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Consumer Acceptability of Crackers Produced from Blend of Sprouted Pigeon Pea, Unripe Plantain and Brewers' Spent Grain and Its Hypoglycemic Effect in Diabetic Rats

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***Abstract*—**Physical, sensory properties and hypoglycemic effect of crackers produced from sprouted pigeon pea, unripe plantain and brewers' spent grain fed to diabetic rats were investigated. Different composite flours were used to produce crackers. Physical and sensory properties of the crackers, the blood serum of the rats and changes in the rat body weight were measured. Spread ratio and break strength of the crackers from different flour blends ranges from 7.01 g to 8.51 g and 1.87 g to 3.01 g respectively. The acceptability of the crackers revealed that Sample A (100% wheat crackers) was not significantly ($p>0.05$) different from Samples C and D. Feeding the rats with formulated crackers caused an increase in the body weight of the rats but a reduced body weight was observed in diabetic rats fed with normal rat feed. The result indicated that cracker produced from the formulated flour blends caused a significant hypoglycemic effect in diabetic rats and led to a reduction of measured biochemical indices. Therefore, this work showed that consumption of crackers from the above formulated flour blend was able to decrease hyperglycemia in diabetic rats.

***Keywords*—**Hypoglycemia, hyperlipidemia, total lipid, triglyceride, total cholesterol.

I. Introduction

BISCUIT is a popular cereal food consumed by the young and old in Nigeria [1]. Crackers are biscuits, which are more or less unsweetened, briny, thin and crunchy. It can be referred to as lean dough because the fat and sugar content of the dough are low relative to the flour contents [2]. Developing countries view composite flour with much interest since it reduces the importation of wheat flour and encourages the use of locally grown crops as flour [3]. Crackers have been reported to be a better use of composite flour than bread because it is convenient, widely utilized, of good eating quality and also a cheaper food product [4].

The presence of hyperglycemia is diagnosed as diabetes mellitus which is described as irregular insulin secretion and imbalance in carbohydrate and lipid metabolism [5]. Diabetes mellitus is becoming the third "killer" of mankind together with cancer, cardiovascular and cerebrovascular diseases [6]. In dietary factors, amount of sugary or starchy food and type of fat intake in the diet play significant roles in enhancing the risk of diabetes. These risk factors occur both jointly and independently [7].

An increase in consumption of legume and plant fibre is presently being recommended for reducing the plasma lipids in people with hyperlipidemia and improving the control of blood glucose in people with compromised glucose tolerance [8].

Pigeon pea (*Cajanus cajans*) is readily obtainable in Nigeria. Metabolic diseases like cancer and diabetes mellitus can be controlled extensively by the addition of germinated pigeon pea to diet. It also has sorting and psychological effect on the patient. Dietary fibre (8.82%), starch (16.2%) and micronutrients are sufficiently present in unripe plantain. The protein and fat content in unripe plantain are low and it has high hypoglycemic effect [9].

Nigeria's total dependence on imported wheat will greatly reduce if plantain is used in the production of baked goods. Spent grain is the most plentiful brewing by-product generated [10]. It is produced in large quantities throughout the year and it is relatively cheap and affordable. Brewer's spent grain (BSG) is not common in the Nigerian market and it also poses an environmental problem when disposed. It can be a basic raw material in the manufacturing of flakes, whole wheat bread and biscuits since it contains high level of protein and fibre coupled with its low cost [10]. Baked goods are enhanced in terms of taste, texture and fibre content through its addition.

The objectives of this study were to determine the physical properties, sensory evaluation of crackers produced from blend of sprouted pigeon pea, unripe plantain and BSG and to assess its hypoglycemic 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

Matured green plantain fruits (*Musa Parasadiaca*) and dried red variety of pigeon pea (*Cajanus cajans*) were purchased from Ogbete Main market in Enugu-Nigeria. BSG was purchased from Nigerian Breweries plc. 9th Mile corner Enugu-Nigeria.

A. Preparation of Germinated Pigeon Pea

The sterilization of pigeon pea was done by soaking in 1% sodium hypochloride for 20 mins preceding the steeping. The grains were thoroughly washed and soaked in water for six

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hours (6 h). Wet jute bags were used in spreading the soaked grain, it was then covered with cotton cloth and left to germinate at room temperature 28 ºC ± 2 for 72 h.

GallenKamp Oven (B.S. Model OV-160, Manchester, U.K.) was used to dry the germinated seed at 50 ºC for 24 h. Separation of rootlets and shoots of the grain (kernels) were done by rubbing off the germinated seed prior to milling and sieving through 100 µm mesh sieve. Before using, the flour was kept in an airtight container and stored at 4 ºC.

B. Preparation of Plantain Flour

Individual fruits were obtained from the mature green plantain fruits bunch, washed, peeled and cut to approximately 2 mm thick using stainless steel knife. A 0.03% sodium metabisulphide solution was used to soak it for 20 mins and dried at 65 ºC for 48 h before a disc attrition mill was used for milling and sieved through 100 µm mesh sieve. The flour was kept in an airtight container and stored at 4 ºC prior to use.

C. Treatment of Spent Grain

Residual sugar and alcohol were removed from the spent grain by a special treatment method of [11] with minor modifications. A suspension of 150 g of BSG was obtained by dissolving 400 ml of water and fermented with yeast *Saccharomyces cerevisiae* (0.8 g) for 1 h to convert its residual sugar to alcohol. Residual alcohol was removed from the fermented suspension through distillation. In order to adjust the pH of the residue to 6.0-7.0, 5 % (W/V) sodium hydroxide solution in a solid-liquid ratio of 1.2:1 was added, and dried to moisture content of 5% and milled into flour without sieving. The flour was kept in an airtight container at 4 ºC prior to use.

D. Composite Flour Formulation

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

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

Samples	WF	SPPF	UPF	BSG
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 modified method of [12] was used as a standard for the preparation of crackers. Table II shows explicitly the formulation of the crackers. A suspension was formed through mixing of yeast with water (25 ºC), and other ingredients. The composite flour was then added and kneaded to form smooth dough. Proofer (Bakbar E81, New Zealand) was then used to proof the dough for 2 h. A dough sheeter (Esmach, Italy) was later used to sheet the dough to about 1.0 mm thickness. The dough was then cut into squares measuring 3x3 cm and 'docked' prior to baking at 160 ºC for 20 mins. 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 Physical Properties

The physical properties were determined as described by [13].

G. Sensory Evaluation of Crackers

Sensory evaluation was carried out with 27 panelists comprising HND students from Institute of Management and Technology Enugu-Nigeria. Testing was done in the sensory laboratory. A 9 point hedonic scale was designed to measure the degree of preference of the samples, with 1 as the lowest and 9 as the highest level of preference [14]. The samples were presented in identical containers, coded with 3-digit random numbers served simultaneously to ease the possibility of the panelists to re-evaluate a sample. Panelists were required to evaluate the taste, crispness, colour, texture and overall acceptance of the crackers.

H. Animal and Diet

The department of animal science, university of Nigeria, Nsukka, Enugu-Nigeria was the purchase point for Adult Wistar – albino rats weighing 150 g-200 g which were used in the experiment. Prior to the experiment, the rats were made to adapt to the laboratory conditions by feeding them adequately for one week with standard food, in their separately and partially constrained metabolic cages. Five rats were used for each group of the study. Thus, 16 hours before the administration of the crackers, they were fasted overnight however allowed free access to water.

I. Induction of Diabetes

The induction of diabetes mellitus was done by the introduction of a single interperitional injection of ice cold alloxan monohydrate freshly dissolved in normal saline (2%) at a dose of 180 mg/kg body weight [15]. Animals in the control groups were injected with single intraperitional injection of normal saline. After 7 days, the fasting blood glucose (FBG) level of the animal was measured and only rats with FBG level more than 220mg/dl were used for the study.

J. Experimental Design

There were six groups of five rats each, altogether thirty rats were involved.

- Group 1 (control 1): Normal control, rats were fed with normal rat feed and were non-diabetes induced.

- Group 2 (control 2): Rats were fed with normal rat feed and were diabetes induced.
- Group 3: Rats were fed with crackers B and were diabetes induced.
- Group 4: Rats were fed with crackers C and were diabetes induced.
- Group 5: Rats were fed with crackers D and were diabetes induced.
- Group 6: Rats were fed with crackers E and were diabetes induced.

At the expiration of few weeks of the feeding, the animals were decapitated after collecting their blood. The international guideline governing animal experiment was carefully followed while performing this experiment.

Body weights of all these rats were taken before and after the experiment.

K. Biochemical Evaluation

Blood samples of each rat were collected in two clean vial bottles from the tip of tail after 16 hours fasting with free access to water. One was used for blood glucose level determination using a glucometer-elite commercial test (Bayer), based on the glucose oxidase method. The blood sugar level was determined on weekly interval for 4 weeks.

The second vial (replicate blood sample) was used for the determination of total serum lipids [16], total cholesterol [17] and the triglycerides [18].

III. Statistical Analysis

Analysis of variance was carried out on the data using statistical package for social science (SPSS), version 15.0. Presentation of result was by means of triplicate determination ± standard deviations. Analysis of variance was used in comparism of means. Differences between means were considered to be significant at $p<0.05$ using the Duncan multiple range test.

IV. Results and Discussion

A. Physical Properties

From Table III, significant differences ($p<0.05$) were noticed in the physical properties of the crackers. Sample C (5.11 g) experienced a higher weight when compared to the other samples this was probably due to the presence of high leguminous content of the composite flour. The increase in the volume of crackers dough after baking is known as the spread ratio. The most desirable crackers are those with higher spread ratio. When compared to the other samples, the control which is 100% wheat had the lowest spread ratio of 7.01, which was significantly ($p>0.05$) different from crackers produced from other composite flour. The low spread ratio value of the control (100% wheat) might be due to the fact that starch polymer molecules are highly bound with the granules [19]. There was an increase in the spread ratio of the other samples which was as a result of the differences in particulate size between the composite flour.

TABLE III
Physical Attributes of Crackers Made with Sprouted Pigeon Pea, Unripe Plantain and BSG Flour Blends

	Diameter (cm)	Thickness (cm)	Weight (g)	Spread ratio	Break strength (g)
A	7.15b±0.11	1.0a±2.13	4.01b±1.14	7.01b±0.04	3.01a±1.12
B	7.66a±0.09	0.9a±1.76	4.81a±0.51	8.51a±2.14	1.98c±2.07
C	7.61a±0.21	0.9a±1.11	5.11a±2.03	8.46a±2.31	2.85b±0.91
D	6.33c±0.34	0.8b±1.71	4.03b±1.02	7.99a±1.11	2.01b±0.01
E	6.31c±1.71	0.9a±2.01	3.81c±1.16	7.15c±0.17	1.87c±0.06

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 cracker samples were significantly ($p<0.05$) different in their breaking strength with the control having the highest value followed by sample C.

B. Sensory Evaluation

The mean scores for the sensory evaluation of sprouted pigeon pea, unripe plantain and BSG composite flour are shown in Table IV. There was no significant ($p>0.05$) difference in taste, but significant ($p<0.05$) differences existed in crispness, colour, texture and overall acceptance.

TABLE IV
Mean scores of Sensory Evaluation of Samples

	Taste	Crispiness	Colour	Texture	Overall acceptance
A	7.51a±0.11	7.01a±2.15	8.20a±0.05	6.81b±0.01	8.41a±1.01
B	7.42a±1.02	6.11b±2.01	7.35b±1.06	7.51a±6.01	7.81b±2.02
C	6.99a±1.22	7.10a±0.51	7.95a±1.23	7.41a±4.11	8.22a±1.02
D	7.54a±0.03	7.21a±1.11	7.50b±2.41	6.91b±0.12	8.05a±2.31
E	7.61a±3.56	7.56a±0.51	6.99c±0.51	7.01a±0.17	7.22b±2.20

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.

Crispiness is perceived when food is chewed between molars and it is expressed in terms of hardness and fracturability. There was no significant ($p>0.05$) difference in terms of crispiness among the samples except Sample B. This means that the other samples except B are as good as the control (100% wheat crackers) in terms of crispiness. The colour showed that Sample A (control) was not significantly ($p>0.05$) different from Sample C but significantly ($p<0.05$) different from other samples.

The overall acceptability of the crackers showed that Sample A (100% wheat crackers) was not significantly different ($p>0.05$) from Samples C and D but was significantly ($p<0.05$) different from the other crackers. This showed that Samples C and D can form a basis for new product development for the production of crackers.

C. Change in the Body Weight of the Rats

There was a drastic loss in body weight of alloxan induced diabetic rat from Table V compared to the weight gain found in the control rats. However, the diabetic rats given the formulated cracker had a significant increase in body weight. The reduction in body weight of the alloxan induced diabetic rats is in agreement with the work done by [20], who reported that during diabetes mellitus, the serum glucose increases, thus leads to lack of sugar in the cells, thereby making the cells to use amino acids and fatty acids as a source of energy thereby causing the proteins and fats in the body to decrease which leads to loss of body weight. In essence when there is insufficient glucose in cell, the animal relied on the stored energy.

TABLE V
CHANGE IN THE BODY WEIGHT OF THE RATS

Group	Rats	Body weight (kg) at week 0	Body weight (kg) at week 4
1 (control 1)	Normal, rats were non-diabetes induced, fed with normal rat feed	$106^{a}\pm1.22$	$117^{a}\pm0.11$
2 (control 2)	Rats were diabetes induced, fed with normal rat	$81^{c}\pm0.31$	$68^{d}\pm2.02$
3	Rats were diabetes induced, fed with crackers B	$80^{c}\pm0.01$	$105^{c}\pm1.31$
4	Rats were diabetes induced, fed with crackers C	$81^{c}\pm1.67$	$106^{c}\pm1.42$
5	Rats were diabetes induced, fed with crackers D	$90^{b}\pm0.42$	$115^{a}\pm0.51$
6	Rats were diabetes induced, fed with crackers E	$88^{b}\pm2.01$	$111^{b}\pm2.11$

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.

D. Serum Parameter

Fig. 1 illustrates the variation in blood glucose of the rats during 4 weeks period of study. The blood glucose levels of diabetic rats fed with formulated crackers meal were significantly ($p<0.05$) lower than those of diabetic rats fed with normal rat feed. The diabetic rats in group 4 fed with Sample C showed the highest decrease in the level of glucose.

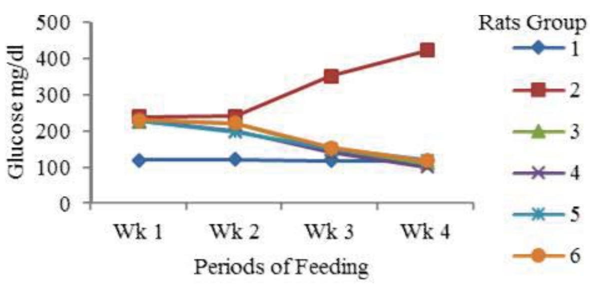

Fig. 1 Glucose levels in plasma of normal and diabetic rats fed with different formulation of crackers

Dietary modification is probably the simplest and cheapest form of diabetes treatment. Fig. 1 revealed that consumption of crackers produced from a blend of sprouted pigeon pea, unripe plantain and BSG lead to a decrease in glucose level. This reduction in glucose level of rat fed the composite flour crackers might be due to the high protein content of the formulated crackers which was higher than the 100% WF crackers (control sample). This finding is in agreement with the work done by [21], which showed that higher legume intakes were associated with lower incidence of diabetes mellitus, lower body mass index (BMI), blood pressure, and serum total cholesterol (TC) compared with cereal intakes. Also inclusion of legumes in the daily diet may have a positive effect in controlling and preventing various metabolic diseases such as diabetes mellitus and coronary heart disease [22]. BSG is considered as a lignocellulosic material rich in protein and fibre, which account for around 20 and 70% of its composition respectively [23]. Incorporation of this spent grain in rat diet prevented increase in plasma total lipid and cholesterol [24] which might be due to elevated stool bulk which might lead to decreased lipid absorption. Reference [25] reported that consumption of soluble dietary fibre may lead to decreased plasma cholesterol and serum glycose.

TABLE VI
EFFECT OF DIFFERENT BLENDS OF COMPOSITE FLOUR CRACKERS ON LIPID, TRIGLYCERIDES AND TC CONCENTRATIONS OF NORMAL AND DIABETIC RATS.

Group	Rats	Total lipid g/dl	Triglyceride mg/dl	TC mg/dl
1 (control A)	Normal control, rats were non-diabetes induced, fed with normal rat feed	$0.91^{b}\pm0.15$	$184^{b}\pm3.23$	$155^{b}\pm1.11$
2 (control B)	Rats were diabetes induced, fed with normal rat feed	$1.54^{a}\pm1.01$	$211^{a}\pm0.14$	$198^{a}\pm2.01$
3	Rats were diabetes induced, fed with sample B	$1.11^{b}\pm0.60$	$131^{d}\pm4.76$	$121^{d}\pm0.33$
4	Rats were diabetes induced, fed with Sample C	$0.99^{c}\pm1.12$	$129^{d}\pm0.33$	$124^{c}\pm4.04$
5	Rats were diabetes induced, fed with sample D	$0.90^{d}\pm0.51$	$135^{c}\pm1.19$	$119^{d}\pm0.51$
6	Rats were diabetes induced, fed with sample E	$1.02^{c}\pm0.22$	$133^{c}\pm1.21$	$120^{d}\pm5.01$

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.

Table VI revealed that the level of total lipid, triglyceride and TC of diabetic rat were 1.54±1.01 g/dl, 211±0.14 mg/dl and 198 ±2.01 mg/dl respectively. The normal control rat had higher levels of total lipid, triglyceride and TC (0.91±0.15 g/dl, 1.84±3.23 mg/dl and 155±1.11 mg/dl respectively).

Hyperlipidemia and hypercholesterolemia are normally associated with diabetes. The high plasma level of total lipid, triglyceride and TC in diabetic animal usually results in alteration in lipid metabolism [26]. This may be as a result of the uninhibited actions of lipolytic hormones in the fat deposit due to the non-existence of insulin [27]. Administration of the alloxan induced diabetic rat with sprouted pigeon pea, unripe plantain and BSG lead to a significant ($p<0.05$) decrease in total lipid, triglyceride and TC. This reduction might be due to the high protein and dietary fibre in the blend of composite flour crackers. The finding is in agreement with the work done by [21] who reported that higher legume consumption lead to decrease in blood sugar and TC than lower consumption of legume. Reference [28] documented that high fibre diets has some effect on hyperlipidemias, diabetes and obesity. Researchers in rats have indicated that dietary fibre interfere with the actions of digestive enzymes thereby retarding the rate at which sugars and fats enter the blood stream [29], [30]. Also, [31] found that soluble dietary fibre of *T. foenum graecum* seed exhibited antidiabetic effects as a result of delay in carbohydrate digestion and assimilation.

V. CONCLUSION

The result from the physical properties and sensory evaluation revealed that sprouted pigeon pea, unripe plantain and BSG crackers compared flavourably with the control (100% wheat crackers). Also, replacing wheat with this composite flour for crackers production could lead to hypoglycemia that will result in decrease in hyperlipidemia in diabetic rats.

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