

Effect of grilled, parboiled and cooked breadfruit on some biochemical indices of normal Wistar rats

Obiajulu Christian Ezeigwe^{1*}, Gift Nonso Okwuenu¹, Chidiebere Malachy Chigbo¹, Peter Okwukwe Ekwunoh², Chisom Miracle Okeke¹, Chidinma Felicia Ezennaya³.

¹Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

²Department of Biochemistry, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.

³Department of Chemistry, Biochemistry and Molecular Biology, Faculty of Science, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Ebonyi State, Nigeria.

ABSTRACT

Background: The extracted seeds of breadfruit can be used for the preparation of delicious and nutritious diets which could either be roasted, parboiled, dehulled, steamed, or grilled. **Aim:** The aim of this study is to determine the effect of fortified feeds on the blood glucose level and some biochemical indices of normal Wistar rats.

Methods: The biochemical parameters (blood glucose levels, lactate dehydrogenase activity {LDH}, and Malondialdehyde levels {MDA}) were determined using standard diagnostic methods. **Results:** The result indicated no significant increase or decrease in blood glucose levels. Lactate dehydrogenase activity showed a significant decrease ($p < 0.05$) in groups fortified with 50% cooked, parboiled, and grilled breadfruit with respect to the control while the Malondialdehyde levels were within the normal range in the fortified groups compared with the control.

Conclusion: The findings from the research showed an improved nutritional quality of the feeds as no toxic or adverse effect was observed on the blood glucose level, lactate dehydrogenase activity, and Malondialdehyde level.

Keyword: Breadfruit, Glucose, Lactate dehydrogenase, Malondialdehyde, Cooked, Grilled, Parboiled.

Date of Submission: 09-03-2023

Date of Acceptance: 22-03-2023

I. INTRODUCTION

Nutrient deficiency plays an important role in micronutrient malnutrition globally and according to Muthaya et al., [1] the major problem of Low- and Middle-income countries (LMICs) is hidden hunger or a chronic lack of essential vitamins and minerals in the diet hence the need for diet fortification. Breadfruit is an evergreen tree that is basically grown for its starchy carbohydrate fruit [2]. According to Tropical Plant Research, Education, and Conservation, 2017 breadfruit is a high-yielding fruit plant as a single tree can produce a minimum of 200 fruit in each growing season.

It is grown in different soil type, especially in well-drained sandy loam or clay loam soil. Breadfruit is majorly planted during the rainy season and irrigated as needed during the first three months of the establishment [2]. Breadfruit is consumed by many due to its valuable nutritional composition. Processing improves some of the nutritional mineral composition of breadfruit. It has been reported that boiled breadfruit contains higher levels of vitamins A, B₂, B₃, B₆, D, E and K compared to roasted and boiled breadfruit [3].

Breadfruit whether ripe or unripe has been known from time immemorial to have several uses. Breadfruit is either cooked, roasted, baked, fried, or boiled before consumption [4]. Cooked breadfruit could be likened to taste like the taste of potatoes or freshly baked bread. The light weight of the breadfruit tree is also used as timber as the woods of breadfruit have been known to be resistant to termites and shipworms [5]. The phytochemicals contained in breadfruit have also been reported by [6,7] to serve as insect repellent. However, this fruit tree that has found versatile importance in various fields with very important nutrients and anti-nutrients and is used for diet fortification [8,9] is studied in this research to determine its effect on some biochemical indices.

II. MATERIALS AND METHODS

Sample Collection and Identification

The breadfruit used for this study was purchased from Orié market, Abagana in Njikoka Local Government Area, Anambra State, Nigeria. The sample was identified and authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is NAUH-77B.

Processing of Sample

The breadfruit was properly washed and mashed with water to remove its slippery nature and was then dried at room temperature for seven days. After the drying, the breadfruit was shared into three portions for processing.

Cooked Breadfruit

The first portion of the breadfruit was parboiled for 45 minutes. The pods were then separated from the chaffs with the help of a corona manual grinding machine. The breadfruit was then cooked using a kerosene stove for a period of 2 hours until it was soft and edible for consumption. Next, the cooked breadfruit was dried at room temperature and pulverized using corona manual grinding machine, and the now powdered cooked breadfruit was stored inside a well-labelled airtight plastic container until use.

Parboiled Breadfruit

The second portion of the breadfruit was parboiled by boiling it inside a pot containing water, on a stove for 45 minutes, till it was partially cooked. The pods were then separated from the chaffs with the help of a corona manual grinding machine, after which it was dried for one week at room temperature. Next, the pods were pulverized using corona manual grinding machine, and the now powdered parboiled breadfruit was stored inside a well-labeled airtight plastic container until use.

Grilled Breadfruit

The third portion of breadfruit was grilled on a frying pan using a stove. The seeds were then separated from the chaffs of the pods and were pulverized using corona manual grinding machine. The now powdered grilled breadfruit was stored inside a well labelled airtight plastic container until use.

Composition of the Rat Feed

The standard feed used was a product of Novum Agric Industries. It was purchased from a Feed dealer in Awka. The ingredients used in the compounding of the standard feed include grains, cereals, vegetables, protein meals, vitamins, minerals, essential amino acids, anti-toxins, enzymes. The composition of the ingredients is as follows: Oil (6%), Protein (16%), Fiber (7%), Ash (10%), Calcium (0.95%), and Phosphorus (0.65%). The feed was fortified with the processed grilled, parboiled, and cooked breadfruit in the following percentages: Using an analytical weighing balance, the feed and respective breadfruits were each measured. To 70g of feed, 30g of grilled breadfruit was added; to 70g of feed, 30g of parboiled breadfruit was added; to 70g of feed, 30g of cooked breadfruit was added; also, to 50g of feed, 50g of grilled breadfruit was added; to 50g of feed, 50g of parboiled breadfruit was added; and to 50g of feed, 50g of cooked breadfruit was added. These formulations were repeated until enough feed was prepared which lasted for a period of one month.

Study Design

A total of 35 Wistar rats weighing between 120g-150g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State, and randomized into seven groups of five rats each and used for the study. They were maintained and housed in cages under standard environmental conditions ($27^{\circ}\text{C} \pm 3^{\circ}\text{C}$, 12-hour light/dark cycle) in Chris Experimental Animal Farm and Research Laboratory, Awka. The rats were weighed, marked, and put into labelled cages. Their random blood glucose levels were also checked. The groupings are as follows:

Group A – Normal Control

Group B – 70% Standard Feed fortified with 30% cooked breadfruit

Group C – 70% Standard Feed fortified with 30% parboiled breadfruit

Group D – 70% Standard Feed fortified with 30% grilled breadfruit

Group E – 50% Standard Feed fortified with 50% cooked breadfruit

Group F – 50% Standard Feed fortified with 50% parboiled breadfruit

Group G – 50% Standard Feed fortified with 50% grilled breadfruit

Feeding of the Experimental Animals

The experimental rats were fed accordingly using the feed prepared for each of the groups. The feeding was done for a period of four weeks after which the rats were fasted and anesthetized with chloroform before blood collection. Blood was collected by cardiac puncture and put in the EDTA bottles and plain bottles for hematological and biochemical analysis respectively. The carcasses were properly disposed by burying.

Random Blood Glucose Concentration

The blood glucose levels of the rats were checked at weekly interval using One Touch Glucometer (Life Scan, USA) and test strips.

Liver Function Test

Serum biochemical indices routinely estimated for liver functions were analyzed. They include: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instructions.

Lipid Peroxidation

Lipid peroxidation was determined by the thiobarbituric acid-reacting substances (TBARS) assay method of Buege and Aust [10]. The reaction depends on the formation of complex between malondialdehyde and thiobarbituric acid (TBA). 0.4ml of serum was collected into the test tubes; 1.6ml of 0.25N HCl was added together with 0.5ml of 15% trichloroacetic acid and 0.5ml of 0.375% of thiobarbituric acid and then mixed thoroughly.

The reaction mixture was then placed in 100°C boiling water for 15 minutes, allowed to cool and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and the optical density recorded at 532nm against reagent blank containing distilled water.

The lipid peroxidation activity was calculated using the formula:

$$\frac{\text{Optical density}}{\text{Time}} \times \frac{\text{extinction co-efficient}}{\text{amount of sample}}$$

Where the extinction coefficient value is $1.56 \times 10^{-5} \text{M}^{-1} \text{CM}^{-1}$

The unit is expressed as $\mu\text{mol}/\text{MDA}/\text{mg}$ of protein.

Lactate Dehydrogenase

Serum lactate dehydrogenase enzyme was determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instructions.

Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean \pm SEM. Statistical analysis of the results obtained was performed by using ANOVA Tests to determine if a significant difference exists between the mean of the tests and control group. The limit of significance was set at $p < 0.05$.

III. RESULTS

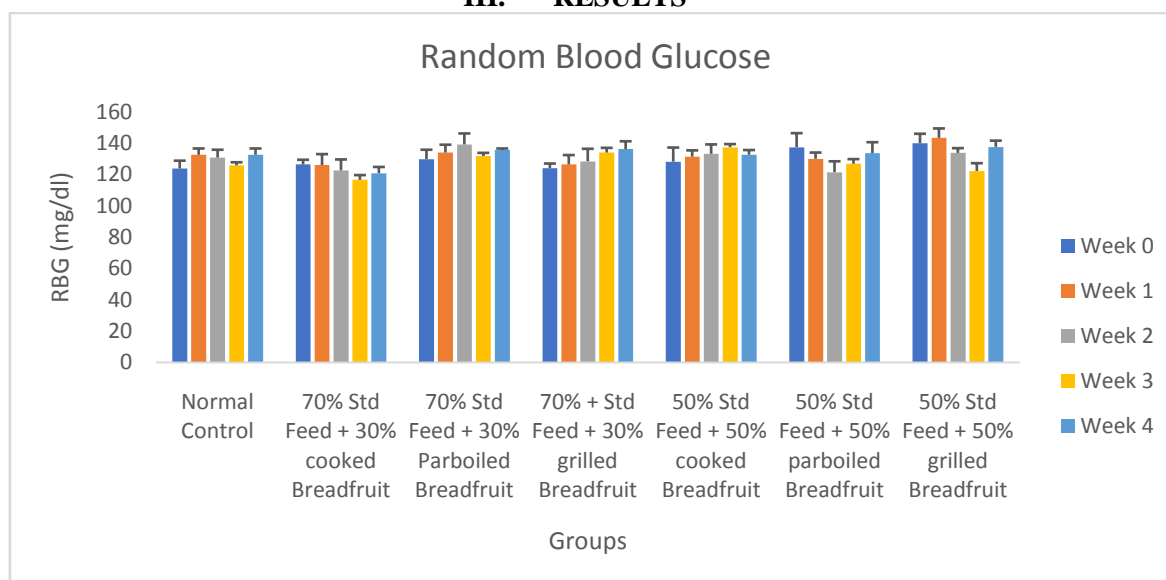


Figure 1: Random blood glucose level of normal rats fed with feed fortified with processed breadfruit. No significant difference was observed in the blood glucose level of all the experimental groups. The normal control group showed a fluctuating increase and decrease throughout the four weeks of the experiment (figure 1). The blood glucose level of the group fortified with 30% cooked breadfruit remained constant till after week 1 before a reduction was observed throughout the remaining weeks of the experiment. The group fortified with 30% of grilled breadfruit showed a continuous increase from week 0 to week 4. An increase in blood glucose level was observed throughout the group fortified with 30% of parboiled breadfruit with highest increase seen in week 2. The blood glucose level of the group fortified with 50% cooked breadfruit increased continuously throughout the week except in week 4 where a reduction was observed. The group fortified with 50% of parboiled breadfruit gradually reduced after week 0 with highest reduction observed in week 2 while the group fortified with 50% grilled breadfruit had an increase in blood glucose level at the first week of the experiment before gradual reduction was observed between week 2 to week 4 with the highest reduction seen in week 3 (figure 1).

Table 2: Results of the weekly random blood glucose of the rats fed with feed fortified with cooked, parboiled and grilled Breadfruit seed expressed as mean \pm SEM.

Groups	Random Blood Glucose (mg/dl)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Normal Control	123.80 \pm 4.67	132.60 \pm 4.39	130.80 \pm 4.75	125.80 \pm 2.13	132.60 \pm 4.18
70% Std Feed + 30% cooked Breadfruit	126.40 \pm 2.52	126.00 \pm 7.91	122.60 \pm 7.54	116.60 \pm 3.01	120.80 \pm 3.69
70% Std Feed + 30% Parboiled Breadfruit	129.80 \pm 5.83	134.00 \pm 5.12	139.20 \pm 6.60	131.80 \pm 1.74	135.60 \pm 9.64
70% + Std Feed + 30% grilled Breadfruit	124.00 \pm 3.18	126.40 \pm 5.84	128.40 \pm 8.48	134.00 \pm 3.02	136.20 \pm 4.73
50% Std Feed + 50% cooked Breadfruit	128.20 \pm 9.21	131.40 \pm 3.54	133.20 \pm 5.92	137.40 \pm 2.42	132.60 \pm 3.14
50% Std Feed + 50% parboiled Breadfruit	137.40 \pm 9.42	130.00 \pm 4.49	121.40 \pm 6.74	126.80 \pm 2.78	133.60 \pm 6.69
50% Std Feed + 50% grilled Breadfruit	140.00 \pm 5.53	143.40 \pm 6.45	133.80 \pm 2.78	122.20 \pm 5.08	137.60 \pm 4.39

Table 8: Effect of feed fortified with cooked, parboiled and grilled Breadfruit seeds on lactate dehydrogenase of Wistar rats expressed as mean \pm SEM.

Groups	LDH (U/l)
Normal Control	355.49 \pm 23.95
70% Std Feed + 30% cooked Breadfruit	364.96 \pm 11.57
70% Std Feed + 30% Parboiled Breadfruit	354.44 \pm 34.71
70% + Std Feed + 30% grilled Breadfruit	323.94 \pm 16.56
50% Std Feed + 50% cooked Breadfruit	238.75 \pm 8.48 ^b
50% Std Feed + 50% parboiled Breadfruit	247.17 \pm 14.61 ^b
50% Std Feed + 50% grilled Breadfruit	212.45 \pm 12.21 ^b

^bSignificant decrease with respect to normal control.

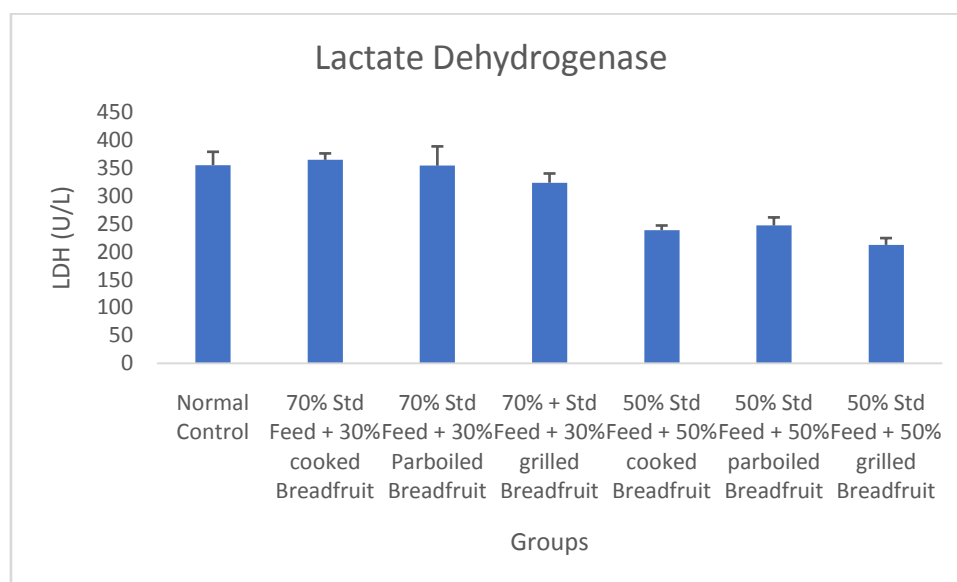


Figure 2: Lactate Dehydrogenase activity of normal rats fed with feed fortified with processed breadfruit.

The result of the Lactate dehydrogenase activity showed a significant decrease ($p < 0.05$) in groups fortified with 50% of cooked, parboiled, and grilled breadfruit with respect to their normal control group. The groups fortified with 30% cooked and parboiled breadfruit indicated a non-significant increase ($p > 0.05$) when compared to the normal control group while the group fortified with 30% grilled breadfruit showed a non-significant decrease ($p > 0.05$) with respect to its control group (figure 2).

Table 9: Effect of feed fortified with cooked, parboiled and grilled Breadfruit seeds on malondialdehyde of Wistar rats expressed as mean \pm SEM.

Groups	MDA ($\mu\text{mol/L} \times 10^{-10}$)
Normal Control	27.14 \pm 14.44
70% Std Feed + 30% cooked Breadfruit	73.12 \pm 60.64 ^a
70% Std Feed + 30% Parboiled Breadfruit	20.44 \pm 6.24
70% + Std Feed + 30% grilled Breadfruit	24.54 \pm 11.65
50% Std Feed + 50% cooked Breadfruit	16.47 \pm 14.51
50% Std Feed + 50% parboiled Breadfruit	17.92 \pm 9.58
50% Std Feed + 50% grilled Breadfruit	24.79 \pm 14.87

^aSignificant increase with respect to normal control.

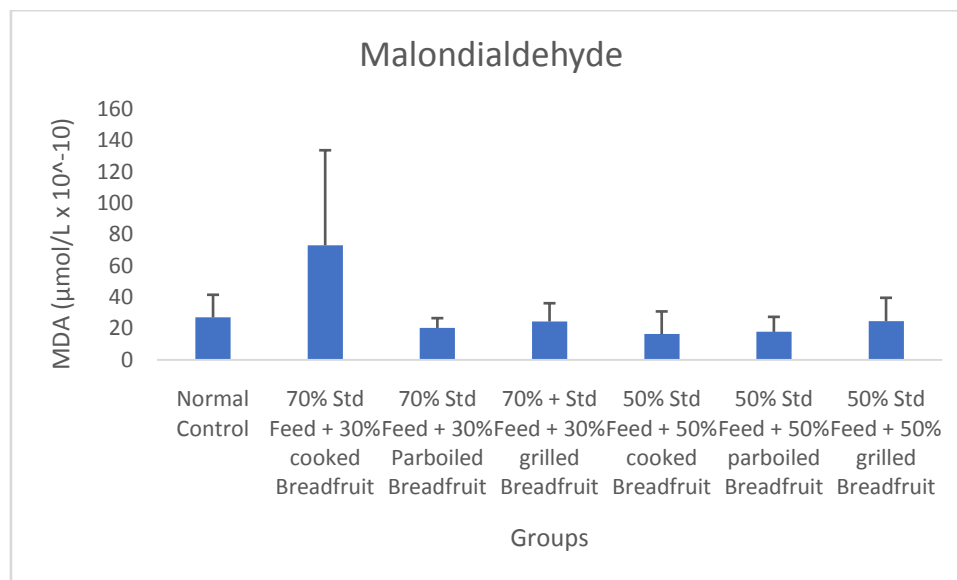


Figure 3:Malondialdehyde level of normal rats fed with feed fortified with processed breadfruit.

The Malondialdehyde level of the experimental animals decreased non significantly ($p>0.05$) in all groups when compared with the control group with exception to the group fortified with 30% cooked breadfruit which showed significant increase ($p<0.05$) with respect to the normal control group (figure 3).

IV. DISCUSSION

Very high ranges of blood glucose could indicate pre-diabetes or diabetes. According to American Diabetes Association [11], glucose is the main source of fuel for the brain and any interference to this source of fuel may cause brain damage, coma and death. The blood glucose level of the experimental animals observed in figure 1 remained within normal ranges as no significant difference was observed in the blood glucose concentration of all the experimental groups. Medicinal plants are useful in the treatment of ailments due to their nutritional and phytochemical composition which in most cases affects the biochemical parameters positively [12]-[14].

Lactate dehydrogenase (LDH) is an enzyme that is expressed extensively in almost all organs and tissues throughout the body such as the pancreas, kidney, blood cells, skeletal muscles, liver and heart because it is released during tissue damage, it is a marker of common disease such as heart failure [15]. LDH catalyzes the reversible conversion of lactate to pyruvate and a high amount of LDH in the blood is an indication of acute or chronic cell damage which can result to complicated heart damage [16]. However, the decreased activity of lactate dehydrogenase observed in the feed fortified with 50% breadfruit suggests that fortification of feeds with breadfruit has no toxic or adverse effect to organs or tissues.

The fortification of feeds with parboiled and grilled breadfruit has no toxic or adverse effect on the Malondialdehyde level of the experimental animals as a non-significant ($p>0.05$) decrease was observed in all groups when compared with the control group with the exception to the group fortified with 30% cooked breadfruit which showed a significant increase ($p<0.05$) when compared with the control group (figure 3). In other studies, carried out by [17,18], no adverse or toxic effect was observed in the Malondialdehyde level of the experimental animals.

V. CONCLUSION

Fortification of feed provides significant benefits to the animal as it helps improve the nutritional quality of the feed thus reducing nutrient malnutrition globally. From our findings, the processing of breadfruit does not pose any health challenges on the blood glucose level, lactate dehydrogenase activity, and malondialdehyde level.

References

- [1]. Muthaya, S.; Rah, J.H.; Sugimoto, J.D.; Roos, F.F.; Kraemer, K.; Black, R.E. The global hidden hunger indices and maps: An advocacy tool for action. PLoS ONE 2013, 8, e67860.
- [2]. Ragone D. 2008. Regeneration guidelines: breadfruit. In: Dulloo M.E., Thormann I., Jorge M.A. and Hanson J., editors. Crop specific regeneration guidelines [CD-ROM]. CGIAR Systemwide Genetic Resource Programme, Rome, Italy. 7 pp.
- [3]. Ezennaya, C. F. and Ezeigwe, O. C. (2023). A comparative study of raw, boiled and roasted *Treculia Africana* (African breadfruit). The Bioscientist Journal 11(1): 15-25.

- [4]. Janick, Jules; Paull, Robert E. (2008). The Encyclopedia of Fruit and Nuts (<https://books.google.com/books?id=cjHCoMQNkcgC&pg=PA476>) . CABI. p. 476. ISBN 978-0-85199-638-7.
- [5]. Little Jr., Elbert L.; Roger G. Skolmen (1989). "'Ulu, breadfruit" (https://web.archive.org/web/20141030071228/http://www2.ctahr.hawaii.edu/forestry/trees/CommonTreesHI/CFT_Artocarpus_altilis.pdf)(PDF). United States Forest Service. Archived from the original (http://www.ctahr.hawaii.edu/forestry/trees/CommonTreesHI/CFT_Artocarpus_altilis.pdf) (PDF) on 30 October 2014.
- [6]. Maxwell A.P. Jones; Jerome A. Klun; Charles L. Cantrell; Diane Ragone; Kamlesh R. Chauhan; Paula N. Brown & Susan J. Murch (2012). "Isolation and Identification of Mosquito (*Aedes aegypti*) Biting Deterrent Fatty Acids from Male Inflorescences of Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg)". *Journal of Agricultural and Food Chemistry*. **60** (15): 3867–3873.
- [7]. Avant, Susan (15 November 2013). "Studies Confirm Breadfruit's Ability to Repel Insects" (<https://www.ars.usda.gov/is/pr/2013/131115.htm>). US Department of Agriculture. Retrieved 14 June 2016.
- [8]. Chinedu, E. Sanni, S., Theresa N. and A. Ebere .2018. Effect of domestic cooking on the starch digestibility predicted glycemic indices, polyphenol content and alpha amylase inhibitory and properties of beans (*Phaseolus vulgaris*) and breadfruit (*Treculia africana*). *Intl. Journal Bio. Macromol.*
- [9]. Nwaigwe, J.O., & Adejumo, B.A. (2015). Qualities of African Breadfruit (*Treculia Africana*) Seed Flour as Influenced by Thermal Processing Methods and Duration, *International journal of technology enhancements and emerging engineering research*, 3(4), 102 - 108.
- [10]. Buege, J.A. and Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*. 52:302-310.
- [11]. American Diabetes Association (Standards of Medical Care in Diabetes. *Diabetes Care*. 2017;40:11-24.
- [12]. Victor Henry Azubuike Enemor, Uchechukwu Chibuzo Ogbodo, Ogechukwu Frances Nworji, Obiajulu Christian Ezeigwe, Chukwuemeka Obumneme Okpala, Gloria Chiamakalheonunekwu. (2020). Evaluation of the Nutritional Status and Phytomedicinal Properties of Dried Rhizomes of Turmeric (*Curcuma longa*). *Journal of Biosciences and Medicine*. 8:163-179.
- [13]. Ezeigwe Obiajulu Christian, Alaabo Prince Ogochukwu, Enemchukwu Benneth Nnanyelugo, Chukwuemeka Ugochi Vivian, Ifedilichukwu Nma Helen, Uhama Kingsley Chukwuka, Naomi Ngozi Nnadi and Iloanya Ebele Lauretta. (2020). Haematological and Biochemical effects of a combination of leaves of *Telfairia occidentalis* and *Mucuna pruriens* in male rats. *International Journal of Biosciences*; 16(1)139-149.
- [14]. BN Enemchukwu, OC Ezeigwe, OF Orinya, EL Iloanya, OO Ebeke, OI Okoro. (2022). Assessment and Evaluation of the antibacterial Activity of essential Oils of *Capsium annum*, *Monodora myristica* and *Ocimum canum* seeds on *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium*. *Journal of Applied Sciences*. 22(4):180-186.
- [15]. Ezeigwe OC, Ani ON, Okafor MC. Toxicological Studies of Citrus aurantifolia Fruit Juice in Wistar Rats. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. 2022a;10(4):38-47.
- [16]. Ezeigwe OC, Uchechukwu CO, Gift NO, Ezennaya CF and Ifedilichukwu NH. Acute and Sub-chronic Toxicological Studies of Citrus aurantium Fruit Juice in Wistar Rats. *Journal of advances in Medicine and Pharmaceutical sciences*. 2022b; 24(3).
- [17]. Arbo MD, Schmitt GC, Limberger MF. Subchronic toxicity of Citrus aurantium L (Rutaceae) extract and p-synephrine in mice. *Regul Toxicol Pharmacol* 2009;54:114–117.
- [18]. Eneh Frank Uchenna, Ezeigwe Obiajulu Christian and Omeje Martha. (2020). Lipid peroxidation activity and phytochemical constituents of extract of groundnut peels. *Journal of Applied Life Sciences International*; 23(1):16-22.

Obiajulu Christian Ezeigwe, et. al. "Effect of grilled, parboiled and cooked breadfruit on some biochemical indices of normal Wistar rats." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 17(3), (2023): pp 37-43.