \pm 1.13 ^a	76.93 \pm 0.21 ^a
B	4.91 \pm 0.21 ^a	15.34 \pm 0.12 ^b	4.11 \pm 0.01 ^b	1.49 \pm 0.11 ^b	4.01 \pm 0.41 ^b	70.14 \pm 0.04 ^b
C	4.01 \pm 1.11 ^b	13.01 \pm 0.04 ^c	4.35 \pm 0.11 ^b	2.08 \pm 0.21 ^c	4.21 \pm 0.21 ^c	72.34 \pm 1.11 ^c
D	3.20 \pm 0.02 ^c	13.92 \pm 0.11 ^d	3.91 \pm 0.04 ^a	2.11 \pm 0.14 ^c	4.32 \pm 0.32 ^d	72.54 \pm 0.02 ^c
E	3.14 \pm 0.03 ^c	12.11 \pm 0.21 ^c	3.43 \pm 0.21 ^d	2.01 \pm 0.13 ^a	3.09 \pm 1.12 ^c	76.22 \pm 0.03 ^d

The values are expressed as mean \pm standard deviation. There is no significant difference between data bearing similar superscripts ($p > 0.05$).

The protein content of the samples ranged between 15.34% to 10.12% with sample A having the lowest value and sample B with the highest value while the carbohydrate content of sample B is the lowest in value. The high value of protein contents in sample B could be attributed to the highest value of sprouted pigeon pea it contained. It has been reported that protein can be supplied to food by supplementing non-leguminous food with legumes since most leguminous plant seeds are rich in digestible protein with good array of amino acid and mineral [18]. The fat content values ranged from 3.43–4.35%. The low fat content of these samples implies longer keeping time because fat contributes significantly in determining the shelf life of food products and as such relatively high fat content could be undesirable in baked food products. This is because fat can promote rancidity in food, leading to development of unpleasant and odorous compounds [19]. Also high fat diet can lead to illnesses such as obesity, heart disease and diabetes [20]. The fiber content ranged from 2.11–4.32% with sample D having the highest value. The high fibre content of Sample D might be due to the highest value of brewers spent grain incorporated into the composite flour blend used in cracker production. Fiber mainly consists of β -glucan, pectins and variety source of gums [21]. By forming a viscous gel matrix, soluble fiber has been shown to be able to bind bile salts, which may reduce blood cholesterol levels. It

also may slow the absorption of glucose from the intestine, thereby requiring less insulin secretion. This is in agreement with the work done by [22] who reported that the addition of BSG in functional food increased its fiber content. High fiber may encourage bowel movement, lower blood cholesterol and help prevent cancer of the colon and Type II diabetes [23].

B. Amino Acid Profile

The results of amino acid compositions of the crackers samples are presented in Table IV. The values obtained showed that the samples contained most of the amino acids that are found in plant proteins. It was observed that the amino acid composition of the crackers samples increased as the percentage of sprouted pigeon pea supplementation increased. The values obtained from Sample A that contain only wheat is lower than those obtained from other samples. This might be because the protein content of legume-based food is higher than that of cereal based food [24].

The lysine content of Sample A is 0.91, and it is lower than other samples. This is expected, as lysine was the first limiting amino acid in cereal-based products. Wheat bread provided less than 20% of the recommended amount. This result is in agreement with [25] who published "a lack of lysine in cereals". Generally, the amino acid composition is higher in Sample B than other samples. This might be due to the highest

value of sprouted pigeon pea supplementation in the sample. Methionine and cystine were the least concentrated in samples B, C, D and E while lysine and Glutamic acid were the highest. The low concentration of methionine and cystine in the sample is in agreement with earlier report, which stated low concentration of methionine and cystine in legume supplemented food [26], [27].

The results showed that samples B, C, D, and E met 66.7% for recommended dietary allowance (RDA) of amino acid for 100gm of the formulated food samples [28].

TABLE IV
AMINO ACID PROFILE OF SPROUTED PIGEON PEA, UNRIPE PLANTAIN AND BREWERS' SPENT GRAIN CRACKERS

Amino acid (g/100g)	Sample A	Sample B	Sample C	Sample D	Sample E
Lysine	0.91	4.01	3.06	3.21	2.99
Histidine	1.21	2.91	2.71	2.05	2.11
Arginine	1.52	3.01	2.91	2.11	2.01
Aspartic acid	0.81	1.25	1.10	1.75	1.81
Threonine	0.91	4.13	3.01	2.81	2.54
Serine	2.03	1.24	0.87	1.09	0.98
Glutamic acid	1.76	4.99	3.91	2.81	2.34
Proline	2.11	2.81	2.15	2.11	2.14
Glycine	1.81	2.72	2.26	2.37	1.01
Alanine	1.57	2.03	1.42	1.41	1.11
Valine	2.21	2.51	2.04	2.21	1.81
Methionine	1.71	1.62	1.01	0.99	0.81
Isoleucine	0.85	3.20	2.54	2.61	1.84
Leucine	2.51	3.84	3.45	2.99	2.71
Phenylalanine	1.41	2.92	2.61	2.72	2.41
Cystine	0.85	1.34	1.33	1.21	2.02

C. Antinutritional Factors

TABLE V
ANTINUTRITIONAL FACTORS PRESENT IN CRACKERS PRODUCED FROM SPROUTED PIGEON PEA, UNRIPE PLANTAIN AND BREWERS' SPENT GRAIN FLOUR BLENDS

Sample	Trpsin inhibitor (mg/100g)	Oxalates (mg/100g)	Tannins (mg/100g)	Saponnins (mg/100g)	Flavonoid (mg/100g)
A	0.04±0.00 ^a	0.12±0.01 ^a	0.08±1.00 ^a	0.09±0.03 ^a	0.21±0.00 ^a
B	0.02±0.01 ^b	0.40±0.01 ^b	0.22±1.02 ^b	0.05±1.00 ^b	0.09±0.00 ^b
C	0.04±1.00 ^a	0.31±0.00 ^c	0.21±1.00 ^b	0.11±0.01 ^a	0.11±0.11 ^b
D	0.04±0.02 ^a	0.22±0.02 ^d	0.11±0.00 ^a	0.15±0.01 ^c	0.21±1.01 ^a
E	0.05±0.01 ^c	0.19±0.11 ^d	0.17±0.02 ^c	0.21±0.21 ^d	0.30±1.02 ^c

The values are expressed as Mean ± Standard deviation. Means with different superscripts in the same column are significantly different ($p < 0.05$). Means are of triplicate determinations.

The levels of anti-nutritional factors found in the crackers are shown in Table V. The values obtained were generally low. The earlier work done by [29] is in concordance with this present work. In his work, he reported low anti-nutritional factors in commonly consumed food articles.

D. Changes in Blood Glucose of Normal and Diabetic Rat

Fig. 1 illustrates the variation in blood glucose of normal control, diabetic control and formulated crackers-treated rat during 4 weeks period of study. The blood glucose levels of diabetic rats were significantly higher compared with those of the control group ($p < 0.05$). After the administration of the

formulated crackers meal, a significant decrease in blood glucose was observed compared with that of the diabetic group fed with the control sample A ($p < 0.05$). The diabetic rats in-group 4 fed with sample C have highest significant decrease in the level of glucose (101mg/dl). Generally all the diabetic rats fed with the formulated cracker showed significant reduction in plasma glucose but different level changes were observed throughout the 4 weeks compared with that of the diabetic rats in group 2 fed with rat feed and control sample A.

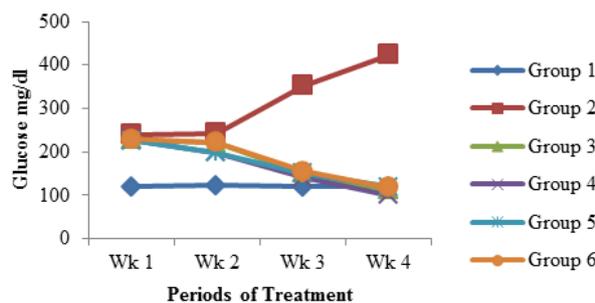


Fig. 1 Glucose levels in plasma of normal and diabetic rats treated with different formulation of crackers produced from blend of sprouted pigeon pea, unripe plantain and brewers' spent grain (Values are mean and standard error of 5 rats/group)

The reduction in plasma glucose might probably be because the formulated crackers contained more dietary fibre than control food, which increased the indigestible carbohydrate portion in small and large intestine, thereby reducing the rate of dietary carbohydrate absorption [30]. Its usefulness in the management of diabetes is thought to be due to dietary fibers ability to slow stomach emptying, modify responses of gastro-intestinal hormones and delay glucose diffusion in the intestinal lumen. The reduction in the plasma glucose might also be due to the high protein content of the formulated crackers, which is higher than the control sample. Moderate protein in diet can also decrease blood glucose level [31].

IV. CONCLUSION

The hypoglycaemic effect of the formulated crackers is probably caused by high dietary fiber and protein content of sprouted pigeon pea, unripe plantain and brewers' spent grain crackers. The usage of these formulated crackers will not only reduce the post-harvest losses associated with pigeon pea and plantain but also will increase the utilization of brewers' spent grain. Therefore, replacing wheat based baked products with other locally available cheap products like sprouted pigeon pea, unripe plantain and brewers spent grain composite flour will exhibit a remarkable blood glucose lowering potential in diabetic rats and could be a means of preventing and managing diabetes.

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