



Expression of Phosphofructokinase-1 Gene in Streptozotocinized Diabetic Rats Fed Fermented and Non-Fermented Maize Diets

Chukwudi Nze¹ and Osaretin Albert Taiwo Ebuehi^{1*}

¹*Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria, Lagos, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author CN performed the laboratory work, analyzed the data and wrote the manuscript. Author OATE designed, supervised the experiments, critically reviewed and edited the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2021/v13i230384

Editor(s):

(1) Dr. Kristina Mastanjevic, Josip Juraj Strossmayer University of Osijek, Croatia.

Reviewers:

(1) José D. Méndez, Mexican Institute of Social Security, Mexico.
(2) Ricardo Henríquez Flores Carrera de Kinesiología, Universidad Central de Chile, Chile.
Complete Peer review History: <http://www.sdiarticle4.com/review-history/66193>

Received 17 January 2021

Accepted 22 March 2021

Published 20 April 2021

Original Research Article

ABSTRACT

Aim: This nutrigenomic research study is to investigate the impact of fermented maize (FM) and non-fermented maize (N-FM) diets on the expression of phosphofructokinase-1 (PFK-1) gene in a diabetic state.

Methodology: The rats were equally grouped into four for the subsequent two weeks after acclimatization; Group 1 contained streptozotocinized-diabetic rats fed with FM diet (DFM), Group 2 contained streptozotocinized-diabetic rats fed with N-FM diet (DNM), Group 3 contained the normal control rats fed with standard rodent chow (NCG) and Group 4 contained diabetic control rats fed with standard rodent chow (DCG). The total phenol, flavonoid and antioxidant capacity (*in vitro*) of the maize diets were analyzed.

Results: Rats fed the N-FM diet had higher concentration of phenols (73.20±0.9 mg/100 g) and flavonoids (82.83±1.02 mg/100 g). The *in vitro* antioxidant assay showed a statistically significant difference between the FM and N-FM diets (p<0.05). After the two weeks period, animals were sacrificed and blood samples obtained for blood chemistry and lipid profile tests. The livers were harvested for antioxidant activity and gene expression assay. The antioxidant assay showed no

*Corresponding author: Email: oebuehi@unilag.edu.ng, ebuehi@yahoo.com;

statistically significant difference among all groups, as well as the blood chemistry and lipid profile. The gene expression assay carried out using two-step Real-time qPCR, showed that PFK-1 gene was more expressed in the DFM group when compared to the DNM and DCG groups.
Conclusion: The FM diet enhanced the expression of PFK-1 gene in streptozotocinized-diabetic rats.

Keywords: Diabetes; phosphofructokinase-1 gene; antioxidants; blood chemistry; fermentation; maize diet; rats.

ABBREVIATIONS

PFK-1 : Phosphofructokinase-1
 ATP : adenosine triphosphate
 FM : fermented maize
 N-FM : non-fermented maize
 F-1, 6-BP : fructose-6-phosphate into fructose-1, 6-bisphosphate
 AMP : Adenosine monophosphate
 F6P : fructose-6-phosphate
 TPC : total phenol content
 GAE : gallic acid equivalent
 QE : quercetin equivalent
 DPPH : 1, 1-diphenyl-2-picrylhydrazyl
 NO : Nitric oxide
 DTNB : 5, 5-dithiobisnitro benzoic acid

fructose-6-phosphate (F6P) by phosphofructokinase-2 (PFK-2). Hence, an abundance of F6P results in a higher concentration of fructose 2, 6-bisphosphate (F-2, 6-BP) [8]. The precise regulation of PFK-1 prevents glycolysis and gluconeogenesis from occurring simultaneously. However, there is substrate cycling between F6P and F-1, 6-BP. Fructose-1, 6-bisphosphatase (FBPase) catalyzes the hydrolysis of F-1, 6-BP back to F6P, the reverse reaction catalyzed by PFK-1. There is a small amount of FBPase activity during glycolysis and some PFK-1 activity during gluconeogenesis. This cycle allows for the amplification of metabolic signals as well as the generation of heat by ATP hydrolysis [8].

1. INTRODUCTION

Diabetes mellitus is characterized by elevated blood glucose levels, the result of perturbations of glucose uptake and metabolism in both the liver and extra-hepatic tissues. In addition to an increase in gluconeogenic activity in liver, a suppression of hepatic glycolysis contributes to the elevation of blood glucose levels [1,2]. There is also evidence that the decline in net glycolytic flux in diabetes may be associated with an increase in glucose/glucose 6-phosphate cycling, whereby glucose is taken up by the liver and phosphorylated, but the glucose 6-phosphate formed is subsequently dephosphorylated and returned to the circulation [3,4,5,6]. Depending on the types of cells where glycolysis occurs, it is regulated at several rate-limiting steps such as glucose uptake, glucose phosphorylation, and/or conversion of fructose-6-phosphate into fructose-1, 6-bisphosphate (F-1, 6-BP) [7].

Phosphofructokinase-1 (PFK-1) catalyzes the first committed and rate determining step of glycolysis, thus representing an essential metabolic control point for carbohydrate utilization. PFK-1 is allosterically activated by a high concentration of adenosine monophosphate (AMP), but the most potent activator is fructose 2, 6-bisphosphate, which is also produced from

Although PFK-1 is the most prominent regulatory enzyme in glycolysis, it is not the only regulatory enzyme. Hexokinase, the enzyme catalyzing the first step of glycolysis, is inhibited by its product, glucose-6-phosphate (G6P) [9]. High concentrations of this molecule signal that the cell no longer requires glucose for energy, for storage in the form of glycogen, or as a source of biosynthetic precursors, and the glucose will be left in the blood. For example, when PFK-1 is inactive, the concentration of F6P rises. In turn, the level of G6P rises because it is in equilibrium with F6P. Hence, the inhibition of PFK-1 leads to the inhibition of hexokinase [10]. However, the liver, in keeping with its role as monitor of blood-glucose levels, possesses a specialized isozyme of hexokinase called glucokinase that is not inhibited by G6P. Glucokinase phosphorylates glucose only when it is abundant because it has about a 50-fold affinity for glucose than hexokinase does. The role of glucokinase is to provide G6P for the synthesis of glycogen, a storage form of glucose, and for the formation of fatty acids. The low glucose affinity of glucokinase in the liver gives the brain and muscles first call on glucose when its supply is limited, whereas it ensures that glucose will not be wasted when it is abundant [9].

Pyruvate kinase's function is to catalyze the last step of glycolysis; thereby, generating the

second ATP of glycolysis and pyruvate. It is able to catalyze this step by transferring the phosphate group from phosphoenolpyruvate to ADP [11].

The reason PFK-1 is regarded as the major regulatory enzyme in glycolysis becomes evident upon noting that G6P is not solely a glycolytic intermediate. G6P can also be converted into glycogen or it can be oxidized by the pentose phosphate pathway. The first irreversible reaction unique to the glycolytic pathway, the committed step, is the phosphorylation of F6P to F-1, 6-BP. Thus, it is highly appropriate for PFK-1 to be the primary control site in glycolysis [9].

The aim of this study is to assess the potency of fermented and non-fermented maize (*Zea mays* L., Poaceae) diets in energy-yielding metabolism in a diabetic state, as it relates to the expression of the PFK-1 gene. The specific objectives include evaluation of the antioxidant capacities of the fermented and non-fermented maize diets, determination of the effects of the maize diets on blood chemistry parameters, including liver function, renal function and lipid profile.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty healthy male albino rats weighing between 120-200 g were purchased for the study. They were randomly divided into four groups. Each group was housed in a polypropylene plastic cage during the two weeks acclimatization period, on a 12 h light and 12 h dark cycle at room temperature (about 25 °C), while being fed a standard rodent chow diet and water *ad libitum* during the period of acclimatization and their body weights measured twice weekly.

2.2 Experimental Diets

The whole maize grains (white variety) used for this study was procured from Ojuwoye market, Mushin, Lagos, Nigeria, and divided into two portions. The first portion was thoroughly washed in clean water, air-dried, steeped in clean water and left standing at room temperature for 4 days, to give room for fermentation to take place. During this period, the water was replaced with fresh water after each day. On the fourth day, the water was decanted and the maize grains, now softened and fermented, was transported to a local corn mill where it was ground to paste. A sizeable piece of Chiffon cloth was draped over a

big plastic bowl and tied up, acting as a sieve during rinsing (of the ground maize), to separate the chaff that may be present in the paste. All the chaff recovered was discarded. The resulting mixture was left standing at room temperature for 3 hours, to allow the paste completely settle at the bottom of the bowl. After this, the water above was decanted and the residual paste poured into a Muslin bag which was tied tight to expel more water from the resulting paste. After the water had been drained to a large extent, the Muslin bag was tied even tighter and left overnight to ensure a near perfect draining of water, and to give the resulting fermented product, its characteristic sour taste. The following day, it was brought out of the bag, ready for consumption.

The second portion however, which is non-fermented, was sorted, cleaned and also taken to the local corn mill, where it was ground to a powdery form.

2.3 Induction of Diabetes

After acclimatization, the animals were subjected to an 18 h overnight fast prior to the induction of diabetes, and their body weights were recorded. 250 g of streptozotocin was dissolved in 10 ml citrate buffer (0.1M, pH 7.4) and dosed in a volume of 0.4 ml/kg intraperitoneal injection (60 mg/kg), which was immediately administered to the animals [12]. Diabetes was confirmed within 48 h after induction, following an 18 h overnight fast. The fasting blood glucose level was determined using a glucometer (ACCU-CHEK Aviva blood glucose meter). Only rats with basal blood glucose level above 200 mg/dL were considered diabetic.

2.4 Experimental Design

For the subsequent two weeks period after acclimatization and induction of diabetes, the animals were randomly re-grouped into 4 groups as stated below:

- I. Group 1 : Streptozotocinized diabetic rats fed with fermented maize diet (n=8) -DFM
- II. Group 2: Streptozotocinized diabetic rats fed with non-fermented maize diet (n=8) -DNM
- III. Group 3: Normal control rats fed with standard rodent chow (n=7)- NCG
- IV. Group 4: Streptozotocinized diabetic control rats fed with standard rodent chow (n=7) – DCG.

This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animals [13] and approved by the Health Research Committee of the College of Medicine, University of Lagos, Lagos.

2.4.1 Determination of phenol, flavonoid contents and antioxidant capacity of fermented and non-fermented maize diets

Aqueous extraction was carried-out by weighing 10 g of each of the two samples, followed by homogenization in 200 ml of water. The mixtures were left standing for 24 h under room temperature and sieved using a Muslin cloth. The resulting filtrate was subjected to further analysis.

2.4.2 Total phenol content

The total phenol content (TPC) was determined using Folin Ciocalteu's method as described by Gillespie and Ainsworth [14], with some modifications. The reaction mixture was prepared by mixing 1ml plant extracts (1 mg/ml), 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in 13 ml of deionized water followed by the addition of 2.5 ml of 7.5% NaHCO₃ solution. The mixture was mixed thoroughly and incubated in a thermostat at 45°C for 45 minutes. The blank solution was also prepared. The absorbance was determined using a spectrophotometer at 760 nm. All the analyses were repeated three times and the mean value of absorbance was obtained. The same steps were repeated for gallic acid (standard solution). TPC was determined by extrapolating calibration line which was construed by gallic acid solution and expressed as gallic acid equivalent (mg GAE) per gram of the extract.

2.4.3 Determination of total flavonoid content

The total flavonoids content of the plant extract was determined by using aluminum chloride calorimetric method based on the methodology reported by Priečina and Karlina [15], with some modifications. 1ml of plant extract was added to 1ml of methanol to mix, followed by addition of 0.5 ml aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM). The mixture was allowed to stand for 30 minutes at room temperature, after which absorbance measured at 415 nm. Quercetin was used to make the calibration curve. The total flavonoid content was expressed in terms of quercetin equivalent (mg

QE/g of sample). All the analyses were repeated three times and the mean value of absorbance was obtained.

2.4.4 Antioxidant capacity determined by DPPH radical scavenging activity

The antioxidant activity of the extracts were quantitatively assessed on the basis of free radical scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the reported method of Pisoschi et al. [16], with slight modification. 0.1 mM solution of DPPH in ethanol was prepared; 1ml of the solution was added to 1 ml of extract in water at different concentrations (25-100 µg/ml). Incubation of the resulting solution was carried out for 30 minutes in dark room at 37°C. Ascorbic was used as positive control under the same assay condition. The absorbance was measured at 517 nm by using a UV-Visible spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The experiments were carried out in triplicate. The percentage inhibition was calculated using the following formula:

$$\text{DPPH Scavenging Activity (\%)} = \left[\frac{A_o - A_s}{A_o} \right] \times 100.$$

Here, "A_o" is the absorbance of the control (no sample, DPPH solution only) and "A_s" is the absorbance in the presence of the standard sample. The IC₅₀ (half maximal inhibitory concentration) value represented the concentration of the compounds that caused 50% inhibition of DPPH radical formation.

2.4.5 Antioxidant capacity determined by Nitric oxide (NO) scavenging activity

2 ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of both samples at various concentrations (25–100 µg/ml), and incubated for 30 minutes at 25°C. After the incubation period, 0.5 ml of the incubated solution is withdrawn and mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 minutes with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. Absorbance was taken at 550 nm and ascorbic acid was used as the standard control. The amount of nitric oxide radical inhibition is calculated following this equation:

% inhibition of NO radical: $[A_0 - A_1] / A_0 \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance of sample after reaction has taken place with Griess reagent.

2.4.6 Blood sample collection and organ harvesting

At sacrifice, the orbital sinus method was employed in blood sample collection. The animals were scuffed with the thumb and forefinger of the non-dominant hand and skin around the eye was pulled till it became taut. This was followed by inserting a capillary tube into the medial canthus of the eye (30° angle to the nose). Slight thumb pressure was exerted to puncture the tissue and enter the plexus/sinus. Once punctured, blood flowed through the capillary tube into the already positioned lithium heparinized blood sample collection bottle. As soon as the required volume of blood was reached, the capillary tube was gently removed and wiped with sterile cotton. Blood collected was immediately centrifuged for the blood chemistry and lipid profile analysis. Hereafter, the livers were gently separated and isolated using scissors and forceps and deposited in separate petri dishes filled with sterilized phosphate buffer saline in preparation for and for antioxidant and gene expression assays.

2.5 Antioxidant Activity

2.5.1 Sample homogenization

Portions of the liver were washed in an ice cold 1.15% KCl solution, blotted and weighed. They were homogenized with 0.1 M phosphate buffer (pH 7.2), putting the organs each into the mortar; laboratory sand was added to it (acid washed sand) and it was blended in the mortar with pestle together. The resulting homogenate was centrifuge at 2500 rpm speed for 15 minutes and it was removed from the centrifuge and the supernatant was decanted.

2.5.2 Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigman [17]. The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCl was used

to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 minutes.

2.5.3 Reduced glutathione (GSH) determination

The reduced glutathione (GSH) content of liver tissue as non-protein sulphhydryls was estimated according to the method described by Sedlak and Lindsay [18]. To the homogenate 10% TCA was added, centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

2.6 Blood Chemistry and Lipid Profile Analysis

Blood samples were centrifuged at 3000 rpm to obtain plasma fractions. Blood chemistry analysis, plasma concentration of total cholesterol, high density lipoprotein (HDL) and triglyceride concentrations were measured using an automatic analyzer, AU-400 Olympus (Randox Laboratories, San Francisco, USA).

2.7 Gene Expression Assay

Minute portions of the liver were excised and immediately placed in an NaOH-sterilized petridish already sitting on ice. The NaOH sterilization was necessary to avoid RNase contamination. The samples were further rinsed in PBS (Phosphate Buffer Saline), to remove traces of blood, after which they were introduced into already standing tubes containing chilled proteinase K. Total RNA extraction was done using Quick-RNA™ Miniprep Plus Kit (Zymo Research, USA). RNA shield™ was added to the solid liver tissue samples. For optimal extraction efficiency, the samples were mechanically homogenized using TissueLyser II (Qiagen). cDNA synthesis was done using FireScript cDNA synthesis kit (SolisBiodyne, Estonia). After thawing (of the kit and RNA template), 2 µl of the RNA template was mixed with 1 µl oligo (dT) primer 65°C (100 µM) and 13 µl of nuclease free water. The resulting mixture was incubated at for 5 minutes and then placed on ice. cDNA was generated from 50°C the RNA template in a reaction catalyzed by reverse transcriptase at in 30 minutes. Caution was taken to ensure that the

volume of cDNA did not exceed 1/10 of the qPCR reaction volume. The 5x HOT FIREPol® EvaGreen® qPCR Supermix (SolisBiodyne, Estonia) was used for the Real-time-quantitative polymerase chain reaction. Forward and reverse primers (10pmol/ μ l respectively), were mixed with 5 μ l cDNA template, 4 μ l 95°C optimized buffer and 10.2 μ l PCR grade H₂O. Initial activation of the cycle was done at for 12 minutes (in one cycle), followed by denaturation of the cDNA template, which was accomplished at 95°C in 15 seconds. Annealing of the primers to their complementary sequence in the cDNA template was achieved at 54°C in 20 seconds, while the elongation step occurred at 72°C in 20 seconds. In this step, the polymerase enzyme sequentially added bases to the 3' of each primer, extending the DNA sequence in the 5' and 3' direction. Denaturation, annealing and elongation steps were carried out in 40 cycles each. The SYBR reporter dye was used, while the relative quantification method (comparative 2^{- $\Delta\Delta$ CT} method) was employed to analyze data retrieved from the reaction.

2.8 Statistical Analysis

Values are expressed as means \pm standard deviation. The differences among the samples were analyzed using one-way ANOVA. A two-tailed unpaired Student's t-test was used to compare the total phenol, flavonoid contents and antioxidant capacities of the fermented and non-fermented maize diets. A value of $p < 0.05$ was considered to be statistically significant. Significant differences are indicated by different lowercase letters. All data generated were analyzed using SPSS version 20.

3. RESULTS

3.1 Initial and Final Mean Body Weights of Normal and Diabetic Rats

As presented on Table 1, animals in the DFM group experienced a statistically significant ($p < 0.05$) decrease in mean body weight (from 151.8 \pm 18.0 to 140.8 \pm 18.4). The same trend continued in the DNM group, which saw a 13% decrease in mean body weight. However, this decrease is not statistically significant. There was also a significant increase of 8.3% in mean body weights of animals in the NCG, while the DCG animals experienced a significant decrease in mean body weights (from 149 \pm 22.9 to 126.2 \pm 24.5), totaling up to 15.3%.

3.2 Total Phenol, Flavonoid Contents and Antioxidant Capacity of Fermented and Non-Fermented Maize Diets

The results from Table 2 below show that the Phenol content of non-fermented maize diet (73.20 \pm 0.9 mg/100 g) is significantly higher ($p < 0.05$) than that of the fermented maize diet (5.28 \pm 0.77 mg/100 g), ditto the flavonoid content (82.83 \pm 1.02 mg/100 g and 7.0 \pm 0.41 mg/100 g respectively). The antioxidant capacity determination revealed a significant difference between the two test diets, as the non-fermented maize diet was shown to have a higher antioxidant capacity (75.82 \pm 0.64 mg/100 g) compared to the fermented maize diet (70.18 \pm 0.37 mg/100 g).

3.3 DPPH Scavenging Activity of Fermented and Non-Fermented Maize Diets

The results of DPPH scavenging activity on Table 3 below reveal that at 25 μ g/ml, there is a significant difference between the DPPH percentage inhibition potential of fermented and non-fermented maize diets (12.24 \pm 0.65 % and 23.40 \pm 0.64% respectively). However, the standard control, ascorbic acid, showed the highest percentage inhibition at the same concentration (45.05 \pm 0.48%).

3.4 Nitric Oxide (NO) Radical Scavenging Activity of Fermented Maize Diets

Results on Table 4 present the findings on the NO radical scavenging assay. A steady increase in percentage inhibition potential was also observed (from 17.92 \pm 1.64% to 84.91 \pm 0.53%), as the concentrations of the extracts rose from 25 μ g/mL through 100 μ g/mL, with the fermented maize diet showing the lowest inhibition percentage. The fermented maize diet was also found to have the highest IC₅₀ value of 73 μ g/mL, followed by the non-fermented maize diet (56.6 μ g/mL) and ascorbic acid (26 μ g/mL). This portrays ascorbic acid as having the highest inhibitory activity, while the other two samples had inhibitory concentration value higher than the 50 mark.

3.5 Liver Antioxidant Activity of Control and Diabetic Rats

The results on Table 5 show that no statistically significant difference was observed in the mean values obtained from the rats in the four groups

subjected to the assay ($p > 0.05$). However, rats in the DCG showed the highest mean concentration (3.3 ± 1.1 and 17.2 ± 2.9) in the SOD activity assay. In the GSH analysis, the DFM rats showed the highest degree of mean concentration (36.3 ± 5.4), followed by rats in the DCG (34.2 ± 2.2).

3.5.1 Blood chemistry of control and diabetic rats

Table 6 presents the findings on the effects of fermented maize and non-fermented maize diets on the blood chemistry parameters measured. The findings reveal that the DCG had the highest alkaline phosphatase (ALP) activity (319.7 ± 29.8) while the DNM group had the lowest (251.6 ± 36.8). The differences observed in all groups were not statistically significant ($p < 0.05$).

The same trend was observed in all the other blood chemistry parameters tested, as shown.

3.5.2 Lipid profile of control and diabetic rats

The results displayed on Table 7 below show that the maize diets did not have any significant effect on the lipid profile of both normal and diabetic rats distributed in all groups.

3.6 Gene Expression Analysis

Table 8 shows the results garnered from the spectrophotometric analysis of mRNA integrity prior to its quantification by gel electrophoresis (as shown in Fig. 1). The results obtained from the relative/comparative quantification ($2^{-\Delta\Delta Ct}$) method employed for the gene expression analysis are displayed in Tables 9, 10 and 11.

Table 1. Initial and final mean body weights of normal and diabetic rats fed fermented and non-fermented maize diets

Group	Initial (g)	Final (g)
DFM	151.8 ± 18.0^a	140.8 ± 18.4^b ($\downarrow 7.2$)
DNM	147.8 ± 36.5^a	128.5 ± 14.6^a ($\downarrow 13.1$)
NCG	148 ± 7.5^a	160.3 ± 7.3^b ($\uparrow 8.3$)
DCG	149 ± 22.9^a	126.2 ± 24.5^b ($\downarrow 15.3$)

Values are expressed as mean \pm standard deviation. Means with different superscript in each row are significantly different at p value < 0.05 . Figures in parenthesis indicate percentage decrease \downarrow or increase \uparrow in body weights. DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow

Table 2. Total phenol, flavonoid contents and antioxidant activity of rats fed fermented and non-fermented maize diets

Sample	Phenol (mg/100 g)	Flavonoid (mg/100 g)	Antioxidant activity (mg/100 g)
Fermented maize	5.28 ± 0.77^a	7.0 ± 0.5^a	70.18 ± 0.37^a
Non-fermented maize	73.20 ± 0.9^b	82.83 ± 1.25^b	75.83 ± 0.64^b

Values are expressed as mean \pm standard deviation of three replicates. Means with different superscript in the same column are significantly different at p value < 0.05

Table 3. DPPH scavenging activity of rats fed fermented and non-fermented maize diets with ascorbic acid as standard

DPPH inhibition (%)				
Concentration ($\mu\text{g/mL}$)				
Sample	25	50	75	100
Fermented maize	12.24 ± 0.65^a	45.49 ± 0.7^a	67.36 ± 0.53^a	77.64 ± 0.85^a
Non-fermented maize	23.40 ± 0.64^b	50.46 ± 0.7^b	64.31 ± 0.467^b	75 ± 0.92^a
Ascorbic acid	45.05 ± 0.48^c	56.55 ± 0.96^c	76.92 ± 0.54^c	89.83 ± 0.36^b

Values are expressed as mean \pm standard deviation of three replicates. Means with different superscript in the same column are significantly different at p value < 0.05

Table 4. Nitric oxide (NO) radical scavenging activity of rats fed fermented and non-fermented maize diets with ascorbic acid as standard

NO inhibition (%)				
Concentration (µg/mL)				
Sample	25	50	75	100
Fermented maize	17.92±1.64 ^a	26.08±0.74 ^a	47.46±0.79 ^a	65.7±0.88 ^a
Non-fermented maize	32.75±0.88 ^b	47.66±1.05 ^b	60.31±0.83 ^b	71.42±0.64 ^b
Ascorbic acid	47.89±0.24 ^c	63.09±0.42 ^c	76.07±0.82 ^c	84.91±0.53 ^c

Values are expressed as mean ± standard deviation of three replicates.
Means with different superscript in the same column are significantly different at *p* value <0.05.

Table 5. Liver antioxidant activity of control and diabetic rats fed fermented and non-fermented maize diets

GROUP SOD	GSH	
(µmol/ml/min/mg Prot.)	(µmol/ml)	
DFM	2.3±0.5 ^a	36.3±5.4 ^a
DNM	2.9± 1 ^a	31.1±4.7 ^a
NCG	3.2±0.8 ^a	28.5±1.0 ^a
DCG	3.3±1.1 ^a	34.2±2.2 ^a

Values are expressed as mean ± standard deviation of three replicates. Means with same superscript in each column are not significantly different. *p* value <0.05 is significant. DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow SOD: Superoxide dismutase, CAT: catalase, GSH: Reduced glutathione, MDA: Malondialdehyde

4. DISCUSSION

4.1 Effect of Fermented and Non-fermented Maize Diets on Mean Body Weight of Normal and Diabetic Rats

The results shown on Table 1 are in consonance with a previous study conducted by Mohal et al. [19]. Animals in the DCG suffered the highest weight loss as shown. It is safe to attribute this to the excessive lipolysis taking place in the adipose tissues, and glycogenolysis in the liver and muscle, characteristic of a diabetic condition, as insulin which is needed to mobilize glucose transporters, which transport glucose into the cells where they are utilized, is deficient. It was also observed that even though the diabetic animals fed with fermented maize diet experienced a significant decrease in mean body weight (7.2%), this difference is quite less when compared to the percentage loss in the mean body weight of animals in the DCG. It is worthy to note that the anaerobic breakdown (fermentation) of starch present in the maize grain, rendered glucose readily available for uptake especially by the Na⁺-glucose co-transporter, since insulin which is responsible for the mobilization of glucose transporters, is deficient. This may be the reason for the lower percentage weight loss observed

in the diabetic animals fed with fermented maize diet.

4.2 Total Phenol, Flavonoid Contents, and Antioxidant Capacity of Fermented and Non-fermented Maize Diets

According to the results on Table 2, we can suggest that the phenolic compounds present in our samples act as antioxidant by acting as reducing agents which convert free radicals into stable compounds [20]. These results are similar to previous studies which demonstrate the antioxidant potentials of plant phytochemicals [21,22]. Phenolics exist in significant quantities as insoluble bound form especially in grains, while this phenomenon is quite opposite in fruits and vegetables [23]. Maize and other grains have been reported to have much more bound than free phenolics [24]. Bound phenolics in maize mainly distribute in the bran fraction [25]. The phenolic profiles of common maize have been reported previously. Adom and Liu [24], found that maize had much higher total phenolic and flavonoid content than other whole grains including rice, wheat, and oats. Ferulic acid and p-coumaric acid, which are hydroxycinnamic acid derivatives, were reported to be the predominant phenolic acids detected in maize and other grains. Phenolics are synthesized in plants as

secondary metabolites during normal growth and development. However, more phenolics can be produced when the plants suffer from stress conditions such as infection, wounding and UV radiation [26]. Due to its diverse health benefits, including anti-oxidant, anti-inflammatory, and anti-cancer activities, ferulic acid is regarded as one of the most important phenolic acids and receiving increasingly more attention [27]. The antioxidant and cardiovascular protective properties of p-coumaric acid makes it to be of

great interest [28]. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, chelators of metal catalyst and singlet oxygen quenchers [29]. Flavonoids are the largest group of phenolic compounds in maize. It has been reported by epidemiological studies that high consumption of flavonoids reduce the risk of chronic diseases including cardiovascular diseases, diabetics, and cancers [30].

Table 6. Blood chemistry of control and diabetic rats fed fermented and non-fermented maize diets

Group	Alkaline phosphatase (IU/L)	Aspartate transaminase (IU/L)	Alanine transaminase (IU/L)	Bilirubin (µmo1/L)	Creatinine (mg/dL)	Urea (mg/ml)
DFM	276.1±38.1 ^a	226.4±93.8 ^a	83.4±0.6 ^a	1.0±0.4 ^a	71.9±19 ^a	6.2±1.2 ^a
DNM	251.6±36.8 ^a	174.2±54.2 ^a	39.2±26.0 ^a	1.1±0.2 ^a	55±9.2 ^a	7.3±1.1 ^a
NCG	268.2±36.8 ^a	216.1±12.7 ^a	79.2±28.5 ^a	1.6±0.2 ^a	81.4±1.6 ^a	7.6±1.1 ^a
DCG	319.7±29.8 ^a	215.4±57.3 ^a	107.5±42.7 ^a	1.4±0.4 ^a	60.1±15.7 ^a	7.4±0.3 ^a

Values are expressed as mean ± standard deviation. Means with same superscript in each column are not significantly different. *p* value <0.05 is significant. DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow

Table 7. Lipid profile of control and diabetic rats fed fermented and non-fermented maize diets

Group	Triglycerides (mg/dl)	High density lipoprotein (mg/dl)	Cholesterol (mg/dl)
DFM	0.6±0.2 ^a	1.1±0.1 ^a	1.1±0.04 ^a
DNM	0.8±0.7 ^a	1.2±0.05 ^a	1.3±0.5 ^a
NCG	0.6±0.3 ^a	1.2±0.3 ^a	1.2±0.1 ^a
DCG	0.3±0.04 ^a	1.1±0.3 ^a	1.3±0.1 ^a

Values are expressed as mean ± standard deviation. Means with same superscript in each column are not significantly different. *p* value <0.05 is significant. DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow

Table 8. mRNA quantification of liver of control and diabetic rats fed fermented and non-fermented maize diets

Group	Concentration (ng/µl)	A 260 (nm)	A 280 (nm)	260/280	260/230
DFM	442.45	11.061	5.937	1.86	2.01
	1211.74	30.294	14.834	2.04	2.23
	601.82	15.045	7.308	2.06	2.09
DNM	569.74	14.243	7.043	2.02	1.92
	1090.54	27.263	13.315	2.05	2.13
	388.91	9.723	5.101	1.91	1.78
NCG	354.32	8.858	4.681	1.89	1.60
	178.54	4.463	2.409	1.85	1.10
	979.32	24.483	11.993	2.04	2.06
DCG	192.35	4.809	2.338	2.06	1.90
	264.36	6.609	3.236	2.03	2.24
	418.95	10.474	5.544	1.89	2.04

DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow

Table 9. Cycle threshold (C_t) values obtained from RT- qPCR assay of rats fed fermented and non-fermented maize diets

	SOD	HPRT
DFM	207	25.884
	28.823	23.982
	19.515	26.177
DNM	40	25.241
	18.705	25.241
	20.290	24.632
NCG	19.831	20.068
	21.36	24.552
	24.772	22.405
DCG	18.535	23.497
	22.051	24.944
	19.114	21.965
Negative control	30.354	27.839
	28.748	32.37

DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow
SOD: Superoxide dismutase, HPRT: Hypoxanthine-guaninephosphoribosyl transferase

Table 10. 2^{-ΔΔCt} Analysis (1) of rats fed fermented and non-fermented maize diets

	HPRT	SOD	ΔCT control (SOD-HPRT)	ΔCT test (SOD-HPRT)	ΔΔCT SOD (ΔCt test-ΔCt control)	Relative fold change (2 ^{-ΔΔCT})
NCG	20.068	19.832	-0.237		0	1
	24.552	21.36	-3.192		0	1
	22.405	24.772	2.367		0	1
DFM	25.884	20.7		-5.184	-4.947	0.03242
	23.982	20.832		-3.15	0.042	1.02954
	26.177	19.515		-6.662	-9.029	0.001914
DNM	25.241	19.4		-5.841	-5.604	0.02056
	22.291	18.705		-3.586	-0.394	0.76102
	24.632	20.298		-4.334	-6.701	0.00961
DCG	23.497	18.535		-4.962	-4.725	0.03781
	24.944	22.051		-2.893	0.299	1.23029
	21.965	19.144		-2.851	-5.218	0.02687

DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow.
SOD: Superoxide dismutase, HPRT: Hypoxanthine-guaninephosphoribosyl transferase



Fig. 1. mRNA quantification by gel electrophoresis

SOD: Superoxide dismutase, HPRT: Hypoxanthine-guaninephosphoribosyl transferase.,,NC: Negative control, PC: Positive control. The numbers 1-4 represent all four groups.

Table 11. 2 ^{-ΔΔCt} Analysis (2) of rats fed fermented and non-fermented maize diets

	HPRT (Group 3) SOD	ΔCT control (SOD-HPRT)	ΔCT test (SOD-HPRT)	ΔΔCT SOD (ΔCt test-ΔCt control)	Relative fold change (2 ^{-ΔΔCT})
NCG 20.068	19.832	-0.237		0	1
24.552	21.36	-3.192		0	1
22.405	24.772	2.367		0	1
DFM 20.068	20.7		0.632	0.869	1.82640
24.552	20.832		-3.72	-0.528	0.69352
22.405	19.515		-2.89	-5.257	0.02615
DNM 20.068	19.4		-0.668	-0.431	0.74175
24.552	18.705		-5.841	-2.665	-0.15877
22.405	20.298		-2.107	-4.474	0.04450
DCG 20.068	18.535		-1.533	-1.296	0.40725
24.552	22.051		-2.501	0.691	1.61440
22.405	19.144		-3.291	-5.658	0.01981

DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow.
SOD: Superoxide dismutase, HPRT: Hypoxanthine-guaninephosphoribosyl transferase

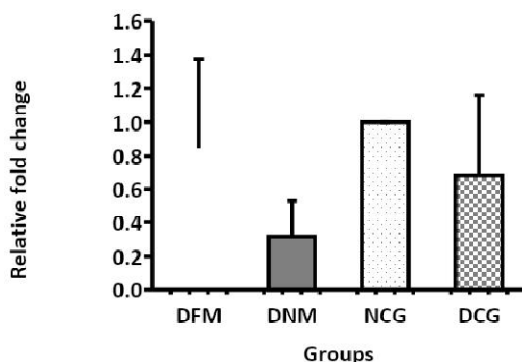


Fig. 2. The relative fold change plot of the ^{-ΔΔCT} analysis (2) the showing the pattern of expression of PFK-1 gene of rats fed fermented and non-fermented maize diets Diabetic rats in the DFM group show a higher level of expression of the PFK-1 gene, only next to the rats in the NCG

DFM: Diabetic rats fed with fermented maize diet, DNM: Diabetic rats fed with non-fermented maize diet, NCG: Normal rats fed with standard rodent chow, DCG: Diabetic control group fed with standard rodent chow

4.3 Antioxidant Activity of rats fed Fermented and Non-fermented Maize Diets

The scavenging ability of DPPH free radical is widely used to analyze the antioxidant potential of naturally derived foods and plants [31]. The results on Table 3 also show that the percentage inhibition was on the increase, even as the concentration of the extracts increased. This is in tandem with previous studies which

demonstrated that DPPH scavenging properties of plant extracts increased with the concentration of extracts [32,33,34]. The calculated inhibitory concentration (IC₅₀) for fermented maize diet, non-fermented maize diet and ascorbic acid turned out to be 52.9 µg/ml, 57.6 µg/ml and 34.9 µg/ml respectively. This shows that ascorbic acid has the highest inhibitory activity, followed by the fermented maize diet since the IC₅₀ of the plant samples is inversely proportional to their antioxidant powers.

The mechanism involved in the scavenging activity of plant extracts may be attributed to the phenolic compounds found in the plant extracts as described in previous studies [22, 34]. Furthermore, the radical-scavenging activity of polyphenols is attributed to its molecular structure, the substitution pattern of the hydroxyl groups, the availability of phenolic hydrogen and the possibility of stabilization of the resulting HO· and NO· radicals via hydrogen donation or through expansion electron delocalization [35]. Previous study suggests that the flavonoid found in the plants exerts the antioxidant action by donating of a hydrogen atom to break the free radical chain [36].

4.4 Liver Antioxidant Activity of Control and Diabetic Rats

Although no statistically significant difference was observed as shown on Table 5, the results on Table 2 suggests that the maize diet have promising antioxidant activities. Enzymatic antioxidants are grouped within the primary and secondary defense systems. The primary

defense is formed by three crucial enzymes capable of preventing the occurrence or neutralizing free radicals: glutathione peroxidase, which donates two electrons that reduce peroxides, catalase that decomposes hydrogen peroxide into water and molecular oxygen, and SOD that turns superoxide anions into hydrogen peroxide [20,37]. The secondary enzymatic defense comprises glutathione reductase and glucose-6-phosphate dehydrogenase. Glutathione reductase turns glutathione into its reduced form, thus recycling it. G6P reforms reductive NADPH [38,39]. Although these two enzymes do not directly neutralize free radicals, they promote the endogenous antioxidants' activity [20]. It has been assessed that enzymatic antioxidants act by decomposing free radicals and in this case damaging oxidative species are converted into hydrogen peroxide and water, while nonenzymatic antioxidants are mainly chain breakers. For instance, it has been reported that tocopherol disrupts a radical oxidation chain after five reactions [40]. The intake of antioxidants from diet is always meant to counterpart the organism's antioxidant defense. Enzymatic natural antioxidants in food (SOD, glutathione peroxidase, and catalase) can be inactivated during processing [41].

4.4.1 Effects of fermented and non-fermented maize diets on blood chemistry parameters of control and diabetic rats

The observations recorded on Table 6 may be due to the fact that the time of evolution of diabetes was not sufficient for these metabolic changes to manifest [42]. A limited number of prospective studies have examined the associations of aspartate transaminase (AST), alanine transaminase (ALT), and other liver markers with risk of subsequent diabetes [43]. In two articles, it was reported that there was no association between ALP concentrations and diabetes risk [44,45], an observation that may be related to the lack of specificity of ALP in indicating liver disease.

4.4.2 Effect of fermented and non-fermented maize diets on lipid profile of control and diabetic rats

Administration of the test diets to the diabetic rats did not show any statistically significant difference to the normal and untreated diabetic control groups in relation to their plasma lipid profiles (Table 7). This is consistent with study conducted by Thompson et al., where animals

that were fed with cafeteria diet with or without addition of micronized maize fibre did not present differences in triglycerides, cholesterol and HDL concentrations, demonstrating that the corn residue has no influence on the plasma lipid profile [46]. Similar results were verified in rats by Vidal-Quintanar et al. [47]. However, the results from a study carried out by Maki et al., showed that approximately 54g/day of corn oil, when consumed as part of a weight-maintenance, low-saturated fatty acid and cholesterol diet by men and women with hypercholesterolemia, resulted in significantly larger reductions from baseline in LDL-Cholesterol, non-HDL-Cholesterol, and the total-Cholesterol/HDL-Cholesterol ratio compared with extra virgin olive oil. Triglyceride concentrations increased with both the corn oil and extra virgin olive oil conditions. However, the degree of triglyceride elevation was smaller with corn oil compared with extra virgin olive oil conditions [48].

4.4.3 Effect of fermented and non-fermented maize diets on expression of PFK-1 gene of control and diabetic rats

The reason for elevation in the levels of PFK-1 gene in the diabetic animals fed with FM diet (DFM) cannot be readily ascertained but can be attributed to a number of factors, including dietary patterns. Diet is an important regulator of gene expression [49]. Dietary patterns may impact gene expression through several mechanisms, for example, certain dietary compounds bind to transcription factors and regulate their activity such as polyunsaturated fatty acids (PUFA) with peroxisome proliferator-activated receptors (PPARs) [50]. Studies regarding energy restricted diets and their effects on gene expression levels have observed down-regulation of genes involved in glycolytic and lipid synthesis pathways [51,52]. The discovery of the galactose operon in bacteria represented a key finding for the study of the regulation of metabolism. That work showed how, by modifying the level of expression of specific enzymes, bacteria can adapt their metabolism to meet their nutritional needs, and it connected, for the first time, changes in enzymatic activity to the transcriptional control of gene expression [53]. It is now commonly accepted that transcriptional regulation also contributes to metabolic homeostasis in complex organisms [54]. The alteration of the mechanisms controlling gene expression (from transcription to translation), may lead to the development of metabolic diseases [54]. Thus, understanding the effect of

nutrients on gene expression may improve our knowledge of metabolic diseases and may offer new therapeutic approaches based on nutritional interventions and individual genetic makeup. For instance, the risk of having a metabolic syndrome caused by a disruption of energy homeostasis is associated with overweight and obesity. This association stresses the link between lipid and glucose metabolism [54]. van Dijk et al., have observed a more pro-inflammatory gene expression profile following a diet high in saturated fat compared to a diet high in monounsaturated fat [55]. Saturated fats can modulate the expression of Toll-like receptors (TLRs) therefore increasing the expression of pro-inflammatory genes [56]. Results observed from the study carried out by Bouchard-Mercier et al., support the scientific evidence regarding the beneficial effects of the consumption of a healthy diet and the deleterious impacts of a western dietary pattern [57]. These results also seem to indicate that gene expression profiles and expression of genes in pathways related to chronic disease are influenced by the presence of a few or more dietary characteristics according to a dietary pattern. In addition, it is imperative to note that many other factors associated with dietary patterns may impact gene expression. For example, physical activity has an effect on gene expression profiles [58], environmental factors [59], racial differences [60], among other factors [61,62,63].

4. CONCLUSION

Data retrieved from this nutrigenomic study further lends credence to the biochemical fact that dietary patterns influence gene expression. In other words, we are what we eat [61,62,63]. The study also reveals the benefits of FM diet especially in a diabetic state, as it is has been shown to enhance the expression of PFK-1 gene which is central to carbohydrate metabolism. However, further studies need to be carried out under a more controlled environment, using specific strains of rats, in order garner more facts behind the enhanced expression of PFK-1 gene in a diabetic state, facilitated by a FM diet.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Health Research Ethics Committee of the

College of Medicine, University of Lagos, Lagos, Nigeria (Approval No. CMUL/HREC / 05/20/618).

ACKNOWLEDGEMENTS

The authors are grateful to technical and laboratory assistance received from Dr. Ayorinde James and Dr. Azuka Okwurawe, of the Nigeria Institute for Medical Research, Yaba, Lagos. The efforts of the laboratory technologists of the Department of Biochemistry, College of Medicine, University of Lagos, Lagos, Nigeria, are thankfully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Davidson MB. Autoregulation by glucose of hepatic glucose balance: permissive effect of insulin. *Metabolism*. 1981;30(3):279-84.
2. Gil J, Carreras J, Bartrons R. Effects of diabetes on fructose 2, 6-P₂, glucose 1, 6-P₂ and 6-phosphofructo 2-kinase in rat liver. *Biochemical and Biophysical Research Communications*. 1986;136(2):498-503.
3. Efendic S, Karlander S, Vranic M. Mild type II diabetes markedly increases glucose cycling in the postabsorptive state and during glucose infusion irrespective of obesity. *The Journal of Clinical Investigation*. 1988;81(6):1953-61.
4. Efendic S, Wajngot A, Vranic M. Increased activity of the glucose cycle in the liver: early characteristic of type 2 diabetes. *Proceedings of the National Academy of Sciences*. 1985;82(9):2965-9.
5. Lickley H, Kemmer F, El-Tayeb K, Vranic M. Importance of glucagon in the control of futile cycling as studied in alloxan-diabetic dogs. *Diabetologia*. 1987;30(3):175-82.
6. Rooney D, Neely R, Beatty O, Bell N, Sheridan B, Atkinson A, et al. Contribution of glucose/glucose 6-phosphate cycle activity to insulin resistance in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 1993;36(2):106-12.
7. Rider MH, Bertrand L, Vertommen D, Michels PA, Rousseau GG, Louis H. 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase: head-to-head with a bifunctional enzyme that controls

- glycolysis. *Biochemical Journal*. 2004; 381(3):561-79.
8. Berg JM, Tymoczko JL, Stryer L. *Biochemistry (Loose-Leaf): Macmillan*; 2008.
 9. Berg JM, Tymoczko J, Stryer L. Glycolysis is an energy-conversion pathway in many organisms. *Biochemistry* 5th ed New York: WH Freeman. 2002.
 10. Dandekar T, Schuster S, Snel B, Huynen M, Bork P. Pathway alignment: Application to the comparative analysis of glycolytic enzymes. *Biochemical Journal*. 1999; 343(1):115-24.
 11. Voet D, Voet JG, Pratt CW. *Fundamentals of biochemistry: life at the molecular level*. Hoboken: Wiley. 2006:1264.
 12. Wang-Fischer Y, Garyantes T. Improving the reliability and utility of streptozotocin-induced rat diabetic model. *Journal of Diabetes Research*. 2018;2018.
 13. Health Nlo. *Guide for the care and use of laboratory animals*, rev. ed. Department of Health and Human Services Publication no(NIH). 1985;85:23.
 14. Gillespie KM, Ainsworth EA. Measurement of reduced, oxidized and total ascorbate content in plants. *Nature Protocols*. 2007;2(4):871.
 15. Pricina L, Karlina D, editors. Total polyphenol, flavonoid content and antiradical activity of celery, dill, parsley, onion and garlic dried in conventive and microwave-vacuum dryers. 2nd International Conference on Nutrition and Food Sciences; 2013.
 16. Pisoschi AM, Cheregi MC, Danet AF. Total antioxidant capacity of some commercial fruit juices: electrochemical and spectrophotometrical approaches. *Molecules*. 2009;14(1):480-93.
 17. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Analytical Biochemistry*. 1978;90(1):81-9.
 18. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*. 1968;25:192-205.
 19. Mohal S, Mondal D, Shamim K. Impact of *Momordica Charantia* (Karela) on the Body Weight in the Streptozotocin-Induced Diabetic Rat. *Bangladesh Journal of Anatomy*. 2011;9(2):106-9.
 20. Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*. 2013;51:15-25.
 21. Kumar S, Kumar R, Dwivedi A, Pandey AK. In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of *Syngonium podophyllum* and *Eichhornia crassipes* leaf extracts on isoniazid induced oxidative stress and hepatic markers. *Bio Med Research International*. 2014;2014.
 22. Kumar S, Mishra A, Pandey AK. Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using in vitro models. *BMC Complementary and Alternative Medicine*. 2013;13(1):120.
 23. Acosta-Estrada BA, Gutiérrez-Urbe JA, Serna-Saldívar SO. Bound phenolics in foods, a review. *Food Chemistry*. 2014;152:46-55.
 24. Adom KK, Liu RH. Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*. 2002;50(21):6182-7.
 25. Lapierre C, Pollet B, Ralet M-C, Saulnier L. The phenolic fraction of maize bran: evidence for lignin-heteroxylan association. *Phytochemistry*. 2001;57(5):765-72.
 26. Beckman CH. Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants?. *Physiological and Molecular Plant Pathology*. 2000;57(3):101-10.
 27. Kroon PA, Williamson G. Hydroxycinnamates in plants and food: current and future perspectives. *Journal of the Science of Food and Agriculture*. 1999;79(3):355-61.
 28. Zhang R, Huang L, Deng Y, Chi J, Zhang Y, Wei Z, et al. Phenolic content and antioxidant activity of eight representative sweet corn varieties grown in South China. *International Journal of Food Properties*. 2017;20(12):3043-55.
 29. Shahidi F, Janitha P, Wanasundara P. Phenolic antioxidants. *Critical Reviews in Food Science & Nutrition*. 1992;32(1):67-103.
 30. Kim M-K, Kim H-a, Koh K, Kim H-S, Lee YS, Kim YH. Identification and quantification of anthocyanin pigments in colored rice. *Nutrition Research and Practice*. 2008;2(1):46-9.

31. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*. 2013;21(2):143-52.
32. Gul MZ, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA. Antioxidant and anti-proliferative activities of *Abrus precatorius* leaf extracts-an *in vitro* study. *BMC Complementary and Alternative Medicine*. 2013;13(1):1-12.
33. Hilmi Y, Abushama MF, Abdalgadir H, Khalid A, Khalid H. A study of antioxidant activity, enzymatic inhibition and in vitro toxicity of selected traditional sudanese plants with anti-diabetic potential. *BMC complementary and Alternative Medicine*. 2014;14(1):149.
34. Mishra A, Sharma AK, Kumar S, Saxena AK, Pandey AK. *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant, and anticancer activities. *Bio Med Research International*. 2013;2013.
35. Tongur T, Erkan N, Ayranci E. Investigation of the composition and antioxidant activity of acetone and methanol extracts of *Daphne sericea* L. and *Daphne gnidioides* L. *Journal of Food Science and Technology*. 2018;55(4):1396-406.
36. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine*. 2012;12(1):221.
37. Rahman K. Studies on free radicals, antioxidants, and co-factors. *Clinical interventions in Aging*. 2007;2(2):219.
38. Gamble PE, Burke JJ. Effect of water stress on the chloroplast antioxidant system: I. Alterations in glutathione reductase activity. *Plant Physiology*. 1984;76(3):615-21.
39. Ratnam DV, Ankola D, Bhardwaj V, Sahana DK, Kumar MR. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *Journal of Controlled Release*. 2006;113(3):189-207.
40. Gupta D. Methods for determination of antioxidant capacity: a review. *International Journal of Pharmaceutical Sciences and Research*. 2015;6(2):546.
41. Pisoschi AM, Negulescu GP. Methods for total antioxidant activity determination: A review. *Biochem Anal Biochem*. 2011;1(1):106.
42. Romero TF, Román GS, Pérez CL, Sánchez TA, Hernández SC. Influence of Diabetes and Gestation in Blood Biochemistry Variables in Wistar Rats. 2018.
43. Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB, Kempf J, et al. Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes*. 2004;53(10):2623-32.
44. Nakanishi N, Suzuki K, Tataru K. Serum γ -glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. *Diabetes Care*. 2004;27(6):1427-32.
45. Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, et al. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes*. 2002;51(6):1889-95.
46. Thompson VBdM, Costa NMB, Martino HSD, Paes MCD. The effect of micronized corn fiber on body weight, glycemia, and lipid metabolism in rats fed cafeteria diet. *Food Science and Technology*. 2018;38(3):462-6.
47. Vidal-Quintanar RL, Hernandez L, Conde K, Vergara-Jimenez M, Fernandez ML. Lime-treated corn husks lower plasma LDL cholesterol in guinea pigs by altering hepatic cholesterol metabolism. *The Journal of Nutritional Biochemistry*. 1997;8(8):479-86.
48. Maki KC, Lawless AL, Kelley KM, Kaden VN, Geiger CJ, Dicklin MR. Corn oil improves the plasma lipoprotein lipid profile compared with extra-virgin olive oil consumption in men and women with elevated cholesterol: results from a randomized controlled feeding trial. *Journal of Clinical Lipidology*. 2015;9(1):49-57.
49. Barnes S. Nutritional genomics, polyphenols, diets, and their impact on dietetics. *Journal of the American Dietetic Association*. 2008;108(11):1888-95.
50. Keller HR, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proceedings of the National Academy of Sciences*. 1993;90(6):2160-4.
51. Franck N, Gummesson A, Jernås M, Glad C, Svensson P-A, Guillot G, et al. Identification of adipocyte genes regulated

- by caloric intake. *The Journal of Clinical Endocrinology & Metabolism*. 2011;96(2): E413-E8.
52. Ong KR, Sims AH, Harvie M, Chapman M, Dunn WB, Broadhurst D, et al. Biomarkers of dietary energy restriction in women at increased risk of breast cancer. *Cancer Prevention Research*. 2009;2(8):720-31.
53. Jacob F, Monod J. Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology*. 1961;3(3):318-56.
54. Haro D, Marrero PF, Relat J. Nutritional regulation of gene expression: Carbohydrate-, fat-and amino acid-dependent modulation of transcriptional activity. *International Journal of Molecular Sciences*. 2019;20(6):1386.
55. van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG, et al. A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. *The American Journal of Clinical Nutrition*. 2009;90(6):1656-64.
56. Lee JY, Hwang DH. The modulation of inflammatory gene expression by lipids: mediation through Toll-like receptors. 2006.
57. Bouchard-Mercier A, Paradis A-M, Rudkowska I, Lemieux S, Couture P, Vohl M-C. Associations between dietary patterns and gene expression profiles of healthy men and women: a cross-sectional study. *Nutrition Journal*. 2013;12(1):24.
58. Catoire M, Mensink M, Boekschoten MV, Hangelbroek R, Müller M, Schrauwen P, et al. Pronounced effects of acute endurance exercise on gene expression in resting and exercising human skeletal muscle. *PLoS one*. 2012;7(11).
59. Singer BH, Ryff CD, Council NR. Environmentally Induced Gene Expression. *New Horizons in Health: An Integrative Approach: National Academies Press (US)*; 2001.
60. Dluzen DF, Hooten NN, Zhang Y, Kim Y, Glover FE, Tajuddin SM, et al. Racial differences in microRNA and gene expression in hypertensive women. *Scientific Reports*. 2016;6:35815.
61. Ebuehi, O.A.T. and Dible, D.C. Hyperglycemic effect on brain cholinergic functions, oxidative stress and protein expression of brain derived neurotrophic factor (BDNF) on cognitive functions in streptozotocin-induced diabetic rats. *Research in Neuroscience* 2015; 4 (1): 1-9.
62. Ebuehi, OAT, Salami, Y, James, A.B and Mgbeadichie, I. N. Nutrigenomic studies of orange juice & vitamin C on gene expressions in neuronal tissues of Sprague Dawley Rats. *African Journal of Biochemical Research*, 7 (2): 13-18. *African Journal of Biochemical Research* 2013; 7 (2): 13-18.
63. Ebuehi, O.A.T. "Nutrients and Neurotransmitters: The chemicals of life in the brain". 10th Inaugural Lecture series, delivered at Main Auditorium, Univ. of Lagos, University of Lagos Press, Lagos, Nigeria, March 21, 2012, pp96

© 2021 Nze and Ebuehi; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/66193>