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The Hyperglycemic Effect of Different Particle Sizes of Composite Flour from Maize, Wheat and African Breadfruit on Adult Diabetic Albino Rats

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Abstract:
This study was carried out to determine the hyperglycemic effect of diets prepared with different particle sizes of composite flour from Maize (*Zea mays*), Wheat (*Triticum aestivum*) and African breadfruit (*Treculia africana*) on adult diabetic albino rats (*Rattus norvegcus*). The samples were milled into different particle flour sizes and used to prepare three different sets of diets (diet A, diet B, and diet C). Diet A represents the fine particle size composite flour, diet B represents medium particle size composite flour, while diet C represents coarse particle size composite flour. The broiler finisher, which serves as the control represents diet D. Sixteen rats were used in this experiment and separated into four groups of four rats each (three experimental groups and one control group). The experimental groups were injected with alloxan to induce diabetes, while the control group was injected with saline solution. Groups 1, 2, 3 and 4 were fed with diets A, B, C and D respectively and their fasting blood glucose level were determined at intervals for a period of twenty-one days. Group 3 had the lowest blood glucose level range (80 to 100 mg/dL) more than diets A and B which has blood glucose level range of 82 to 109 mg/dL and 103 to 124 mg/dL respectively. This is an indication that the particle size of diet C is the best in the management of diabetes among the other diets. Therefore, the particle size of diet C is more effective in the management of diabetes than that of diets A and B. However, the three diets were able to reduce the blood glucose level to normal.

Keywords: African Breadfruit, Albino Rats, Hyperglycaemic, Maize, Wheat

1. Introduction

Diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced (Shoback et al., 2011). Maize (*Zea mays*) is an important food crop not only because it is consumed worldwide, but also due to its nutritive value (Mboya et al., 2011). Maize provides more carbohydrates than wheat and sorghum, and it is a good source of phosphorus. It also contains small amounts of calcium, iron, thiamine, niacin and fat (Mboya et al., 2011). Furthermore, maize tends to provide high yield per unit of land, which makes maize a key crop in ensuring its availability and promoting food security to the consumers (Brandes, 1992). Wheat (*Triticum aestivum*) is the most important stable food crop for more than one third of the word population and contributes more calories and proteins to the world diet than any other cereal crops (Abd-EL-Haleem et al., 1998). It is nutritious, easy to store and transport and can be processed into various types of food (Shewry, 2007). Wheat is considered a good source of protein, minerals, B-group vitamins and dietary fibre. It is an excellent health building food and can be used to prepare bread, produce biscuits, noodles, confectionary products (Kumar et al., 2011). African breadfruit (*Treculia africana*) is a rich source of carbohydrates that gives enough energy and calories throughout the day (Zerega et al., 2004). It is also rich in B-complex vitamins particularly vitamins B1 (thiamine), B6 (pyridoxine), and B3 (niacin), and also contains minerals such as iron, copper, magnesium, and phosphorus that are important for the body's proper functioning (Zerega et al., 2004).

It is well known that the management of diabetes is labour-intensive and the care is often micro- and macro-vascular complications prohibitive (Silverstein and Cogen, 2006). This may lead to poor control with acceleration of micro- and macro-vascular complications. Although, previous studies have been conducted on the control and management of diabetes, but a research is yet to be conducted on the hyperglycemic effect of different particle sizes of composite flours from maize, wheat and African breadfruit on diabetes.

This study will provide sufficient information on the management of diabetes which in turn will improve control of diabetes with reduction in micro- and macro-vascular complications. This research will bring to limelight the hyperglycemic effect of different particle sizes of composite flours from maize, wheat and African breadfruit on diabetic patients.

2. Research Methods

Material

The raw materials used in this study include maize (Zea mays), wheat (Triticum aestivum), African breadfruit (*Treculia africana*), bone meal, salt, vegetable oil, fish meal and onion. These raw materials were purchased from Umuahia main market, Umuahia North, Abia State Nigeria. The albino rats used for this experiment were procured from Veterinary Medicine Department, University of Nsukka, Nigeria.

The instruments used were hammer milling machine (Tiger-extuda 6.5 horsepower), electric oven (model-mc-1110KGallenkamp), and glucometer (Accu-Chek Active Glucometer)

Methods

This experiment was carried out in a Completely Randomized Design (CRD). Out of the sixteen albino rats, twelve administered diabetic rats were placed under treatment of three different diets, while the remaining four were placed on the control treatment. Each group of experimental rats (1, 2 and 3) was fed with respective diets (A, B and C).

Research Procedures

Production of Maize Flours

Maize kernels were sorted manually, washed and boiled with clean water in a pot for five minutes, drained using stainless steel and then dried in an electric oven until it was properly dried. The dried samples were milled using hammer milling machine into different particle (fine, medium and coarse) flour sample sizes.

Production of Wheat Flour

The wheat seeds were sorted manually, washed with clean water, soaked overnight in clean water using a clean container and drained using perforated stainless steel. The samples were dried in an electric oven until it was properly dried. The dried samples were milled using hammer milling machine into fine, medium and coarse flour samples sizes**.**

Production of African Breadfruit Flour

The African breadfruit seeds were sorted manually, washed with clean water, boiled in clean water for 5 minutes, drained using stainless steel and dried in an electric oven. The dried samples were milled using hammer milling machine into fine, medium and coarse flour samples sizes.

Particle Size Determination

The particle sizes of the composite flours were determined using the method as described by Sonaye and Bayi (2012). Approximately 50 g of each sample was poured into the top sieve which was the largest screen opening of 1.8mm. Each lower sieve in the column was smaller opening than the above. At the base was a round pan called the receiver. The column was placed in a mechanical shaker. The smaller, shakes the column usually for 15 – 20 minutes. When the shaking was completed, the materials on each sieve were weighed. The percentage of aggregate passing through each sieve and the percentage retained can be calculated using equation below.

$$\%\text{cumulative retained} = \frac{W\ sieve}{W\ total} \times 100$$

Note:
W sieve = weight of aggregate in the sieve
W total = total weight of the aggregate

Then, the cumulative percentage of aggregate retained in each sieve can be calculated by adding up the total amount of aggregate retained in each sieve and the amount in the previous sieves. The cumulative percentage passing of the aggregate is calculated by subtracting the percentage retained from 100% as shown below:

$$\%\text{cumulative passing} = 100 - \%\text{ cumulative retained.}$$

Diet Formulation

Three sets of diet (diet A, diet B and diet C) were used for this research work. Diet A was prepared using fine particle size composite flours. Diet B was prepared using medium particle size composite flours and Diet C was prepared using coarse particle size composite flours. The diets were formulated using "Trial and Error" method as described by Olomu (1995). All the necessary ingredients used were weighed in the right proportions as shown in Table 1. The ingredients were mixed thoroughly for each diet. Eight hundred milliliters of boiling water was added into diet A and mixed thoroughly to form dough. Six hundred fifty milliliters of boiling water was added to diet B and mixed thoroughly to form dough. Four hundred milliliters of boiling water was added to diet C and mixed thoroughly to form dough. The three sets of dough obtained were kneaded and used to form pellets manually. The pellets obtained were dried in an electric oven until they were dried completely. The dried pellets were stored in three different sets of air-tight containers for further use.

Table 1: Experimental Diet Formulation

Ingredient	Inclusion level (%)
African breadfruit flour	44
Wheat flour	24
Maize	18
Fish	7
Oil	2.5
Bone meal	2.5
Onion	1.0
Salt	1.0
Total	100

Proximate Composition of the Formulated Diet

Proximate compositions of the formulated diet were determined in duplicates for moisture, ash, crude fiber, fat and protein as described by AOAC (1990). The carbohydrate content was determined by difference.

Animal Feeding Experiments

The animals were grouped into four sets. Group 1, 2, and 3 were experimental groups, while group 4 was the control. Each group was made up of four rats. All the animals were fed with broiler finisher during the seven days period of acclimatization as described by Tenpe and Yeole (2009). All the animals were fed with different diets per day for twenty – one days (three weeks). Group 1 was fed with diet A which was formulated with fine particle size flours. Group 2 was fed with diet B which was formulated with medium particle size flours. Group 3 was fed with diet C which was formulated with coarse particle size flours. Finally, group 4 was fed with broiler finisher which served as control diet.

Injection of Alloxan Monohydrate Solutions

The method described by Vinuthan et al. (2007) was used to dissolve the alloxan in sterile normal saline prior to injection. Group 1, 2, and 3 were intravenously injected alloxan monohydrate solution for them to be diabetic, while group 4 was injected only saline solution (NaOH). The quantity of alloxan monohydrate solution injected depends on the individual weight of the rats. The individual rats were weighed using sensitive electronic weighing balance (model – TP – A200/0.01g) and ordinary weighing balance. These solutions were injected after seven days of acclimatization, and allowed to stay for four days before checking their blood glucose level.

Collection of Blood Glucose Level

The method described by Sheriff et al. (2011) was used in determination of blood glucose level of the animals. Blood samples were collected at four days interval. The tail of the animals were aseptically cleaned and blood sample taken by puncturing the base of the tail of the animal then allowed to drop at the edge of a test strip fixed into a glucometer and the value displayed automatically on the glucometer and recorded.

Data Analysis

The results obtained were subjected to analysis of variance (ANOVA) with the mean values compared by Duncan's test at 5% significance level. Statistical analysis was performed using Statistical Package for Social Scientists (SPSS), version 16.0, on a personal computer (SPSS, 1995).

3. Results and Discussion

Proximate Composition

The results of the proximate composition (Table 2) of the formulated diets reveal that there was significant difference ($p<0.05$) among the samples in terms of moisture content. The moisture content of the samples ("fine", "medium" and "coarse") ranged from 7.93 – 8.61%. Sample "fine" had the highest moisture content (8.61%), while sample "coarse" had the lowest moisture content (7.93%). This could be as a result of the differences in the particle sizes of the flour samples used in the formulated of the diets. The values obtained in this study conform to the maximum allowable limit of 14% for moisture content in flour (Austin, 1984). High moisture content, will promote the growth of microorganisms which causes odour and off flavor (Austin, 1984). Also, the higher the moisture content of food material, the shorter the shelf life and vice versa (Nnam, 2002).

Table 2: Proximate Composition (%) of the Formulated Diets.

Diets	Moisture Content	Fat Content	Fiber Content	Protein Content	Ash Content	Carbohydrate
Fine	$8.61^{a}\pm0.13$	$6.61^{c}\pm0.14$	$1.00^{c}\pm0.14$	$38.33^{b}\pm0.74$	$3.70^{c}\pm0.00$	$41.76^{a}\pm0.88$
Medium	$8.01^{b}\pm0.19$	$6.79^{b}\pm0.14$	$1.60^{b}\pm0.14$	$34.65^{c}\pm0.99$	$8.19^{a}\pm0.01$	$40.75^{b}\pm1.18$
Coarse	$7.93^{c}\pm0.07$	$7.00^{a}\pm0.00$	$1.81^{a}\pm0.14$	$40.43^{a}\pm0.74$	$6.50^{b}\pm0.00$	$36.34^{c}\pm0.80$

a-c means in the same column with different superscripts are significantly different ($P<0.05$).

The fat content of the diet samples ranged from 6.61 – 7.00%, with sample "coarse" having the highest fat content (7.00%), while sample "fine" had the lowest fat content. This could be as a result of the grainy particle size of sample "coarse". These values did not exceed the less than 20% limit of saturated fat recommended for diabetic patients (Bellow and Nicholas, 2005). Thus, the level of fat present in the food is adequate for experimental rats as excess fat content could lead to cardiovascular disease and stroke (ADA, 2012). Fat content in food plays some vital roles in maintaining healthy skin and hair, insulating body organs against shock, maintaining and promoting healthy cell function (Mozaffarian et al., 2006). This helps to protect vital organs until such time as the offending substances can be metabolized and removed from the body by such means as excretion, urination and hair growth.

The fibre content of the samples ranged from 1.00 – 1.81%. Sample "coarse" had the highest fibre content which was significantly different ($p < 0.05$) from other diets, while sample "fine" had the lowest fibre content. This could be as a result of the differences in the particle sizes of the flour samples used in formulation of the diets. Fibre, which refers to the indigestible carbohydrate component that is present in food (Allen, 1997), is characterized by low or no nutritional value, but because of its effect on the digestive system, it is considered to help solve such problems as diabetes and high levels of blood cholesterols (Wolever,1990). However, these values are good when compared to the recommended total fibre intake of 10 – 25g per day which is between 40 – 100% fibre intake for diabetic patients (Bellow and Nicholas, 2005).

The result of the protein content of the diet samples ranged from 34.65 – 40.43%. Sample "coarse" had the highest protein content (40.43%) which was significantly different ($p < 0.05$) from other diet samples, while sample "medium" had the lowest protein content (34.65%). This could be as a result of the ingredients added during the formulation of the diets. These values are higher than the recommended protein intake of 15 – 20% for the general population as well as those with diabetes (ADA, 2012). According to Bellow and Nicholas (2005), these high values will not be detrimental to the diabetic patient if the kidney (renal) function is normal. The variation in these results could be as a result of other ingredient added during the diet formulation. However, the presence of protein helps to slow down the rate of digestion because of its content, thereby reducing the blood glucose level and management of food-based nutritional problems such as diabetes, obesity, and so on (Wolever, 1990).

The ash content of the diets ranged from 3.70 – 10.20%. Sample "medium" had the highest ash content (10.20%) which was significantly different ($p < 0.05$) from other diet samples, while sample "fine" had the lowest ash content (3.70%). This could be as a result of different particle sizes of the flour samples used in formulation of the diets. The ash content represents the total mineral contents present in food and thus, serves as a viable tool for nutritional evaluation (Lienel, 2002). The variation in these results could be as a result of other ingredient added during diet formulation or differences in the sizes of flour particles. However, these values conform with the findings of Lienel (2002), who stated that the ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%.

The values of the carbohydrate content of the diet samples showed that sample "fine" had the highest carbohydrate content (41.76%), while sample "coarse" had the lowest carbohydrate content (36.34%). The differences in these results could be as a result of different particle sizes of flour samples. These values are within the recommended calories to be obtained from carbohydrate, which according to ADA (2012) is between 40 – 65%. ADA (2012) also recommended that sucrose-rich food should be substituted for other carbohydrates in the meal plan of diabetic patients. Thus, a diet consisting of very low amounts of daily carbohydrate for several days will usually result in higher levels of blood ketone bodies than an isocaloric diet with similar protein content (Westman, 2002). However, these results of carbohydrate content also conform to the findings of Brand-Miller and Foster-Powell (2005), who stated that the high and low carbohydrate flour blends produced moderate carbohydrate values. This indicates that the carbohydrate contents of the formulated diets were moderate.

Blood Glucose

The results of the fasting blood glucose level of the experimental rats are presented in Table 3. The normal blood glucose level of the rats ranged from 94 – 124 mg/dl. From the results obtained, the initial blood glucose levels of the experimental rats were normal. This is in agreement with the findings of American Diabetes Association (ADA, 2006), which stated that the normal blood glucose level for non – diabetics is within the range of 80 – 120 mg/dl. On the first day (day 1), the fasting blood glucose level of the experimental rats after the induction of diabetes ranged from 320 – 429 mg/dL, while that of the control ranged from 108 – 112 mg/dL. This could be as a result of the alloxan that was injected in those experimental rats. These results concur with the findings of Cetin and Kara (1994), who stated that blood glucose level higher than 200 mg/dL is considered to be diabetic, but symptoms may not start to become noticeable until even higher values such as 250 – 300 mg/dL or more. On the second day (day 2), group 1 showed a significant decrease in their fasting blood glucose level, followed by group 2. However, group 3 showed a minimal decrease in their fasting blood glucose level. On the third and fourth day (day 3 and 4), group 1 and 2 showed a tremendous fluctuation in the increase and decrease of their fasting blood glucose level when compared to group 3 which continued to decrease gradually. This fluctuation in the increase and decrease of their fasting blood glucose level could be as a result of the feeding rate of the rats or from their digestive systems. However, the fasting blood glucose level of the control group remained normal. On the fifth day (day 5), the fasting blood glucose level of group 1 ranged from 92 – 109 mg/dL, group 2 ranged from 103 – 124 mg/dL, group 3 ranged from 80 – 100 mg/dL, while that of the control (group 4) ranged from 101 – 111 mg/dL. The results of the groups on the fifth day showed the most lowered fasting blood glucose level with group 3 when compared with the other experimental groups (groups 1 and 2) and even the control group. However, these results conform to the normal range of 80 – 125 mg/dL fasting blood glucose level of non-diabetics (ADA, 2006). The values obtained from members of group 3 which were fed with diet of 46% cumulative particle size tend to agree with the findings of Englyst and Hudson (1996), that grainy food particles lower the rate of digestion thereby resulting in low blood glucose response in individual. However, the values obtained from group 2 and 3 fed with diets of 25.6% and 8.4% cumulative particle sizes respectively have results which are marginally within normal range. This is an indication that the formulated diet C met the requirement of diabetic diet and can be used to manage diabetes. The control group maintained a normal glucose level.

Table 3: Fasting Blood Glucose Level of the Albino Rats

Diet	Rat Number	Normal BGL (mg/dL)	Fasting Blood Glucose Level (mg/dL)				
			Day 1	Day 2	Day 3	Day 4	Day 5
A	A1	94^{d}+0.01	$429^{a}\pm0.01$	$103^{c}\pm0.01$	$117^{c}\pm0.01$	$121^{b}\pm0.01$	$94^{b}\pm0.01$
	A2	$115^{c}\pm0.01$	$403^{d}\pm0.01$	$92^{d}\pm0.01$	$124^{a}\pm0.01$	$126^{a}\pm0.01$	$92^{d}\pm0.01$
	A3	$120^{b}\pm0.01$	$405^{c}\pm0.01$	$124^{a}\pm0.01$	$119^{b}\pm0.01$	$111^{d}\pm0.01$	$94^{b}\pm0.01$
	A4	$124^{a}\pm0.01$	$420^{b}\pm0.01$	$113^{b}\pm0.01$	$114^{d}\pm0.01$	$120^{c}\pm0.01$	$109^{a}\pm0.01$
B	B1	$109^{a}\pm0.01$	$329^{c}\pm0.01$	$163^{a}\pm0.01$	$115^{c}\pm0.01$	$130^{a}\pm0.01$	$123^{b}\pm0.01$
	B2	$109^{a}\pm0.01$	$339^{a}\pm0.01$	$87^{c}\pm0.01$	$125^{a}\pm0.01$	$127^{b}\pm0.01$	124^{a}0.01
	B3	$102^{b}\pm0.01$	$330^{b}\pm0.01$	$84^{d}\pm0.01$	$123^{b}\pm0.01$	$120^{c}\pm0.01$	$103^{d}\pm0.01$
	B4	$100^{c}\pm0.01$	$320^{d}\pm0.01$	$96^{b}\pm0.01$	$123^{b}\pm0.01$	$130^{a}\pm0.01$	$113^{c}\pm0.01$
C	C1	$124^{a}\pm0.01$	$423^{a}\pm0.01$	$300^{a}\pm0.01$	$115^{b}\pm0.01$	$102^{c}\pm0.01$	$99^{b}\pm0.01$
	C2	$117^{b}\pm0.01$	$381^{d}\pm0.01$	$243^{b}\pm0.01$	$127^{a}\pm0.01$	$124^{a}\pm0.01$	$100^{a}\pm0.01$
	C3	$124^{a}\pm0.01$	$394^{c}\pm0.01$	$191^{d}\pm0.01$	$82^{d}\pm0.01$	120^{b}0.01	$80^{d}\pm0.01$
	C4	$102^{c}\pm0.01$	$420^{b}\pm0.01$	$204^{c}\pm0.01$	$107^{c}\pm0.01$	$87^{d}\pm0.01$	$92^{c}\pm0.01$
D	D1	$102^{a}\pm0.01$	$112^{c}\pm0.01$	$101^{d}\pm0.01$	$109^{b}\pm0.01$	$94^{c}\pm0.01$	$101^{d}\pm0.01$
	D2	$98^{c}\pm0.01$	$108^{b}\pm0.01$	$107^{c}\pm0.01$	$117^{a}\pm0.01$	$87^{d}\pm0.01$	$103^{c}\pm0.01$
	D3	$94^{d}\pm0.01$	$111^{d}\pm0.01$	$110^{a}\pm0.01$	$100^{c}\pm0.01$	$96^{b}\pm0.01$	$111^{a}\pm0.01$
	D4	$101^{b}\pm0.01$	$109^{a}\pm0.01$	$109^{b}\pm0.01$	$100^{c}\pm0.01$	$101^{a}\pm0.01$	$110^{b}\pm0.01$

a-d means in the same column with different superscripts are significantly different ($P<0.05$). A = Fine particle size composite flour diet, B = Medium particle size composite flour diet, C = Coarse particle size composite flour diet, BGL = Blood Glucose Level, D = Normal feed (broilers finisher), A1 - A4 = Group 1 experimental rats, B1 - B4 = Group 2 experimental rats, C1 - C4 = Group 3 experimental rats, D1 - D4 = Group 4 experimental rats (control).

The results of the particle size analysis of the composite flours are presented in Table 4. From the results of the particle size analysis obtained, it was shown that the aggregate, percentage and cumulative particle sizes retained of the composite flour samples were 4.00 – 15.00 g, 8 – 30% and 46% respectively for sample C, while sample B had 6.20 – 18.40 g, 12.4 – 36.8% and 25.6% respectively, and sample A had

10.30 – 20.10 g, 20.6 – 40.2% and 8.4% respectively. These was as a result of different particle sizes of the flour samples. According to Sonaye and Bayi (2012), flour is known to be a heterogeneous mixture of particles of different densities and shapes. Also, sieving, sedimentation and photo-extinction has been adopted in measuring particle sizes (Sonaye and Bayi, 2012). The results also show that sample C is grainier than samples A and B. Hence, its effect on glucose level of group 3 experimental rats was better than those in group 1 and 2. This result is in line with the findings of Salvin (2004), who stated that whole grains affect glucose and insulin responses, partly due to their slow digestibility. According to Salvin (2004), the greater the intake of whole grains, the lower fasting insulin levels will be.

Table 4: Particle Size Analysis of the Composite Flours.

Diet Sample	Sieve size (mm)	Aggregate particle retained (g)	Particle retained (%)	Cumulative particle retained (%)
Fine	1.8	10.3	20.6	
	1.6	15.4	30.8	8.4
	1.4	20.1	40.2	
Medium	1.8	6.2	12.4	
	1.6	12.6	25.2	25.6
	1.4	18.4	36.8	
Coarse	1.8	4.0	8	
	1.6	8.0	16	46
	1.4	15.0	30	

Conclusion

In conclusion, the initial symptoms observed after the induction of diabetes such as body weakness, dehydrated tail, emaciation, loss of weight, rough hair coat, which are indications of diabetes began to disappear after the introduction of the diets on the experimental rats. With the fasting blood glucose level of diet C and its cumulative aggregate particle size grains, it can be concluded that diet C (which had the least fasting blood glucose level range and the highest cumulative aggregate particle size values) is the best diet among the diets (A, B and C). Therefore, the results obtained from these diets were good in the management of diabetes, but diet C which is the coarse particle size diet has much effect because its grainy particles lower the rate of digestion which results to a lowered blood glucose level in individual. Further research needs to be done on this work on the aspect of optimizing other raw materials which will yield the best effect on the hyperglycemic effect on diabetic patients. Also, diabetes should be induced on both the control and experimental rats to determine the effect when fed them with normal feed and the different diets respectively.

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