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Biochemical Parameters, Activity Levels of Marker Enzymes and Serum Electolytes of Wistar Albino Rats Fed Mango (*Mangifera indica*) Pulp Formulated Diets

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Authors' contributions

This work was carried out in collaboration among all authors. Author PNI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ENO and BAA managed the analyses of the study. Author BAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Biochemical parameters, activity levels of marker enzymes and serum electrolytes of Wistar albino rats fed naturally ripe (control), unripe and artificially ripened mango pulp formulated diets were investigated. Ripe and unripe fruits were collected and used for the study and artificially ripened mangoes were obtained by wrapping unripe fruits with dark polyethylene bag; treatment with calcium carbide and by dipping into hot water. After ripening, the mango samples were air-dried, pulverized and used to formulate the 10%, 20%, 30% diets fed to rats for 28 days and their plasma collected for biochemical investigations. At 10% level of incorporation, plasma protein concentration in the control (65.63±0.12 mg/dl) was significantly higher ($p< 0.05$) than values for unripe groups (55.50±1.91 mg/dl) and calcium carbide (56.97±1.27 mg/dl) while Albumin concentration was highest in the unripe (41.33±3.58 mg/dl) but lowest in the calcium carbide (33.50±0.69 mg/dl) but at 20 and 30% levels, no significant difference was observed for both parameters. Billirubin was significantly higher in the calcium carbide groups but lowest in the control groups at all levels of

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incorporation while Creatinine and Urea revealed no significant difference at all levels. Marker enzyme assay showed significant variations only in the concentrations of Alkaline phosphatase and Gamma glutamyl transterase at the 30% level of incorporation only. Electrolyte assay revealed that at 30% level of incorporation, significantly lower levels of potassium were observed in the calcium carbide (3.43±0.06 mg/dl) group compared to the control (3.80±0.17 mg/dl).Thus, artificial ripening of fruits may adversely affect Biochemical parameters, activity levels of marker enzymes and serum electrolytes.

Keywords: Artificial ripening; mango; enzyme markers; biochemical parameters; electrolyte profile.

1. INTRODUCTION

The role of fruits is not limited to seed dispersal [1] but they also play very vital and indispensible role in human nutrition supplying the much needed growth supporting nutrients such as vitamins, minerals, complex starches, proteins, lipids and important phytochemicals essential for maintaining optimal health [2,3]. Among the fruits, mango (*Mangifera indica*) in the *Anacardiaceae* family generally found in tropical and subtropical regions is known as the king of fruits [4] in terms of marketing and consumption which cuts across every age group because it has a nutritious, energizing and revivifying taste [5].

Information from emerging data has posited that adequate consumption of fruits can improve vision and prevent diseases including age related ailments such as osteoarthritis, osteoporosis and dementia [6]. Also, [7] reported that insufficient intake of fruits such as mango may be a contributory factor to the higher rates of diseases such as cardiovascular diseases, Prostrate and colon cancer. Furthermore, [8,9,10] have submitted that depression among adolescents, adults, and the elderly as well as poor mental health may not be unconnected to inadequate consumption of fruits such as mango.

Due to unavailability during off seasons, fruit consumption is not as high as it should be. Availability is hampered due to the rapid ripening, softening, deterioration and subsequent spoilage of the fruits. More so, fruits once ripened are susceptible to diseases which consequently, limit their storage, handling and transportation [11]. Therefore to curb or minimize post harvest losses and to maximize profit, many climacteric fruits such as mango are harvested prior to ripening and ripening is induced artificially [12]. Post-harvest storage of mango fruits is a major challenge in a technologically evolving country such as Nigeria [13]. Fruit ripening is a natural process that initiates a complexity of biochemical alterations which gives rise to colour and pigment formation, starch breakdown, textural changes, aroma formation and fruit abscission [14]. In order to meet the high demand for seasonal fruit and to maximize profit, fruit vendors harvest even immature green fruits and subject them to various ripening methods [15]. According to [16] many chemical substances such as calcium carbide, acetylene and ethereal are used to induce ripening. More so, many artificial methods such as pit smoking, dipping into hotwater, wrapping in dark cellophane bags amongst others are employed in artificial ripening [17]. Information from emerging data suggests that the use of artificial ripening agents elicits deleterious effects to human health and may induce many health conditions such as abdominal pain, diarrhea, vomiting, headache, dizziness and insomnia which may progress to memory loss and even cerebral oedema if not checked [18,19]. The rising insistent call for food safety has propelled numerous research work geared towards investigating possible risks associated with the use of artificial methods in fruit ripening [20]. Owing to the importance of fruits to the wholeness and wellbeing of man, this study was carried out to investigate possible changes in Biochemical parameters, activity levels of marker enzymes and serum electrolyte indices of rats fed mango fruit formulated diets, occasioned by these artificial ripening methods.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Samples

Ripe and unripe mango fruits were collected from Okwuato Community in Aboh Mbaise Local Government Area of Imo State, Nigeria. The ripe and a set of the unripe fruits were cleaned, sliced, air dried, ground and stored in cellophane bags while the rest of the unripe fruits were cleaned and given the following treatments; a group was left in the sun for four hours after which they were tied in a clean empty dark poly bag for three days to induce ripening. A second

group was put in a plastic bucket containing ground calcium carbide (2 g of calcium carbide/100 g of mango) for 24 hours to induce ripening while the third group was soaked in hot water (100°C) for 5 mins; cleaned and covered with a thin cloth. After ripening was induced in all groups, the fruits were sliced, air dried, ground and stored in separate cellophane bags prior to analysis [17].

2.2 Rat Feeding Studies

Ninety Wistar albino rats weighing between 100 g and120 g were obtained from the Animal House of the Department of Anatomy, University of Lagos and kept in clean plastic cages under a 12hr light and dark cycle and housed in the Animal House of the Department of Pharmacology, University of Lagos. All experimental animals were handled in accordance with [21] and were approved by Institutional Research Ethics Committee at the University of Lagos. The rats were acclimatized to the laboratory environment for a period of one week. After which they were randomly assigned into five groups of 18 rats per group. Group one (control) were divided into 3 subgroups of 6 animals per group and sub group one were fed 10% naturally ripe mango pulp formulated diet, subgroup two were fed 20% naturally ripe mango pulp formulated diet and sub group three were fed 30% naturally ripe mango fruit formulated diet. Similarly groups two, three, four and five were subdivided and fed 10%, 20% and 30% unripe, polybag, calcium carbide and hot water induced ripened mango formulated diets respectively. The rats were fed ad-libitum for four weeks and left over feed and water were discarded daily. At the end of the feeding studies, 4 ml of blood was collected from nine rats per group (3 rats per sub group for the 10%. 20% and 30% diet groups) by ocular puncture through the optical vein and was transferred into lithium heparin bottles. The blood samples were spun with a Bucket Centrifuge at 4,000 rpm for 5 minutes to ensure proper separation of the plasma from the packed cells. The plasma obtained was kept in plain vial for biochemical analysis.

2.3 Biochemical Assay

The Serum levels of total protein, albumin, direct bilirubin, creatinine urea, AST, ALT, ALP, GGT, were determined using assay kits from Randox laboratories, U.K, by adopting the standard procedures described by [22] while sodium ion (Na+), potassium ion (K+) and chloride ion concentrations were determined using the methods of [23]. All investigations were carried out in the Chemical Pathology laboratory, University of Lagos Teaching Hospital, Idiaraba, Lagos.

2.4 Statistical Analysis

The data were analyzed by the analysis of variance (ANOVA). The difference between the various extracts and animal groups were compared using the Duncan multiple range test. The results are expressed as mean ± standard deviation. Significance was accepted at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

The results of biochemical parameters of Wistar albino rats fed naturally ripened, unripe and artificially ripened mango pulp *(Mangifera indica*) formulated diets is shown in Table 1. Results revealed that at 10% level of incorporation, there was no significant difference in plasma protein concentration obtained for the control (65.63±0.12 mg/dl) polybag, (69.87±9.58 mg/dl)) and hotwater (65.50±0.00 mg/dl), but each was significantly higher than values obtained for the calcium carbide and unripe groups while at 20 and 30% levels of incorporation no significant difference was observed in all groups.
Albumin concentration at 10% level of incorporation was highest in the unripe (41.33±3.58 mg/dl) but lowest in the calcium carbide (33.50±0.69 mg/dl)) but at 20 and 30% levels, no significant difference was observed. Values obtained for billirubin concentration followed a similar pattern at all levels of incorporation and revealed that billirubin was highest in the calcium carbide groups but lower in the control.

There was no significant difference ($p \geq 0.05$) at all levels of incorporation and in all groups in the concentration of creatinine and urea as shown in Table 2.

The result of the marker enzymes assay revealed that Aspartate transaminase levels at 10% and 30% showed a similar pattern as values were highest in the hotwater groups but lowest in the polybags while at 20%, no significant difference ($p \geq 0.05$) was observed between the control and the test groups but the levels of AST in the polybag (19.00±0.00 µ/l) was significantly lower than the values for calcium carbide (25.20±0.00 µ/l) and hotwater (26.03±8.60 µ/l)

groups. At 10 and 30% no significant difference (p≥0.05) was observed in the levels of Alanine transaminase (ALT) but at 20%, values for the unripe (26.20±2.42 µ/l) group, there was significantly higher levels than that of the polybag (20.00±3.46 µ/l) group. At 10% level of incorporation there was no significant difference in the level of Alkaline phosphatase (ALP) but at 20% level of incorporation, ALP level of the calcium carbide (193.47±7.39 µ/l) was significantly higher (p≤ 0.05) than those of unripe (124.93±28.52 µ/l) and hotwater (145.20±56.12 µ/l) groups only while at 30% level, values obtained for the control was significantly different from all the groups which ranged from 278.93±5.77 µ/l in calcium carbide to 116.13±5.77 µ/l in the polybag. At 10 and 20% level of incorporation, there was no significant difference in the level of Gamma glutamyl transterase (GGT) but at 30%, the concentration of (GGT) was significantly higher (p≤ 0.05) in the calcium carbide (44.93±1.62 mg/dl) compared to the control (32.00±0.00 mg/dl) and the rest of the group.

Results obtained for electrolyte concentrations shown in Table 4 revealed that at 10% level of incorporation, potassium concentration ranged from 3.90±0.35 mg/dl in hotwater to 3.53±0.06 mg/dl in polybag but at 20% level, no significant difference (p≤ 0.05) was observed while at 30% level of incorporation, the potassium level was highest in the control (3.80±0.17 mg/dl) but lowest in the calcium carbide (3.43±0.06 mg/dl) group whereas for sodium and chloride, no significant difference (p≤ 0.05) was observed at all levels of incorporation of formulated diet.

3.2 Discussion

The results from Biochemical study indicated a statistically significant decrease in serum total protein in the rats fed the 10% calcium carbide (56.97±1.27 mg/dl) artificially ripened mango pulp formulated diet, compared with the control (65.63±0.12 mg/dl) but compared to the unripe (55.50±1.91 mg/dl) no difference was observed indicating that the ripening method may probably not be an ineffective ripening method [24]. The reduction in protein may be attributed to increased proteolysis [22] or probably reduction in the synthesis of protein which maybe occasioned by the presence of a toxicant which may consequently have resulted into a shift in nitrogen metabolism [13]. Reduction may also be attributed to protein degradation which may have been initiated by inhibition of ribosomal activity possibly due also to a toxicant as reported by [25]. The observed decrease agrees with the information from emerging data suggesting that toxicants such as arsenic and phosphorus present in calcium carbide may leak into the fruit [26,27] in the course of ripening. Protein is a vital food nutrient necessary for the synthesis, structure, function, and repair of body tissue Elevated total protein is an indication of tissue damage whereas a decrease indicates depletion in the protein reserve and this may signal hepatic toxicity [28].

The plasma albumin level increased significantly in the unripe group of the rats fed 10% mango formulated diets compared with the control. This could be attributed to dehydration or probably liver function impairment since the liver is responsible for the metabolism of protein [29]. The hepatic cells are solely responsible for the synthesis of plasma albumin which is very vital in the regulation of blood pressure and the binding and transportation of cellular components such as water, cations, hormones, billirubin, thyroxine as well as drugs [30].

Increase in bilirubin concentration as seen in the artificially ripened mango groups especially in the group fed calcium carbide ripened mango at the three levels of incorporation of diet in this research masterpiece, may be suggestive of a hepatobiliary disorder occasioned by the complete or partial blockage of the bile ducts due to increased tension on the bile ducts owing probably to the presence of toxicants or may be due to infections which consequently led to the accumulation of plasma bilirubin [31,32]. Bilirubin is formed from the lysis of the heme component of red cells. A bilirubin test is important in establishing the underlying factor for jaundice. Hyperbilirubinema may result in the buildup of bilirubin in the brain which is a risk factor in neurological disorder. Elevated Billirubin may be attributed to increase in heamolysis, ineffective erythropoiesis and resorption of a hematoma. Hyperbillirubinaemia is prevalent in parenchymal liver disease and billiary obstruction [33]. In the current study, the highest level of bilirubin was noticed in the rats fed calcium carbide ripened mango formulated diet which differed significantly from the control and also from the other artificial ripening methods. This suggests that calcium carbide increases the level of billirubin more than the other test groups lending support to earlier report of hepatic dysfunction induced by toxicants such as arsenic found in calcium carbide [34,35].

Table 1. Biochemical parameters of wistar albino rats fed riped, unriped and artificially riped mango *(Mangifera indica*) pulp formulated diets

	Total protein (mg/dl)			Albumin (mg/dl)			Direct billirubin (mg/dl)		
Groups	**10%**	**20%**	**30%**	**10%**	**20%**	**30%**	**10%**	**20%**	**30%**
Control	65.63±0.12^{a}	67.30±12.12^{a}	61.80±4.50^{a}	35.47±2.37^{b}	39.80±4.50^{a}	40.43±5.13^{a}	0.67±0.12^{c}	0.60±0.00^{c}	0.77±0.12^{c}
Unripe	55.50±1.91^{b}	58.01±2.54^{a}	60.80±8.83^{a}	41.33±3.58^{a}	40.33±5.31^{a}	39.37±2.19^{a}	0.73±0.06^{b}	0.83±0.06^{b}	1.03±0.06^{b}
Polybag	69.87±9.58^{a}	59.90±1.91^{a}	61.00±1.91^{a}	38.60±0.00^{b}	38.80±0.17^{a}	40.40±0.00^{a}	0.93±0.12^{a}	0.67±0.12^{b,c}	0.90±0.00^{b}
Calcium carbide	56.97±1.27^{b}	58.43±0.64^{a}	61.73±0.64^{a}	36.50±0.69^{b}	37.33±2.71^{a}	41.40±0.00^{a}	1.33±0.12^{b,c,}	1.67±0.23^{a}	1.97±0.00^{a}
Hot water	65.50±0.00^{a}	63.23±1.96^{a}	60.20±0.00^{a}	37.76±0.98^{b}	38.63±4.79^{a}	41.17±1.33^{a}	0.87±0.12^{b,c}	0.63±0.06^{b,c}	1.00±0.00^{b}

Values are mean ± SD of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level

Table 2. Creatinine and urea of wistar albino rats fed riped, unriped and artificially riped mango *(Mangifera indica*) pulp formulated diets

Groups	Creatine (mg/dl)			Urea (mg/dl)		
	10%	**20%**	**30%**	**10%**	**20%**	**30%**
Control	1.00±0.00^{a}	1.27.±0.06^{a}	1.17±0.06^{a}	39.47±2.19^{a}	39.53±2.31^{a}	45.00±0.00^{a}
Unripe	1.10±0.17^{a}	1.23±0.12^{a}	1.22±0.35^{a}	39.47±2.19^{a}	41.33±1.15^{a}	42.47±0.46^{a}
Polybag	1.10±0.35^{a}	1.19±0.00^{a}	1.10±0.35^{a}	39.37±1.10^{a}	40.87±2.31^{a}	41.33±1.15^{a}
Calcium carbide	0.90±0.17^{a}	1.26±0.17^{a}	1.20±0.17^{a}	40.70±2.25^{a}	40.00±0.00^{a}	43.73±1.27^{a}
Hot water	1.20±0.17^{a}	1.30±0.00^{a}	1.19±0.17^{a}	40.73±2.19^{a}	40.87±2.31^{a}	44.53±1.15^{a}

Values are mean ± SD of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level

Table 3. Activity levels (µ/l) of marker enzymes of wistar albino rats fed riped, unriped and artificially riped mango *(Mangifera indica*) pulp formulated diets

Groups	Aspartate transaminase			Alkaline transaminase			Alkaline phosphatase			Gamma glutamyl transferase		
	10%	20%	30%	10%	20%	30%	10%	20%	30%	10%	20%	30%
Control	21.80±2.09[a]	20.93±2.48[a,b]	16.63±1.09[a]	18.00±1.73[a]	20.67±2.19[a,b]	20.33±3.75[a]	125.00±21.13[a]	156.67±10.85[a,b]	187.60±15.93[c]	30.83±1.62[a]	37.93±1.67[a]	32.00±0.00[b]
Unripe	23.33±4.62[a]	21.53±2.31[a,b]	19.63±2.82[a]	21.17±4.04[a]	26.20±2.42[a]	20.33±2.31[a]	172.13±27.48[a]	124.93±28.52[b]	220.93±0.92[b]	33.33±2.89[a]	33.73±1.27[a]	32.53±3.69[b]
Polybag	19.00±0.00[a]	19.00±0.00[b]	18.33±4.04[a]	16.67±0.29[a]	20.00±3.46[b]	20.73±1.96[a]	125.33±10.85[a]	155.40±23.90[a,b]	116.13±5.77[e]	35.33±4.62[a]	35.26±1.61[a]	33.00±2.42[b]
Calcium carbide	19.12±0.29[a]	25.20±0.00[a]	16.30±0.52[a]	17.33±1.44[a]	21.80±4.50[a,b]	21.53±4.10[a]	157.60±31.52[a]	193.47±7.39[a]	278.93±5.77[a]	35.43±3.21[a]	38.00±0.00[a]	44.93±1.62[a]
Hotwater	23.63±1.15[a]	26.03±8.60[a]	15.67±2.31[a]	18.40±4.16[a]	21.87±1.96[a,b]	23.20±0.35[a]	130.93±33.72[a]	145.20±56.12[b]	163.60±21.13[d]	36.63±3.41[a]	36.73±2.19[a]	37.07±0.81[b]

Values are mean ± SD of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level

Table 4. Serum electrolyte levels of wistar albino rats fed riped, unriped and artificially riped mango *(Mangifera indica*) pulp formulated diets

Groups↓	Potassium (mg/dl)			Sodium(mg/dl))			Chloride (mg/dl)		
	10%	20%	30%	10%	20%	30%	10%	20%	30%
Control	3.73±0.12[a,b]	3.50±0.00[a]	3.80±0.17[a]	140.23±0.41[a]	144.00±7.27[a]	138.67±5.77[a]	99.30±2.42[a]	100.80±2.25[a]	98.37±0.64[a]
Unripe	3.70±0.17[a,b]	3.59±0.12[a]	3.60±0.00[b]	139.93±7.51[a]	139.53±1.15[a]	137.47±4.73[a]	91.00±2.94[a]	100.07±0.23[a]	96.90±0.00[a]
Polybag	3.53±0.06[b]	3.71±0.16[a]	3.53±0.06[b]	135.80±6.06[a]	138.60±2.77[a]	137.27±2.54[a]	94.83±1.79[a]	97.23±4.21[a]	97.87±1.15[a]
Calcium Carbide	3.80±0.18[a,b]	3.67±0.26[a]	3.43±0.06[b]	138.80±2.60[a]	137.77±4.09[a]	136.13±5.77[a]	97.47±0.58[a]	100.00±0.00[a]	100.00±0.00[a]
Hotwater	3.90±0.35[a]	3.60±0.17[a]	3.57±0.06[b]	140.30±0.00[a]	139.20±6.23[a]	142.67±4.62[a]	96.63±3.18[a]	96.73±1.27[a]	99.93±0.12[a]

Values are mean ± SD of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level

Results obtained for creatinine and urea revealed that no significant difference was observed compared to the control and in all groups of rats at all levels of incorporation of diet. creatinine and urea analysis are carried out to evaluate kidney function [36]. Creatinine is produced from creatine as a waste product of normal breakdown of muscle tissue which is filtered out through the kidneys without re-absorption and then excreted in the urine. Blood level of creatinine is elevated when this infiltration is deficient, thus creatinine levels are used as a test of renal function [37]. Since no significant difference was observed for both parameters it implies that the kidney was not yet negatively affected. This report agrees with earlier report [38] who submitted no significant difference in both parameters.

The marker enzymes of hepatic function showed a varied response of the experimental rats. Aspartate transaminase (AST) and Alkaline transaminase (ALT) were slightly elevated though not statistically significant in most of the artificial ripening groups. This finding agrees with previous report of [15] and [13].

When compared to the control, a significant increase in the level of Alkaline phosphatase (ALP) was observed in the calcium carbide and unripe mango groups while the hot water and polybag groups reduced significantly in the rats fed 30% formulated diet. ALP are a set of vital metallo enzymes needed for the hydrolysis of ester bonds and the cleaving of phosphorus thereby creating an alkaline environment suitable for cellular functions [39]. They are abundant in the hepatic parenchyma, osteoblasts, intestinal mucosa, placental cells and renal epithelium. Significantly elevated activity level of ALP implies liver impairment as reported by [40] While the reduction as observed in the rats fed 30% polybag ripened mango formulated diet could be deduced as a potential risk factor in retarded growth as the enzyme is essential not just for the formation but also for calcification of the tissues of the bone. Similarly, Aspartate transaminases (AST) and Alanine transaminases (ALT) are liver enzymes responsible for amino acid metabolism. They are vital enzymes in investigating liver diseases and myocardial infarction. Increased plasma activity levels of AST (present in the same amount in the heart, skeletal muscles and liver) and ALT (most prevalent in the liver) are indicative of cardiac and hepatic tissue damage [41] since damage results in the drainage of these enzymes from intercellular stores into the plasma [42] therefore they serve as accurate indicators of liver or cardiac injury. Increased activity levels of ALP and a concomitant increase in billirubin concentration has been attributed to cholestastic liver injury; a decrease in bile flow due to impaired secretion by hepatocytes or may be due to obstruction of bile flow through intra or extra hepatic bile ducts owing to an elevated central venous pressure [43]. The observed significant elevation in the level of this enzyme (ALP) in the rats that were fed calcium carbide ripened mango formulated diet is yet another pointer to the health risks associated with the use of calcium carbide in fruit ripening which is in agreement with previous reports [44]. [45] reported that a concomitant elevation of the liver marker enzymes indicates hepatic disorder whereas an ALP increase that is inversely proportional to the increase in AST indicates a cholestatic disorder. The study revealed a slight though statistically insignificant increase in AST but it is possible that prolonged consumption of calcium carbide ripened mango fruits beyond 28days could result in findings that are statistically significant thus indicating liver impairment.

The various electrolytes analyzed showed that there was statistically significant difference only in potassium levels of the rats fed all groups of 30% artificially ripened mango formulated diet when compared with the control. Electrolytes produce positively and negatively charged ions. Electrolyte measurements are important tools in investigating conditions that lead to electrolyte disorder like dehydration, renal dysfunction, and diseases associated with the lungs, endocrine (glandular) or heart ailments. Alterations in electrolyte amounts can be attributed to numerous factors and ailments. The kidney controls the acid-base level of the body. Imbalance is indicative of renal dysfunction resulting in the accumulation of extracellular fluid; a disease condition known as oedema [46]. Accumulation of body fluid is determinant of many factors including dietary intake, rate of water absorbance and elimination from the body by the kidney as well as the regulating hormone (aldosterone) which is secreted by the adrenal cortex. Aldosterone retains sodium and enhances the depletion of potassium. Oedema may result in numerous health conditions such as cramps, irregular heartbeat and may lead to death [13].

4. CONCLUSION

Findings have revealed that artificial ripening of fruits caused various alterations in Biochemical parameters, activity levels of marker enzymes

and serum electrolytes especially for animals that were fed calcium carbide ripened mango formulated diets, therefore artificial ripening by these methods should be discouraged.

ETHICAL APPROVAL

All experimental animals were handled in accordance with the US National Institute of Health Guidelines for the Care and Use of Laboratory Animals (2011) and were approved by Institutional Research Ethics Committee at the University of Lagos.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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